

*HERPETOLOGIA
PETROPOLITANA*

**Proceedings
of the
12th Ordinary General Meeting
of the
Societas Europaea Herpetologica**

12 – 16 August 2003
Saint-Petersburg, Russia



Edited by
Natalia Ananjeva and Olga Tsinenko

Saint-Petersburg, 2005

HERPETOLOGIA PETROPOLITANA

Editors: Natalia Ananjeva and Olga Tsinenko

Proceedings of the 12th Ordinary General Meeting
of the Societas Europaea Herpetologica
August 12 – 16, 2003,
St. Petersburg, Russia

Published in St. Petersburg – Moscow, June 2005

Papers should be cited as (e.g.):

in: Ananjeva N. and Tsinenko O. (eds.), *Herpetologica Petropolitana. Proc. of the 12th Ord. Gen. Meeting Soc. Eur. Herpetol., August 12 – 16, 2003, St. Petersburg, Russ. J. Herpetol.*, **12**(Suppl.), pp.

Published by SEH

with the support of the Zoological Institute of Russian Academy of Sciences,
Program of the Department of Biological Sciences of Russian Academy of Sciences

“Fundamental Bases of Biological Resources Management,”

Program of the Presidium of Russian Academy of Sciences

“Scientific bases of the conservation of biodiversity in Russia,”

and program “Grants of President of Russian Federation supporting Leading Scientific Schools” NS 1647.2003.4

Cover photo: Toad-headed agama, *Phrynocephalus mystaceus* (Pallas, 1776)

Photograph: Nikolai Orlov

ISSN 1026-2296

ISBN 5-93881-044-2

Foreword

It was a particular proud for us and for Zoological Institute of the Russian Academy of Sciences that the SEH followed our invitation to organize the 12th Ordinary General Meeting (OGM) in St. Petersburg in August 2003. It was of special importance that it was held in conjunction with 2nd Ordinary Meeting of A. M. Nikolsky, s Herpetological Society under the Russian Academy of Sciences and North Eurasian Reptile Specialist Group of SSC/IUCN that should promote mutual enrichment and further development of cooperation between both societies in the field of fundamental and conservation research.

Approximately 340 delegates from 33 countries attended five days of meeting (August 12 – 16). Because of combined meeting of two societies there were much more participants than in the last previous OGM SEH (176 persons in France, 9th OGM SEH, in 1998, 148 persons in Cretea, Greece, 10th OGM SEH, in 1999 and 86 persons in Slovenia, 11th OGM SEH in 2001). Zoological Institute meeting hall was too small for housing this herpetological forum. Therefore, the main sites of the 12th OGM were beautiful historical buildings of the St. Petersburg Branch of the Russian Academy of Sciences, St. Petersburg State University, and Zoological Institute.

Herpetologica Petropolitana includes great part of the contributions presented as talks or posters during the 12th Ordinary General Meeting of the Societas Europaea Herpetologica held in St. Petersburg, August 12 – 16, 2003.

All manuscripts received were subjected to a reviewing process and were sent to reviewers.

List of scientific and linguistic reviewers:

Natalia Ananjeva, Leo Borkin, Miguel A. Carretero, David Cundal, Igor Danilov, Mathieu Denoël, Jeff Ettling, Jakob Hallermann, Georgy Lada, Luca Luiselli, Robert Macey, Claude Miaud, Tatyana Platonova, Andrew Snider, Anton Stumpel, Oksana Tishenko, Miguel Vences.

Their help was inestimable; we express our gratitude to all of them.

We are also thankful for assistance under preparation of this volume to Larissa Iohanssen and Roman Khalikov.

**St. Petersburg
March 28, 2005**

**Natalia Ananjeva,
Olga Tsinenko**

HISTORY AND ANNIVERSARY DATES OF RUSSIAN HERPETOLOGY IN ST. PETERSBURG

Natalia B. Ananjeva¹

The origin of herpetology in St. Petersburg as well as the beginning of Russian fundamental research in the fields of zoology, in general, and herpetology, in particular, can be assigned to the time of Peter the Great. The first museum in Russia, the *Kunstammer* (The Cabinet of Naturalia), was established by Peter the Great in 1714 in the capital of the Russian Empire. Its aim was to separate everything “that belongs to the animal kingdom” from other collections of the *Kunstammer* such as the Chamber of Rarities. The first exhibition of the Zoological Museum, the predecessor of the Zoological Institute, was opened in St. Petersburg on 4 July 1832. This date is now regarded as the time of the foundation of the Zoological Institute of the Russian Academy of Sciences (Fundamental Zoological Researches, 2004).

During the period of exploration and organized scientific expeditions, the Russian Academy of Sciences aimed to study the natural resources of the unbounded territories of Russia, and a number of prominent foreign scientists were invited to Russia for these research expeditions. One of the most prominent scientists was Peter S. Pallas, whose activities resulted in the first inventory of biological resources of the country. This period was also a time of exploration and description in herpetology. Many new collections were obtained owing to the first expeditions of the Academy of Sciences and purchases abroad, including a part of the second Seba collection, purchased at an auction in 1752 (Juriev, 1981). Among the first expeditions of interest to herpetologists was Peter S. Pallas’s trip to eastern Russia and Siberia (1768 – 1773), and many herpetological studies can be traced to this scientist. The historical center of Russian herpetology was the Zoological Museum (now Institute) in St. Petersburg. One of the results of Pallas’s great expedition was the first inventory of amphibians and reptiles of Russia, including descriptions of numerous new species. Most of the collections (including amphibians and reptiles) were taken to the Crimea where he finished writing his classical book, *Zoographia Rosso-Asiatica* (Svetovidov, 1987), which is often regarded as the complete inventory of herps. Unfortunately, most of his material is no longer available; some specimens were transferred later to the Zoological Museum of the Humboldt University in Berlin and the National Museum of Natural History in Paris.

Further development in the study of herpetology in St. Petersburg is associated with the official recognition of the Zoological Museum as an independent institution in

1832, whereas it was previously considered only a part of the *Kunstammer*. The first museum director, Johann F. Brandt (from 1830 to 1879), was well known for his herpetological activities; he described several new species of Asian reptiles and greatly enlarged the collection from various expeditions within the Russian Empire and abroad (particularly from Baron Georg Heinrich von Langsdorff in Brazil and through exchanges with other museums). Brandt and Alexander A. Strauch, who replaced him in the post of the museum director (from 1879 to 1890), both of whom were academicians, were founders of the Russian school of herpetology.

From the very outset, the St. Petersburg school of herpetology appeared to be an authoritative international center, with research activities extending far outside the geographical borders of the Russian Empire. Alexander A. Strauch was an especially important figure in the history of Russian and, indeed, world herpetology (Adler, 1989; Ananjeva, 1998). He had serious scientific communications with most of the leading herpetologists of the day, and used these contacts to increase the herpetological collection through purchase and exchange with other museums. A very significant source of new and interesting materials was from the first expedition of the famous explorer Nikolai M. Przewalski in the vast unknown and remote regions of Central Asia. By 1882, the herpetological collection included 5889 catalogue numbers representing 1222 species of reptiles (119 species of turtles, 14 crocodiles, 596 lizards, and 493 snakes) and 1285 catalogue numbers of 283 species of amphibians (9 caecilians, 224 frogs, and 50 salamanders). This collection was widely representative for its time (Strauch, 1889).

As a result of his own careful study of turtles, crocodiles, lizards, snakes, and salamanders, Strauch wrote several monographs and world synopses (Strauch, 1866, 1869, 1870, 1873, 1887, 1890). His review of the snake fauna of the Russian Empire (1873) and results of his identification and description of the amphibians and reptiles (1876) from Przewalski’s first expedition to Central Asia are of special value for Russian and world herpetology.

The next stage in the development of herpetology in St. Petersburg was closely related to activities of the leading Russian and Soviet herpetologist, Alexander M. Nikolsky (1858 – 1942), who was a successor to Alexander Strauch as curator of a combined division of fishes, amphibians and reptiles responsible for herpetological collection at the Zoological Institute.

¹ Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, 199034 St. Petersburg, Russia; E-mail: azemiops@zin.ru.

A student of St. Petersburg University, Nikolsky defended his masters dissertation in 1887 and doctoral dissertation in 1889. In St. Petersburg he was appointed curator of the university collection in 1887 and later held the position of Head of the Department of Fish, Amphibians, and Reptiles (1896 – 1903) (Mazurmovich, 1983; Adler, 1989; Ananjeva, 1998; Ananjeva and Darevsky, 2004). Nikolsky was the author of several important monographs with information of continued importance, including synopses of the herpetofaunas of Turkestan (1899) and the Caucasus (1913), and the first comprehensive book, in three volumes, on amphibians and reptiles *Fauna of Russia and Adjacent Countries* (1915, 1916, 1918). Another important contribution of Nikolsky was the study, identification, and description of the rich herpetological material collected in Iran by Nikolai Zarudny, who was one of the first zoologists to study the diverse fauna of Persia (Anderson, 1999). In 1896 – 1904 N. A. Zarudny conducted four famous expeditions to Persia (1896, 1898, 1900 – 1901, 1903 – 1904) supported by the Russian Academy of Sciences, the Russian Geographic Society, and the St. Petersburg Zoological Museum. It is interesting to note that in 1885, Nikolsky and Zarudny carried out a joint trip to the Transcaspien region and northeastern Persia.

Nikolsky's contemporary, the well-known herpetologist Jacques von Bedriaga, was informally associated with the Zoological Museum. His most famous and important contribution was a large monograph (in quarto and nearly 800 pages; 1898 – 1912) devoted to the study of the huge herpetological collections made by Przewalski and other Russian explorers during their Central Asian expeditions. Famous naturalists, collectors, and explorers associated with the Zoological Institute such as S. G. Gmelin, E. Eichwald, and E. Ménétriés, have made great contributions to the study of the Caucasus region.

Successors of these great zoologists to the position of Head and Curator of the Department of Herpetology at the Zoological Institute, USSR Academy of Sciences, were Sergei A. Chernov (1930 – 1960) (Photos 1 and 2) and Ilya S. Darevsky (Photos 2, 4 and 5) (1961 – 1995). Beginning in 1996, the curatorship of the Department of Herpetology and the administration of the Laboratory of Herpetology and Ornithology passed to Natalia B. Ananjeva (Scarlatto, 1982; Adler, 1989; Ananjeva, 1998; Ananjeva and Darevsky, 2004).

It was a great pleasure for us that the SEH congress was held in St. Petersburg in 2003, which was an important anniversary for three leading Russian and Soviet herpetologists, Sergei A. Chernov (100th Anniversary), Pavel V. Terentjev (100th Anniversary), and Lev I. Khosatzky (90th Anniversary).

Sergei A. Chernov (1903 – 1964) (Photos 1 and 2), a student of A. M. Nikolsky, was born in Khar'kov in the Ukraine, and graduated from the University of Khar'kov in

1926. In Khar'kov he was a student of A. M. Nikolsky. Chernov was appointed curator of the Department of Herpetology of the Zoological Institute at the USSR Academy of Sciences, succeeding Sergei F. Tsarevsky. The museum was reorganized to the Zoological Institute in 1930. Chernov, who became one of the leading Soviet herpetologists during his 30-year curatorship (1930 – 1960), conducted extensive herpetological field work in the Transcaspien region (1932), in the Caucasus (1937 – 1939), and in Tajikistan (1942 – 1944). The results were published as a series of articles and several monographs, including regional synopses of the herpetofauna of Armenia (1937, 1939) and Tajikistan (1959). His most widely known work was the book *Field Guide to the Reptiles and Amphibians of the USSR*, co-authored with Pavel V. Terentjev (three editions in 1936, 1940, and 1949) that was translated into English in 1965. During the last years of his life, Chernov studied the taxonomy of Palearctic snakes, revising the snakes earlier referred to the genera *Contia* and *Agkistrodon*. He began a large project on the skull structure of Palearctic snakes that unfortunately was left incomplete. An extensive osteological collection that was made by Chernov himself during his work on this project is stored at the Department of Herpetology. Despite an atmosphere of ideological barriers and “the iron curtain” in the Soviet Union, Chernov tried to keep contacts with the international herpetological community that is reflected in his correspondence stored in the archives of the Zoological Institute. He retired in 1960 because of serious health problems; his former student, Ilya Darevsky, was appointed Curator of the Department of Herpetology after him.

Pavel V. Terentjev (1903 – 1970) (Photos 1 and 2) a leading herpetologist and author of the world's first textbook of herpetology, worked at the Department of Herpetology at the Zoological Institute during World War II, including the time of the Siege of Leningrad (1942 – 1944). He was born in Sevastopol', Crimea, and graduated from Moscow State University in 1926 (Borkin, 2003). He studied taxonomy and biogeography, mostly of Anura, but also conducted ecological, biometric, and evolutionary research (Adler, 1989; Scarlatto, 1982; Borkin, 2003). He was the Head of the Department of Vertebrate Zoology at Leningrad State University (1954 – 1964) and was a professor of zoology for many years (1934 – 1942, 1944 – 1951; 1954 – 1964). His profound influence on his numerous students was very significant owing to his wide scientific interests in many fields and general discipline of biology. Terentjev also had an appointment at the Zoological Institute in 1951 and 1965 – 1968. His University scientific career continued in 1968 – 1970 when he was appointed the Head of Cabinet of biological statistics (biometria in Russian) at the University in the Department of Genetics. His former student, Vladimir Ishchenko (2004), believed that Terentjev's courses of statistics for biologists and herpetology that he attended in 1959 were most likely the first courses in these disciplines in



Photo 1. Pavel V. Terentjev (left) and Sergei A. Chernov (right).



Photo 2. Staff of Department of Herpetology including Ph. D. students, and visiting fellows. November 1957.

Sitting (from left to right): Sergei A. Chernov (curator of herpetology), Pavel V. Terentjev (Leningrad University, professor), Lev I. Khosatzky (Leningrad University, docent); **standing** (from left to right): Kirill B. Yuriev (Ph.D. student), Ilya S. Darevsky (Postdoc fellow), Lyudmila N. Lebedinskaya (collection manager), Günther Peters (Ph.D. student from Berlin), Abdulla M. Alekperov (Ph.D. student from Baku, Azerbaijan).



Photo 3. Lev I. Khosatzky January 1977.

the Soviet Union. It is generally known that Terentjev was a pioneer and early proponent of using statistics in zoology.

Terentjev was an author of *Field Guide to the Reptiles and Amphibians of the USSR* (together with Chernov), manuals on the frog (1950), and the first textbook in herpetology (1961). It is important to note Terentjev's contribution in the organization of the All-Union Herpetological Committee. This Committee (the predecessor of Nikolsky's Herpetological Society) was affiliated with the Department of General Biology of the USSR Academy of Sciences, and was led by Terentjev since 1962. He organized the first and second All-Union herpetological meetings that were held in Leningrad in 1964 and 1967.

Lev I. Khosatzky (1913 – 1992), (Photos 2 – 4) the younger contemporary of Chernov and Terentjev, was also associated with both Leningrad State University and the Zoological Institute of the Russian Academy of Sciences. He had a number of posts during his life, but most of his scientific career was associated with the Department of Vertebrate Zoology at Leningrad State University. He completed



Photo 4. Leningrad Herpetologists from Zoological Institute and Leningrad State University (from left to right): L. A. Nesson, V. A. Cherlin, N. B. Ananjeva, L. I. Khosatzky, I. S. Darevsky, L. Y. Borkin, N. L. Olrov. June 1979.

his academic training at Leningrad State University in 1936, and continued his career as a postgraduate student under Professor Kashkarov's supervision, who was the Head of the Vertebrate Zoology Department at that time.

After the Second World War and demobilization, Khosatzky had a special position at the Zoological Institute (1945 – 1948) to prepare his doctoral dissertation (the second and highest degree in the Soviet Union). Afterwards, he returned to the Department of Vertebrate Zoology at the University where he worked as a docent until his retirement in 1983. His research field was quite extensive and covered paleontology, morphology and variation of fossil and recent turtles, ecology and ecological physiology, and the zoogeography and conservation of amphibians and reptiles. His publication list consists of 264 titles (Borkin, 2003), most of which were written in Russian. He was a true teacher whose broad biological interests in many respects determined the future research field of his numerous university students. Khosatzky's paleontological activities were closely connected with both the Department of Vertebrate Zoology of Leningrad State University and the Department of Herpetology of the Zoological Institute of the Russian Academy of Sciences. The paleoherpetological collection at the Zoological Institute was founded as a result of his work. He mainly studied Neogene turtles of the USSR, and described some of the common turtles from the Late Cretaceous of Mongolia. Some of his papers and monographs (for example, Khosatzky and Redkozubov, 1989) dealt with the Neogene herpetofauna of Moldavia. Khosatzky was the graduate supervisor for Ananjeva and Borkin during their study at the University. I. Khosatzky also trained many paleo- and neoherpeto-

logists, among them N. Ananjeva, L. Borkin, K. Juriev, L. Nesson, V. Borkhvardt, N. Golubev, A. Zakharov, V. Cherlin, I. Gaizhauksene, M. Ivakhnenko, V. Kotlyarevskaya, V. Kuznetzov, G. Peters, O. Redkozubov, V. Tofan, E. Kovalenko, G. Cherepanov, and many others.

Lyudmila N. Lebedinskaya (1906 – 1989) (Photos 2 and 5), the permanent collection manager and secretary of the Department of Herpetology from 1942 to 1978, worked for 36 years with all three outstanding herpetologists, S. Chernov, P. Terentjev, and L. Khosatzky. She protected the collections of the Zoological Institute, including the herpetological specimens, during the Second World War and the Siege of Leningrad (together with P. Terentjev).

The scientific, teaching, and organizational activities of three prominent zoologists and herpetologists whose centenaries (Chernov and Terentjev) and 90th Anniversary (Khosatzky) coincided in 2003 with the year of the 12th SEH Congress, contributed greatly to the development of herpetology in the Soviet Union. Their efforts allowed the creation of a scientific center and did not interrupt academic lineages in herpetology. Data on the continuity between M. Bogdanov and A. Nikolsky at the St. Petersburg Imperial University (Altig, 1989: 183) should be supplemented with academic ties at Leningrad State University, consisting of doctoral dissertations (candidate in USSR and Russia) under the supervision of Terentjev and Khosatzky. It is significant to note the close and fruitful contacts between the Department of Vertebrate Zoology at Leningrad University and the Department of Herpetology at the Zoological Institute; Terentjev and Khosatzky worked at the Zoological Institute during different periods of time. The following generations



Photo 5. From left to right: Natalia Ananjeva, Leo Ja. Borkin, Lyudmila N. Lebedinskaya, Vasilyi A. Kireev (Ph.D. student from Élista city, Kalmykia), and Ilya S. Darevsky. 1971.

of Soviet and Russian herpetologists received systematic training from them when studying zoology at Leningrad University.

This project is supported by Grant of President of Russian Federation for leading scientific schools NS 1647.2003.4 and by grant RFFI 05-04-48165 to Natalia B. Ananjeva

REFERENCES

- Adler K.** (1989), "Herpetologists of the past," in: Adler K. (ed.), *Contributions to the History of Herpetology. Vol. 5*, Soc. Study Amphib. Reptiles, pp. 5 – 178.
- Altig R.** (1989), "Academic lineages of doctoral degrees in herpetology," in: Adler K. (ed.), *Contributions to the History of Herpetology. Vol. 5*, Soc. Study Amphib. Reptiles, pp. 179 – 202.
- Ananjeva N.** (1998), "Institutional profile — Department of Herpetology, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia. History and current research," *Herpetol. Rev.*, **29**(3), 136 – 140.
- Ananjeva N. and Darevsky I. S.** (2004), "Herpetological research in Zoological Institute, Russian Academy of Sciences," in: Stepanyanz S. D. (ed.), *Fundamental Zoological Research. Theory and Methods. Proc. Int. Conf. "170th Anniversary of Zoological Institute,"* October 23 – 25, 2002," KMK, pp. 27 – 36 [in Russian].
- Anderson S.** (1999), *The Lizards of Iran*, Society for the study of amphibians and reptiles, Ithaca (NY).
- Borkin L. Ja.** (1999), "Lev Isaakovich Khosatzky. Life and works," in: *Actual Problems of Herpetology and Toxinology. Issue 3*, Institute of Ecology of Volga River Basin, Russian Academy of Sciences, Tol'yatti, pp. 3 – 40.
- Borkin L. Ja.** (2003), "Lev Isaakovich Khosatzky: bibliography (1941 – 1999)," in: *Actual Problems of Herpetology and Toxinology. Issue 6*, Institute of Ecology of Volga River Basin, Russian Academy of Sciences, Tol'yatti, pp. 10 – 36.
- Borkin L. Ya.** (2003), "Moscow period of life of P. V. Terentjev," in: Rossolimo O. L. and Dunayev E. A. (eds.), *Moscow Herpetologists*, Zoological Museum, Moscow State University, KMK, Moscow, pp. 385 – 422.
- Chernov S. A.** (1937), *The Field Guide of the Snakes, Lizards, and Turtles of Armenia*, Academy of Sciences [in Russian].
- Chernov S. A.** (1939), "Herpetological fauna of Armenian SSR and Nakhichevan ASSR," in: *Zool. Coll. Papers of Armenian Branch of Academy Science of USSR*, Vol. 1, pp. 79 – 194 [in Russian].
- Chernov S. A.** (1959), "Reptilia," in: *The Fauna of Tajik SSR. Proc. of the Tajik SSR Academy of Sciences*, Issue 48, Stalinabad [in Russian].
- Fundamental Zoological Researches. Theory and Methods* (2004), Zoological Institute, Russian Academy of Sciences, KMK, Moscow – St. Petersburg [in Russian]
- Itschenko V. G.** (2003), "Pavel Victorovich Terentjev (Teacher)," in: Rossolimo O. L. and Dunayev E. A. (eds.), *Moscow Herpetologists*, Zoological Museum, Moscow State University, KMK, Moscow, pp. 422 – 435.
- Juriev K. B.** (1981), "Albert Seba and his contribution to the development of herpetology," *Trudy Zool. Inst. AN SSSR*, **101**, 109 – 120 [in Russian].
- Khosatzky L. I. and Redkozubov O. I.** (1989), *Neogene Turtles of Moldavia*, Shtiinza, Kishinev [in Russian].
- Mazurmovich B. N.** (1983), *Alexander Mikhailovich Nikolsky*, Nauka, Moscow [in Russian].
- Nikolsky A. M.** (1915), *The Fauna of Russia and Adjacent Countries. Reptilia. Vol. I. Chelonia and Sauria*, Academy of Science, Petrograd [in Russian].
- Nikolsky A. M.** (1916), *The Fauna of Russia and Adjacent Countries. Reptilia. Vol. II. Ophidia*, Academy of Science, Petrograd [in Russian].
- Nikolsky A. M.** (1918), *The Fauna of Russia and Adjacent Countries. Vol. III. Amphibia*, Academy of Science, Petrograd [in Russian].
- Scarlato O. A.** (ed.) (1982), *Zoological Institute of the Academy of Sciences of the USSR. 150 Years*, Nauka, Leningrad [in Russian].
- Strauch A.** (1866), "Synopsis der gegenwartig lebenden Crocodyliden, nebst Bemerkungen uber die im Zoologischen Museum der Kaiserlichem Akademie der Wissenschaften vorhandenen Repräsentanten dieser Familie," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **X**(13), 1 – 120.
- Strauch A.** (1869), "Synopsis der Viperiden, nebst Bemerkungen uber die geographische Verbreitung dieser Giftschlangenfamilie," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **XIV**(6), 1 – 143.
- Strauch A.** (1870), "Revision der Salamandriden-Gattungen nebst Beschreibung einiger neuen oder weniger bekannten Arten dieser Familie," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **XVI**(4), 1 – 107.
- Strauch A.** (1873), "Die Schlangen des Russischen Reichs, in systematischer und zoogeographischer Beziehung geschildert," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **XXI**(4), 1 – 287.
- Strauch A.** (1876), "*Part III. Reptilia and Amphibia*," in: Przewalsky N. M., *Mongolia and the Tangut Country. Vol. 2*, Imper. Russ. Geogr. Soc., St. Petersburg [in Russian], pp. II, IV, 1 – 55.
- Strauch A.** (1887), "Bemerkungen uber die Geckoniden-Sammlung in zoologischen Museum der Kaiserlichem Akademie der Wissenschaften zu St. Petersburg," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **XXXV**(2), 1 – 72.
- Strauch A.** (1889), *Das Zoologische Museum der kaiserlichen Akademie der Wissenschaften zu St. Petersburg in seiner Funfzigjarigen Bestehen*, St. Petersburg [in Russian].
- Strauch A.** (1890), "Bemerkungen uber die Schildkrotensammlung im zoologischen Museum der Kaiserlichem Akademie der Wissenschaften zu St. Petersburg," *Mem. A'Acad. Imp. Sci. St.-Petersbourg. VII Ser.*, **XXXVIII**(2), 1 – 127.
- Svetovidov A. N.** (1978), *The Types of the Fishes Species Described by P. S. Pallas in "Zoographia Rosso-Asiatica"*, Nauka, Leningrad [in Russian].
- Terentjev P. V. and Chernov S. A.** (1936), *The Brief Field Guide of Amphibians and Reptiles of USSR*, Uchpedgiz [in Russian].
- Terentjev P. V. and Chernov S. A.** (1940), *The Field Guide of Amphibians and Reptiles of the USSR. 2nd Edition*, Leningrad [in Russian].
- Terentjev P. V. and Chernov S. A.** (1949), *The Field Guide of Amphibians and Reptiles of the USSR. 3rd Edition*, Sovetskaya Nauka, Moscow [in Russian].

**SYSTEMATICS,
PHYLOGENY,
BIOGEOGRAPHY,
AND GENETICS
OF AMPHIBIANS
AND REPTILES**

PLURAL NAMES EARN AND NEED PLURAL VERBS

L. Bauer¹

Keywords: Taxonomy, Nomenclature, Herpetology, Batrachology,.

INTRODUCTION

It is an omission of the International Code of Zoological Nomenclature — really an omission to be regretted and to be corrected in future editions — that family names are not expressly mentioned as words in plural form. Clearly they are and understanding will be furthered by realizing that indeed they are.

Names of families and higher categories are words in plural form. As such names indeed do represent plural contents, verbs should not be used in singular. Zoologists not always seem to be aware of this and especially in herpetology I noticed that the official family names often appear with wrong verb forms.

As mostly North American and other AngloSaxon publications often show this aberration, people whose mother tongue is not English erroneously might consider exactly an aberrant form only to be correct. The use of -ids instead of -idae makes no difference, apart from a would be cosmetic English look.

MATERIAL AND METHODS

After having been bewildered every so often when using mainly AngloSaxon herpetological literature I compared some other zoological disciplines as well. To make my point I compared codification of botany and zoology. And from grammar I tried to find out what custom might underlie such erroneous use.

In botany the situation has been clearly defined by wording in the nomenclatural code, which I possess in several editions, published after botanical congresses, normally with four or five years in between. May the ICZN follow. Follow in wording badly needed clarification and in more frequently being issued.

RESULTS

International Code of Botanical Nomenclature adopted by the Sixteenth International Botanical Congress, St Louis, Missouri, July-August 1999.

16.1: The name of a taxon above the rank of family is treated as a noun in the plural and is written with an initial capital letter.

18.1: The name of a family is a plural adjective used as a noun...

Mary Irish and Gary Irish, *Agaves, Yuccas and Related Plants*, Portland: 2000.

Page 14: ...the Agavaceae can be said to occur in a wide range of habitats...

Page 25: The Agavaceae have perfect flowers...

Botanical family names end in -aceae. Therefore -aceae is nomenclaturally identical with and completely comparable to -idae.

Stephen J. Gould, *Wonderful Life*, London: Penguin, 1991.

From page 112 I cite Leif Størmer: The Trilobitomorpha are...

Page 158, Gould himself: The Crustacea are an enormous and diverse group...

J. E. Webb, J. A. Wallwork and J. H. Elgood, *Guide to Living Reptiles*, London: 1978.

Page 39: The Cryptodira include the great majority...

Page 79: The Squamata are probably...

Page 118: The Scolecophidia include...

Page 118: The Henophidia, containing several families, show a transition...

Page 118: The Henophidia are clearly remnants...

Page 138: The Elapidae have...

Lester Short and Jennifer Horne, *Toucans, Barbets and Honeyguides*, Oxford: 2001.

Page 15: The Ramphastidae or toucans are...

Some herpetologists might write “Ramphastids are” but Ramphastidae actually is the same.

The Ramphastidae are the toucans and in this combination they cannot but correctly use a plural verb form. Otherwise they sometimes seem to have difficulties.

Page 20: Trachyphonini — the tribe Trachyphonini Prum, 1988, is monotypic. {OK}

Page 21: Lybiini — the Lybiini Sibley and Ahlquist, 1985, contains the rest. {in error}

Page 23: Calorhamphini — the Calorhamphini Prum, 1988, is a monotypic Asian tribe. {??}

Page 23: Megalamaitini — the Megalamaitini Blyth, 1852, comprise the other... {OK}

Page 24: Capitonini — the first of the two tribes in the neotropics is Capitonini {OK}

¹ IL CORBAU, Sassigné, F-58700 Montenoison, France.
E-mail: l.bauer@wanadoo.nl; peruviridis@wanadoo.fr

Page 24: Semnornithini — the Semnornitini Prum, 1988, have a bony crest {OK}

Page 28: Prodotiscini — the Prodotiscini Roberts, 1922, is a tribe containing... {??}

Page 29: Indicatorini — the {tribe, LB} Indicatorini Swainson, 1837, is comprised of... {??}

{??} in this construction the situation appears slightly unclear; better: ...form a tribe ...

Indeed I really do admire this book as well as others referred to, so let me state that remarks like these review great work done, and should not be read as negatively intended criticism.

Claus Nielsen, *Animal Evolution. Second Edition*, Oxford: 2001.

Page 35: The mitochondria have...

First citation of course is not a family name nor phylum or whatsoever, but the form clearly demonstrates what it is all about: mitochondria is the plural form of mitochondrion. Names of higher categories may be plural forms of a word ending in -um.

Page 48: The phylum Placozoa comprises... The phylum is subject.

Page 114: The Gnathostomulida apparently all have...

Page 169: Annelids are... {Annelida would express exactly the same}

Page 172: The Echiura resemble annelids...

Page 210: The Mandibulata also appear to be...

Also some other interesting citations demonstrate what reasons there are to make my point.

Pages 89 and 271: Protonephridia are... Who does immediately realise this is anatomy?

Protonephridia might be organisms in stead of organs. What difference then? Nothing!

Several of these cited places come from the middle of pages text. I do have the impression that editorial influence withheld standard verbs with phylum names in opening sentences.

Let me check some different words again.

Page 304: The spermatozoa have the head extended... Interestingly the sperm cells are compared with animals but might -zoa (in this book we encounter Placozoa, Eumetazoa, Gastraeazoa, Bryozoa, Rhombozoa) as animal taxa — singular: taxon — induce especially editors but authors as well to promote either singular verb forms or different word forms?

They do. Vide page 48 and page 51: The Eumatazoans...

Paul L. Th. Beuk (ed.), *Checklist of the Diptera of The Netherlands*, Utrecht: 2002.

In this checklist we find

The family Sciaridae is found on every continent...*

*Correct: The family as a word is subject!

Sciaridae are small flies...

Pediciidae are long and slender...

Tipulidae are medium- to large-sized...

Dixidae are medium-sized...

Stratiomyidae are very variable ...

Conopidae are parasitoids...

Braulidae are well-known bee-parasites.

Chloropidae are small flies... Chloropidae occur on meadows...

Nycteribiidae are obligate blood-sucking ectoparasites of bats.

Hypodermatidae are obligatory parasites of mammals.

Oestridae are obligatory parasites of mammals.

This illustrates the correct way to grammatically handle family [higher category] names.

DISCUSSION

It is an idiosyncrasy of English grammar (idiom) — making it a very interesting language — that several words in singular form (police, people, kin, crew) come with a standard verb, i.e., not third person singular, when it is felt or realized that such words refer to some collectivity of several individuals, say plural contents.

In zoological use of family names the verb also has to be used standard. I am unable to grasp that at present exactly in English, at least in herpetology and often in other zoological texts as well, plural form and plural contents of taxa above the genus group are buried in erroneous use of verbs in third person singular.

If we refer to Webb and colleagues, who also compiled a Guide to Living Amphibians, we cannot help but get the impression that in understanding of taxonomy and nomenclature no advance has been gained. On the contrary: modern language use in several herpetological publications hampers understanding.

It is not grave of course if one uses erroneous forms in ones own writings and even in print there seldom will arise problems. But — apart from unnecessary difficulties in understanding by colleagues — the grave thing might be and apparently already does occur that editors try to “correct” already correct wording.

If one uses a term like “the family” verbs should appear in singular, if one wishes to use verbs in third person singular a comparable construction becomes essential. But it appears unnecessary in scientific publications to substitute -ids for -idae: ids is just a formal deviation from idae and has no different meaning.

It would be a great victory of common sense and philosophical understanding if the ICZN in future editions should reach the maturity to state the plural character of names for every taxon above the genus group — id est family group names and also of otherwise not regulated names given to all higher categories.

A REPLACEMENT NAME FOR *Lophopus*

L. Bauer¹

Keywords: *Hyla*, *Quinzhyla*, replacement name, *Lophopus* Tschudi, 1838.

INTRODUCTION

It is clear that *Hyla marmorata* Daudin is the type by monotypy of *Lophopus* Tschudi, 1838. This name *Lophopus* in Amphibia is preoccupied by *Lophopus* Duméril, 1837 in Polyzoa.

See Bokermann and ASW.

Hyla marmorata belongs to a complex of smallish treefrogs with the aberrant chromosome number of $2N = 30$ instead of $2N = 24$. For some twenty years now I am convinced that this group is sufficiently distinct from typical *Hyla* to be nomenclaturally separated and I decided indeed they should be recognized as such.

As a new name is needed, in 1985 already I concluded that the best way seems to choose a replacement name for *Lophopus* Tschudi and therefore I coined *Quinzhyla*.

The type species so becomes *Hyla marmorata* Daudin (= *Lophopus marmoratus* Tschudi) and the name takes the date of Tschudi's publication. First species group of course is that around *Quinzhyla marmorata*.

Definition. Fairly small neotropical treefrogs with haploid chromosome complement of 15. Differing from typical Hylineae also in the, almost *Rhacophorus*-like, full webbing of toes. The species often are lichen-like cryptically coloured and have a glandular body outline.

Etymology. The name is referring to the haploid chromosome number in latin languages (quinze, quince, quindecem) contaminated with hyla.

¹ IL CORBAU, Sassigné, F-58700 Montenoison, France.
E-mail: l.bauer@wanadoo.nl; peruviridis@wanadoo.fr.

DISCUSSION

Some twenty years ago the name *Quinzhyla* appeared in photocopy only in very restricted circulation. But with the same etymology diverse alternatives remained possible and I did hesitate to choose.

Quindechyla is closer to correct use of Latin, as can be Quindecyla and Quindehyla but they lack euphonious character, whereas *Quinzhyla* has a better look, a more agreeable image.

Quincehyla, Quinchyla, Quinhyla, and Quinzyla also came in the picture, but — although I do like the eight letter forms — I choose *Quinzhyla* from a purely cosmetic point of view.

Now in my name *Quinzhyla* the first part is from French origin, which was one of the reasons to hesitate: combining a modern language form with an existing name of classical origin must not be common practice. That is why this name remained in drawers. But I could not resist.

So I decided to take the word as it suits me. The replacement name is *Quinzhyla*.

REFERENCES

- Bokermann W. C. A. (1964), "Notes on the frogs of the *Hyla marmorata* group...", *Senck. biol.*, **45**(3/5), 243 – 254.
Amphibian Species of the World, Database 1998 – 2002.
<http://research.amnh.org/cgi-bin/herpetology/amphibia>.

GENOME SIZE VARIATION IN THE BALKAN ANURANS

L. J. Borkin,¹ S. N. Litvinchuk,² J. M. Rosanov,² G. Džukić,³ and M. L. Kalezić^{3,4}

Keywords: genome size, DNA flow cytometry, Anura, *Bombina*, *Bufo*, *Hyla*, *Pelobates*, *Rana*, Serbia and Montenegro, Macedonia.

Serbia and Montenegro harbor 16 anuran species arranged in five genera and five families (Džukić and Kalezić, 2004). In the area, many anurans are distributed at their geographic (southern or northern) limits. Zoogeographically, the country belongs to the southern part of the European or Boreal Region (the Central European Province), in contact with the Mediterranean Region (the Balkan Province) at the south (Borkin, 1999; Džukić and Kalezić, 2004). Therefore, the study of anurans of Serbia and Montenegro is of obvious importance in terms of systematics, geographic variation, and zoogeography. The goal of present paper was to evaluate the genome size variation in some Balkan anurans in comparison with that of frogs and toads from eastern Europe, taken mostly from the Euro-

pean part of the former USSR, with some samples from Romania and Hungary.

In a total, we examined 118 specimens from 27 samples of 13 species and subspecies collected across Serbia and Montenegro, as well as from two samples from Macedonia (Table 1; Fig. 1). The amount of DNA per nucleus (genome size) was measured by flow cytometry. Red blood cells of the grass frog (*Rana temporaria*) taken in St. Petersburg and Pskov Provinces were used as a reference standard. The details of the technique have been published previously (Borkin et al., 2001).

Two species of fire-bellied toads, *Bombina bombina* and *B. variegata*, are distributed in Serbia and Montenegro. The latter species is presented by two subspecies. We found that the average genome size in *B. bombina* was higher than in *B. v. variegata* at the level of 5.5%, and value ranges of both taxa did not overlap (Table 1; Fig. 2). Such differences between these species, which are known to hybridize in contact zones, were recorded in the Ukrainian Transcarpathians as well (Khalturin et al., 1996, 2001).

Importantly, another subspecies of the yellow-bellied toad, *B. v. scabra*, proved to be closer to *B. bombina* rather than to *B. v. variegata* (Table 1; Fig. 2). Genome size values in both subspecies of *B. variegata* did not even overlap, unlike the case with *B. v. scabra* and *B. bombina*. The separation between *B. v. variegata* and *B. v. scabra* was also supported by bioacoustic, as well as electrophoretic and mitochondrial DNA data (Vasara et al., 1991; Szymura et al., 2000). Based on our small sample sizes (from 3 to 6 individuals), two groups of *B. v. scabra* might be recognized. The first one would consist of the south Serbian (Vucje) and Macedonian (Trojaci) samples having a little higher genome size values (the limits were between 21.43 and 21.78 pg), whereas the second group would contain the Montenegro samples with smaller genome size rang-



Fig. 1. The map of Macedonia, Serbia and Montenegro with samples of anurans studied.

¹ Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, St. Petersburg 199034, Russia; E-mail: lacerta@zin.ru

² Institute of Cytology Russian Academy of Sciences, Tikhoretsky Pr., 4, 194064 St. Petersburg, Russia; E-mail: slitvinchuk@yahoo.com

³ Institute for Biological Research "Siniša Stanković," Belgrade, Serbia and Montenegro.

⁴ Institute of Zoology, Belgrade, Serbia and Montenegro.

TABLE 1. Genome size variation (pg) in 13 species and subspecies of the Balkan anurans.

	Locality	Country/Republic	<i>n</i>	Mean	S.D.	Min	Max
<i>Bombina bombina</i>							
1	Obrez	Serbia	5	21.86	0.08	21.77	21.98
7	Jagodina	Serbia	5	21.82	0.06	21.75	21.91
9	Todorovce	Serbia	6	21.85	0.03	21.79	21.88
			16	21.84	0.06	21.75	21.98
<i>Bombina variegata scabra</i>							
10	Vucje	Serbia	5	21.55	0.07	21.43	21.61
11	Trojaci	Macedonia	4	21.71	0.09	21.59	21.78
13	Biogradskoe Lake	Montenegro	3	21.33	0.07	21.28	21.41
14	Bukumirskoe Lake	Montenegro	6	21.34	0.05	21.27	21.40
15	Jankovichi-Krsh	Montenegro	6	21.27	0.03	21.21	21.31
			24	21.43	0.17	21.21	21.78
<i>Bombina variegata variegata</i>							
6	Brza Palanca	Serbia	6	20.71	0.04	20.66	20.75
<i>Bufo bufo</i>							
4	Trešnja	Serbia	3	13.27	0.03	13.24	13.30
13	Biogradskoe Lake	Montenegro	4	13.15	0.03	13.11	13.17
			7	13.20	0.07	13.11	13.30
<i>Bufo viridis</i>							
5	Banatska Palanka	Serbia	2	9.66	—	9.65	9.66
<i>Hyla arborea</i>							
14	Bukumirskoe Lake	Montenegro	2	9.98	—	9.91	10.04
<i>Pelobates fuscus fuscus</i>							
1	Obrez	Serbia	5	8.97	0.03	8.94	9.01
7	Jagodina	Serbia	1	8.77	—	—	—
5	Banatska Palanka	Serbia	4	8.94	0.05	8.89	9.00
3	Pancevo	Serbia	3	9.02	0.02	9.00	9.04
8	Aleksinac	Serbia	3	8.83	0.05	8.79	8.88
			16	8.93	0.09	8.77	9.04
<i>Pelobates syriacus balcanicus</i>							
5	Banatska Palanka	Serbia	5	7.91	0.03	7.86	7.93
7	Jagodina	Serbia	2	7.88	—	7.87	7.89
12	Nakolec	Macedonia	3	7.94	0.01	7.93	7.95
			10	7.91	0.03	7.86	7.95
<i>Rana graeca</i>							
10	Vucje	Serbia	1	15.41	—	—	—
13	Biogradskoe Lake	Montenegro	3	15.30	0.05	15.24	15.33
			4	15.33	0.07	15.24	15.41
<i>Rana esculenta</i>							
2	Obedska Bara	Serbia	17	14.96	0.07	14.86	15.10
<i>Rana lessonae</i>							
1	Obrez	Serbia	2	14.12	—	14.10	14.14
2	Obedska Bara	Serbia	4	14.16	0.02	14.14	14.19
			6	14.15	0.03	14.10	14.19
<i>Rana ridibunda</i>							
2	Obedska Bara	Serbia	1	16.07	—	—	—
16	Gornj Ceklin	Montenegro	1	15.95	—	—	—
			2	16.01	—	15.95	16.07
<i>Rana shqiperica</i>							
17	Virpazar	Montenegro	6	14.34	0.12	14.21	14.49

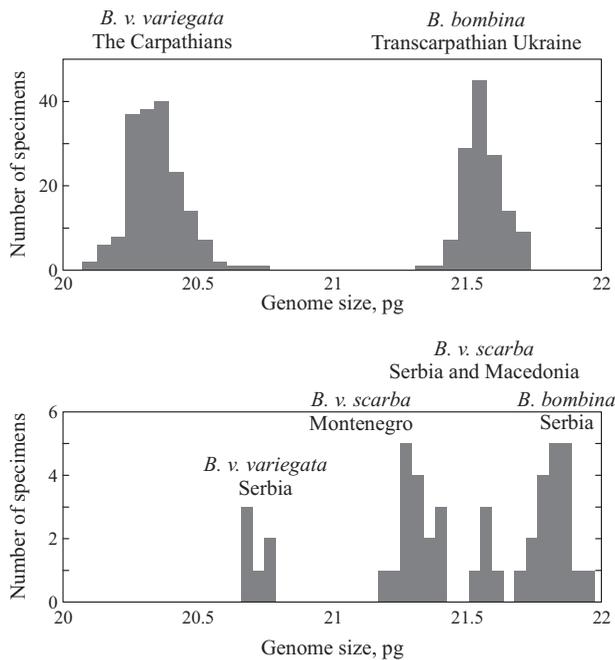


Fig. 2. Genome size variation in European fire-bellied toads (*Bombina*).

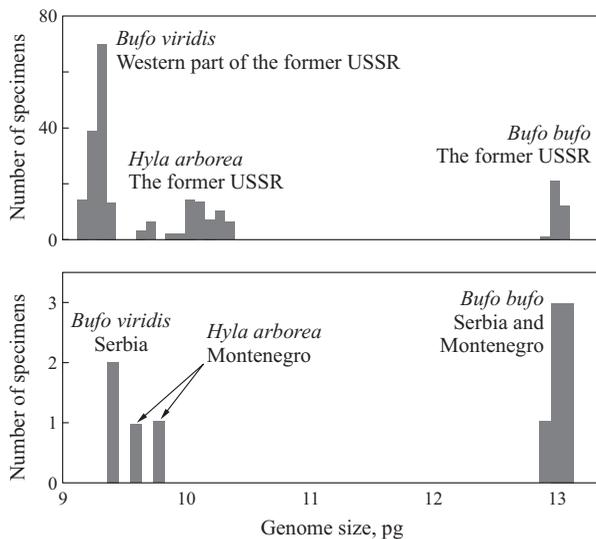


Fig. 3. Genome size variation in three species of toads (*Bufo*) and tree-frogs (*Hyla*).

ing from 21.21 to 21.41 pg (Fig. 2). However, the confirmation of such groups needs additional evidence based on larger sampling.

The genus *Pelobates* is also represented in the Balkans by two species, which are significantly differed by genome size (Table 1). It is important because in Serbia both species can occur syntopically, for instance, in Banatska

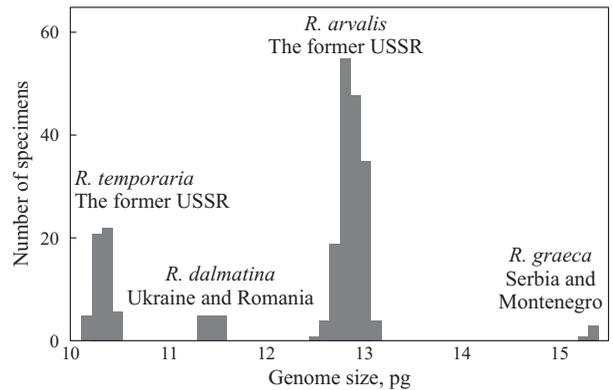


Fig. 4. Genome size variation in brown frogs (*Rana temporaria* group).

Palanka (Rot-Nikčević et al., 2001) unlike in the Caucasian region (e.g., Borkin, 1998). In *P. syriacus*, the Balkan samples were characterized by smaller amount of nuclear DNA (7.86 – 7.95 pg) in comparison with Azerbaijan spadefoot toads (8.02 – 8.39 pg, Khalturin et al., 2003). These differences would support the validity of the Balkans and Asian subspecies (see Ugurtas et al., 2002).

Several years ago, using DNA flow cytometry, we found two cryptic forms of *P. fuscus fuscus* in eastern Europe. The averaged genome size in the western populations was equal to 8.83 pg, ranging from 8.65 to 9.06 pg, whereas the eastern populations had higher values (9.32 pg, within the range 9.18 – 9.43 pg). The reality of both forms was confirmed by allozyme analysis (Borkin et al., 2001, 2003; Khalturin et al., 2003). According to genome size (8.77 – 9.04 pg; Table 1), five Serbian samples can be assigned to the western kind of *P. f. fuscus*, which was formerly recorded in Moldavia, Ukraine, Belarus', Latvia, and the western part of European Russia.

We failed to find any differences in genome size between the Balkan samples of *Bufo bufo* (Serbia and Montenegro, Table 1) and common toads collected in European part of the former USSR (Fig. 3).

The amount of nuclear DNA seemed to be the same in Serbian green toads and numerous samples of *B. viridis viridis* from the western part of the former Soviet Union (Table 1; Fig. 3).

In the case with tree frogs (*Hyla arborea arborea*) we can draw analogous picture: no differences between two specimens from Montenegro and samples from the former USSR, although the Balkan sample was too small (Table 1; Fig. 3).

Green frogs of the Balkan region were the subject of numerous recent papers because of a quite complicated situation in terms of their systematics and distribution, with cryptic speciation in *ridibunda*-like frogs (Dubois

and Ohler, 1994). In Serbia and Montenegro four species are currently recognized. Three members of the hybridogenetic complex (*Rana lessonae*, hybridogenetic *R. esculenta*, and *R. ridibunda*) collected in northern Serbia (Danubian plain region) had quite different genome sizes (Table 1), which were similar to that of the same frog species from European part of the former USSR, respectively. Curiously, all three frogs occurred syntopically at Obedska Bara, and, moreover, two samples of *R. esculenta* were represented by females only. Such a kind of population system with unisexual diploid hybrids seems to be a unique one in Europe.

Rana shqipERICA, which was described from Skadarско Jezero, near Virpazar, Montenegro (Hotz et al., 1987), is endemic to the Adriatic Balkans, with quite restricted distribution in southern Montenegro and Albania. According to genome size (Table 1), this species is closer to *R. lessonae* rather than to other Balkan green frogs. Thus, our data seem in concert of suggestion that the both taxa may be sister species (Plötner, 1998).

Among the Balkan brown frogs (*Rana temporaria* group) we examined genome size in two samples of *R. graeca*, endemic to the Balkan region. The comparison with three other species (*R. arvalis*, *R. dalmatina*, and *R. temporaria*), collected from European part of the former USSR and Romania, demonstrated obvious differences between all these species. Moreover, *R. graeca* proved to be characterized by the highest genome size (Fig. 4).

The research was supported by the Russian Foundation for Basic Research grant (No. 02-04-49631), Russian Federal Program "Integration" grants (E 0121, E 3248), Russian Government grant "Scientific Schools" (NSh 1647.2003.4), and partly funded by the Serbian Ministry of Science, Technologies and Development (grant No. 1623, "Integrative Study of Amphibians and Reptiles from the Central Balkans").

REFERENCES

- Borkin L. J.** (1998), "Amphibia," in: Ananjeva N. B., Borkin L. J., Darevsky I. S., and Orlov N. L., *Zemnovodnye i Presmykayushchiesya [Amphibians and Reptiles]*, ABF, Moscow, pp. 19 – 174 [in Russian].
- Borkin L. J.** (1999), "Distribution of amphibians in North Africa, Europe, Western Asia, and the former Soviet Union," in: Duellman W. E. (ed.), *Patterns of Distribution of Amphibians: a Global Perspective*, The Johns Hopkins Univ. Press, Baltimore – London, pp. 329 – 420.
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., and Milto K. D.** (2001), "Cryptic speciation in *Pelobates fuscus* (Anura, Pelobatidae): evidence from DNA flow cytometry," *Amphibia-Reptilia*, **22**(4), 387 – 396.
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., Khalturin M. D., Lada G. A., Borissovsky A. G., Faizulin A. I., Kotserzhinskaya I. M., Novitsky R. V., and Ruchin A. B.** (2003), "New data on the distribution of two cryptic forms of the common spadefoot toad (*Pelobates fuscus*) in eastern Europe," *Russ. J. Herpetol.*, **10**(2), 111 – 118.
- Dubois A. and Ohler A.** (1994), "Frogs of the subgenus *Pelodyx* (Amphibia, Anura, genus *Rana*): a catalogue of available and valid scientific names, with comments on name-bearing types, complete synonymies, proposed common names, and maps showing all type localities," *Zool. Polonica*, **39**(3 – 4), 139 – 204.
- Džukić G. and Kalezić M. L.** (2004), "The biodiversity of amphibians and reptiles in the Balkan Peninsula," in: Griffiths H., Kryštufec B., and Reed M. (eds.), *Balkan Biodiversity. Pattern and Process in the European Hotspot*, Kluwer, Dordrecht, pp. 167 – 192.
- Hotz H., Uzzell T., Günther R., Tunner H., and Heppich S.** (1987), "*Rana shqipERICA*, a new European water frog species from the Adriatic Balkans (Amphibia, Salientia, Ranidae)," *Notulae Naturae*, **468**, 1 – 3.
- Khalturin M. D., Borkin L. J., Litvinchuk S. N., and Rosanov J. M.** (1996), "Hybridization between *Bombina bombina* and *Bombina variegata* in the Ukrainian Transcarpathians: electrophoretic and genome size data," in: *Biologia płazów i gadów. Materiały Konferencyjne. IV Ogólnopolska Konferencja Herpetologiczna*. Kraków. 26 – 27 września 1996, Wydawnictwo Naukowe WSP, Kraków, pp. 45 – 46.
- Khalturin M. D., Rosanov J. M., Litvinchuk S. N., and Borkin L. J.** (2001), "Hybridization between fire-bellied toads *Bombina bombina* and *B. variegata* in the Ukrainian Transcarpathians," in: *Proc. of the 1st Meeting Nikolsky Herpetol. Soc. "The Problems of Herpetology"*, Pushchino – Moscow, pp. 312 – 313.
- Khalturin M., Litvinchuk S. N., Borkin L. J., Rosanov J. M., and Milto K. D.** (2003), "Genetic variation in two cryptic forms of the common spadefoot toad, *Pelobates fuscus* (Pelobatidae, Anura, Amphibia) differing in genome size," *Tsitologiya*, **45**(3), 308 – 323 [in Russian, with English summary].
- Plötner J.** (1998), "Genetic diversity in mitochondrial 12S rDNA of western Palearctic water frogs (Anura, Ranidae) and implications for their systematics," *J. Zool. Syst. Evol. Res.*, **36**, 191 – 201.
- Rot-Nikčević I., Sidorovska V., Džukić G., and Kalezić M. L.** (2001), "Sexual size dimorphism and life history traits of two European spadefoot toads (*Pelobates fuscus* and *P. syriacus*) in allopatry and sympatry," *Annales Ser. Hist. Nat. Koper*, **11**, 107 – 120.
- Szymura J. M., Uzzell T., and Spolsky C.** (2000), "Mitochondrial DNA variation in the hybridizing fire-bellied toads, *Bombina bombina* and *B. variegata*," *Mol. Ecol.*, **9**, 891 – 899.
- Ugurtas I., Ljubisavljević K., Sidorovska V., Kalezić M. L., and Džukić G.** (2002), "Morphological differentiation of the Eastern spadefoot toad (*Pelobates syriacus*) populations," *Israel J. Zool.*, **48**, 13 – 32.
- Vasara E., Sofianidou T., and Schneider H.** (1991), "Bioacoustic analysis of the yellow-bellied toad in northern Greece (*Bombina variegata scabra* L., Anura, Discoglossidae)," *Zool. Anz.*, **226**(5/6), 220 – 236.

VARIABILITY OF ADVERTISEMENT CALLS AND RELEASE CALLS OF GREEN FROGS IN THE MOSCOW OBLAST', RUSSIA

K. I. Chernyshov¹

Keywords: green frog, release calls, advertisement calls.

INTRODUCTION

Research on anuran bioacoustics has been intensively carried out during last 25 years. In the course of this period, a considerable amount of data was accumulated on qualitative as well as quantitative call parameters of many frog species. Frog calls seem to be largely species-specific, allowing for their use in regional faunistical explorations. New species and subspecies of frogs have been described based on their bioacoustic differentiation (Schneider and Sofianidou, 1985).

Comparative studies of acoustic signals in the amphibia are made difficult by the fact that the calls of these poikilothermic animals depend on environmental parameters. Dependence of call variables on temperature is known and, in general, is well-studied for many groups (Radwan and Schneider, 1988; Schneider et al., 1988), but other variables, such as body size and sex of the animal, or weather, level of motivation, season and interspecific hybridization also can play important roles (Green, 1982; Malmos et al., 2001). For example, influence of body size on various parameters of advertisement calls was revealed for the genera *Rana*, *Bombina*, *Alytes*, and *Bufo* (Egiasarian and Schneider, 1990; Nevo and Schneider, 1983).

The aim of the present work was an attempt to contribute to the understanding of the influence of temperature, body size and sex on different call types for two frog species: *Rana ridibunda* Pall. and *R. lessonae* Cam.

MATERIAL AND METHODS

Material was collected in July 1988 from ponds around the Zvenigorod biological station [Faculty of Biology, Moscow State University (MSU), Odintsovo raion, Moscow Oblast', Russia]. Calls of adult sexual mature animals were recorded in chorus and individually. In addition we captured 21 individuals of *R. ridibunda* and 40 individuals of *R. lessonae* of different sexes and size categories for which we recorded release calls (at water tempera-

tures ranging between 6 to 22°C). Recording of signals were made with a dictaphone Forward LF-182A HONG-MAN or a tape recorder Panasonic RX-M50, at a tape speed of 9.25 cm/sec and at a distance of about 40 cm from animal. Comparison and analysis of sound signals of frogs were made at PC employing "Avisoft-SASLabPro" software.

The studied animals were divided into the size classes "small" and "large." Snout-vent lengths of these categories were as follows: *R. ridibunda*: 84.0 – 93.0 and 74.1 – 76.6 mm for "large" and "small" males, respectively, and 120.0 – 150.2 and 75.0 – 80.0 for "large" and "small" females, respectively; *R. lessonae*: 58.7 – 60.7 and 54.3 – 55.0 mm for "large" and "small" males, respectively, and 62.1 – 67.0 and 58.1 – 61.6 mm for "large" and "small" females, respectively.

RESULTS AND DISCUSSION

Advertisement calls. With increasing temperature, the main parameters of the advertisement calls of both species change as follows, based on our analysis of recordings from frog choruses: intercall interval and call duration decrease (due to a decrease in the duration of intervals between single pulses); single pulse duration decreases, the number of pulses per call increases (Tables 1 and 2, Figs. 1 and 2). Some increase in energy maxima of the dominant frequencies is observed in *Rana ridibunda* calls, except the third frequencies maximum. Oppositely, *Rana lessonae* demonstrates the decrease of frequencies maximum values with the increase of temperature.

Release calls. Data on release calls (analysis of individual recordings) were analyzed separately for eight groups in *R. ridibunda*, depending on sex, size and temperature (Table 4). Data for *R. lessonae* were divided only into four groups, depending on sex and body size and refer all to a temperature of ~16°C (Table 3).

The increase of temperature induces the release call of *Rana lessonae* (we compared frogs equal in sex and of the same size) a decrease in call duration in all groups, except

¹ Faculty of Biology, Moscow State University, Leninskie Gory 1/12, 119899 Moscow, Russia; E-mail: kir-ch@mail.ru

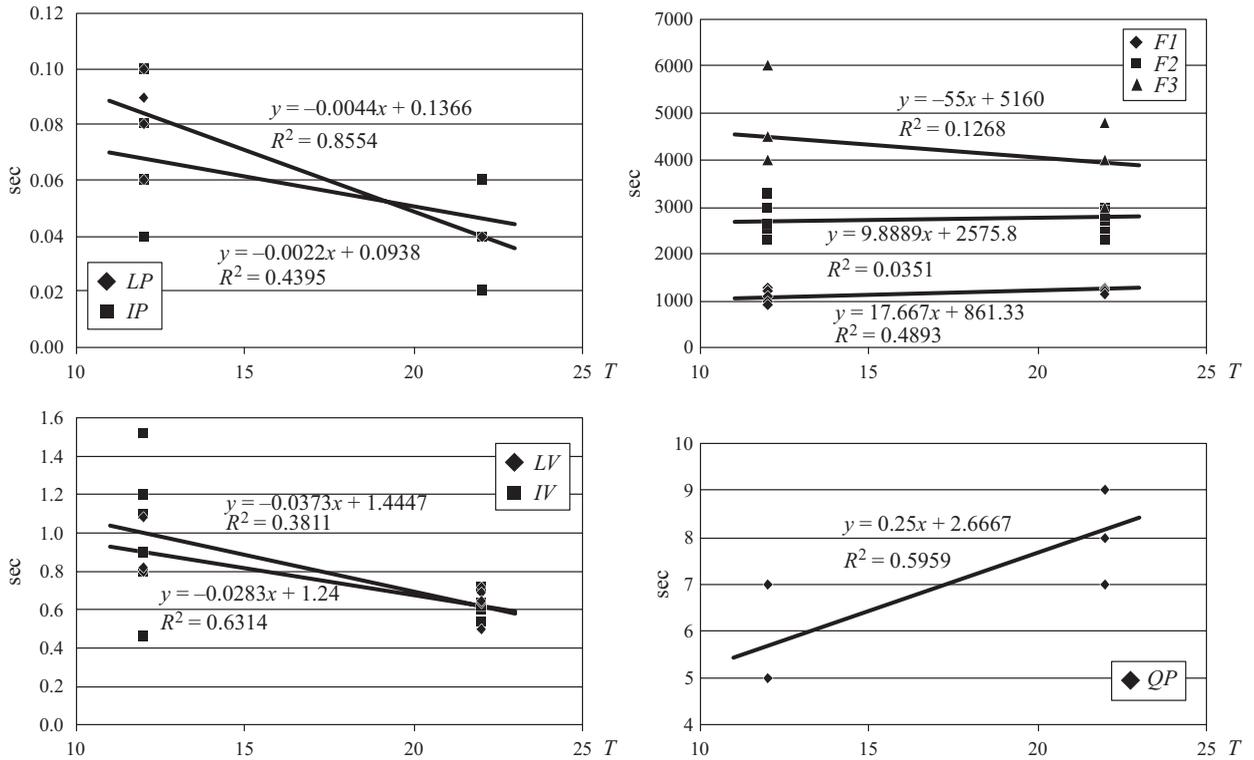


Fig. 1. Advertisement call of *R. ridibunda*. The influence of temperature on the components of call. R , coefficient of approximation. For other abbreviations, see Table 1.

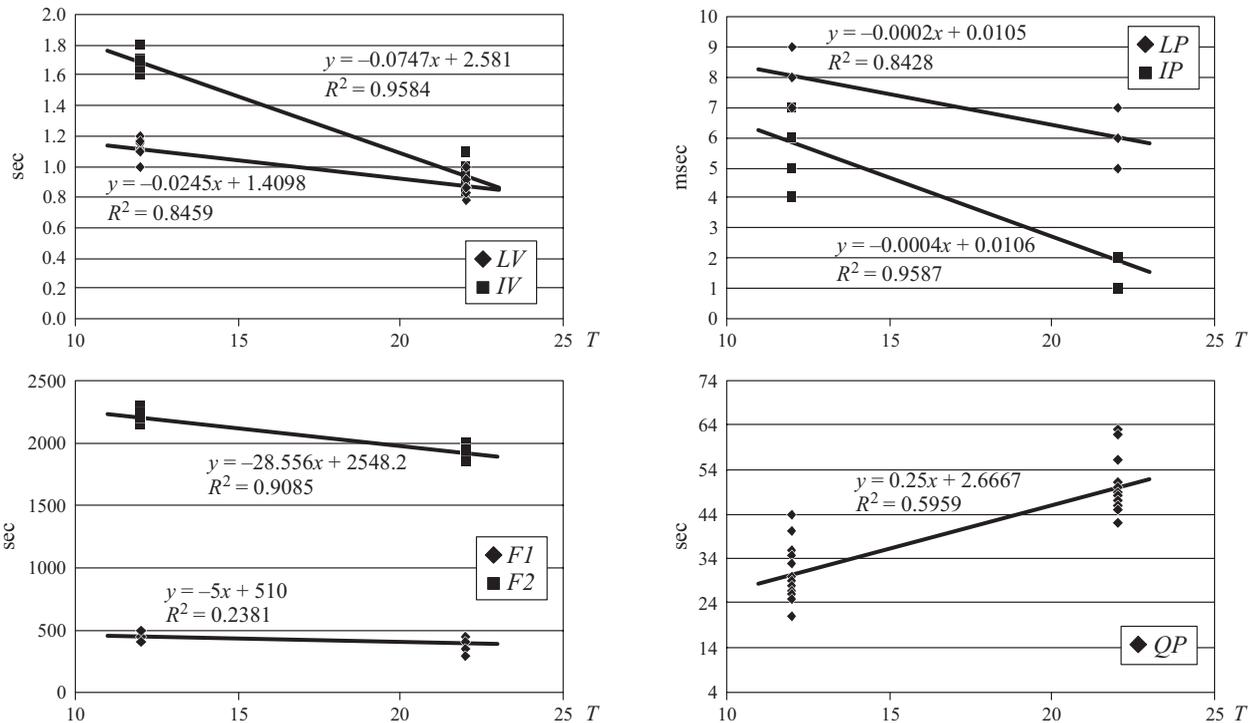


Fig. 2. Advertisement call of *R. lessonae*. The influence of temperature on the components of call. R , coefficient of approximation. For abbreviations, see Fig. 1 and Table 1.

TABLE 3 (continued)

	QP			F1			F2			F2					
	lm	sm	if	Sf	lm	sm	lf	sf	lm	sm	lf	sm	lf	sf	
M	13.583	8.000	19.600	13.000	858	1042	1100	840	2231	2406	2113	1069	4550	—	
S _x ²	2.601	14.609	78.114	29.029	6602.564	38623.188	76818.182	99888.889	70828.804	164850.543	388958.333	69959.239	2857.143	—	
S _x	1.613	3.822	8.838	5.388	81.256	196.528	277.161	316.052	266.137	406.018	623.665	264.498	53.452	—	
S _d	0.329	0.780	2.282	1.347	22.536	40.116	80.009	99.944	54.325	82.878	311.833	53.990	18.898	—	
M ± tS _x , P = 0.95	0.021	0.049	0.143	0.084	1.413	2.516	5.017	6.267	3.407	5.197	19.554	3.386	1.185	—	
F-test, size	5.616	2.691			5.850	1.300			2.327	5.560				—	
	P < 0.01			P < 0.05			P < 0.01			P < 0.01			P < 0.01		
t-test, size	6.593	0.324			0.381	0.433			1.766	1.725				—	
	P < 0.00			P > 0.05			P > 0.05			P > 0.05			P > 0.05		
F-test, sex	30.027	1.987			11.635	2.586			5.492	2.356				—	
	P < 0.01			P > 0.05			P < 0.01			P < 0.05			P < 0.01		
t-test, sex	0.355	0.353			0.484	0.321			0.196	13.522				—	
	P > 0.05			P > 0.05			P > 0.05			P < 0.001			P < 0.01		

F-test and t-test values are cited for the estimation of level of significance of size influence on the call parameters (size), the sex of animals is equal; and sex influence (sex), the size of animals is equal. lm, “big” males; sm, “small” males; bf, “big” females; sf, “small” females. For other abbreviations see Table 1. Temperature was constant (16°C).

TABLE 4. Main Parameters of Release Calls for *R. ridibundae*

T	LV						LGP								
	8 – 10			15			8 – 10			15					
	lm	sm	if	Sf	lm	sm	lf	sf	lm	sm	lf	sm	lf	sf	
M	0.480	0.580	0.515	0.220	0.270	0.410	0.310	0.250	0.061	0.106	0.170	0.065	0.048	0.059	
S _x ²	0.002	0.000	0.002	0.001	0.001	0.006	0.003	0.001	0.000	0.000	0.001	0.000	0.000	0.000	
S _x	0.027	0.011	0.045	0.025	0.025	0.064	0.041	0.030	0.017	0.015	0.018	0.011	0.012	0.019	
S _d	0.008	0.003	0.010	0.005	0.005	0.015	0.011	0.006	0.003	0.004	0.007	0.002	0.002	0.004	
M ± tS _x , P = 0.95	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
F-test, T	2.516	24.359	1.218	1.643					2.134	2.984	4.315			0.687	
	P < 0.05			P > 0.05			P > 0.05			P < 0.05			P > 0.05		
t-test, T	21.295	10.973	14.279	4.020					1.041	12.657	13.435			1.835	
	P < 0.001			P < 0.001			P < 0.001			P < 0.001			P > 0.05		
F-test, size	7.469	4.419			8.206	3.276			1.542	8.630		1.102		0.727	
	P < 0.01			P < 0.01			P < 0.01			P > 0.05			P > 0.05		
t-test, size	11.143	27.625			8.793	4.932			8.852	11.458		5.348		2.890	
	P < 0.001			P < 0.001			P < 0.001			P < 0.001			P < 0.001		
F-test, sex	1.329	2.247			4.072	6.601			5.438	2.447		2.689	0.564		
	P > 0.05			P < 0.05			P < 0.01			P < 0.01			P > 0.05		
t-test, sex	2.701	65.240			3.366	9.912			13.444	5.582		1.461	6.390		
	P < 0.01			P < 0.001			P < 0.01			P < 0.001			P > 0.05		

TABLE 4 (continued)

<i>T</i>	<i>QP</i>																														
	8 – 10					15					8 – 10					15															
	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if						
<i>M</i>	6.429	8.556	7.600	8.000	4.003	5.556	6.714	20.643	667	600	575	667	600	1050	505	900	575	1067													
<i>S</i> _x ²	15.341	26.278	20.800	4.800	0.000	7.278	6.527	14.401	2500.000	83333.333	0.071	5000.000	50.000	20000.000	3333.333	25000.000	2500.000	3333.333													
<i>S</i> _x	3.917	5.126	4.561	2.191	0.006	2.698	2.555	3.795	50.000	288.675	0.267	70.711	7.071	141.421	50.000	141.421	50.000	57.735													
<i>S</i> _d	1.086	1.709	2.040	0.894	0.003	0.899	0.683	1.014	25.000	166.667	0.071	50.000	5.000	100.000	25.000	100.000	25.000	33.333													
<i>M</i> ± <i>tS</i> _x , <i>P</i> = 0.95	0.068	0.107	0.128	0.056	0.000	0.056	0.043	0.064	1.568	10.451	0.004	3.135	0.314	6.271	1.568	6.271	1.568	2.090													
<i>F</i> -test, <i>T</i>	460219.780	3.611	3.187	3.000		50.000	4.167	35000.000	1.500																						
	<i>P</i> < 0.01	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.01	<i>P</i> > 0.05																						
<i>t</i> -test, <i>T</i>	0.470	0.345	0.147	1.801		1.395	0.854	0.244																							
	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.01		<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05																							
<i>F</i> -test, size	1.713		4.333		2.206	33.333		70000.000	400.000									1.333													
	<i>P</i> > 0.05		<i>P</i> > 0.05		<i>P</i> > 0.05	<i>P</i> < 0.01		<i>P</i> < 0.01	<i>P</i> < 0.05									<i>P</i> > 0.05													
<i>t</i> -test, size	0.216		0.070		11.392	0.375		17.995	3.945									8.924													
	<i>P</i> > 0.05		<i>P</i> > 0.05		<i>P</i> < 0.01	<i>P</i> > 0.05		<i>P</i> < 0.01	<i>P</i> > 0.05									<i>P</i> < 0.01													
<i>F</i> -test, sex	1.356	5.475			1.979	35000.000		16.667	50.000									6.000													
	<i>P</i> > 0.05	<i>P</i> > 0.05			<i>P</i> > 0.05	<i>P</i> < 0.01		<i>P</i> > 0.05	<i>P</i> > 0.05									<i>P</i> > 0.05													
<i>t</i> -test, sex	0.160	0.069			1.886	0.656		1.463	1.395									1.614													
	<i>P</i> > 0.05	<i>P</i> > 0.05			<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05	<i>P</i> > 0.05									<i>P</i> > 0.05													

<i>T</i>	<i>F2</i>															<i>F3</i>														
	8 – 10					15					8 – 10					15														
	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if	lm	sm	lf	sf	if					
<i>M</i>	1267	1433	1057	2825	2825	1150	2025	571	1625	1750	2700	1667	1667	1667	1667	1525	1525	2325	–	–	–	–	–	–	–	–				
<i>S</i> _x ²	163333.333	13333.333	22637.363	1250.000	405000.000	1250.000	23736.264	1250.000	83333.333	1250.000	20000.000	83333.333	1250.000	83333.333	1250.000	1250.000	1250.000	1250.000	–	–	–	–	–	–	–	–	–			
<i>S</i> _x	404.145	115.470	150.457	35.355	636.396	35.355	154.066	35.355	35.355	353.553	141.421	288.675	288.675	288.675	288.675	35.355	35.355	35.355	–	–	–	–	–	–	–	–				
<i>S</i> _d	233.333	66.667	40.211	25.000	450.000	25.000	41.176	25.000	25.000	250.000	100.000	166.667	166.667	166.667	166.667	25.000	25.000	25.000	–	–	–	–	–	–	–	–				
<i>M</i> ± <i>tS</i> _x , <i>P</i> = 0.95	14.632	4.180	2.522	1.568	28.218	1.568	2.582	1.568	1.568	15.677	6.271	10.451	10.451	10.451	10.451	1.568	1.568	1.568	–	–	–	–	–	–	–	–				
<i>F</i> -test, <i>T</i>	2.480	10.667	1.049	1.000		100.000		66.667																						
	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05		<i>P</i> > 0.05																						
<i>t</i> -test, <i>T</i>	0.216	5.599	1.206	33.941		0.896		2.540																						
	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> < 0.05		<i>P</i> < 0.05																						
<i>F</i> -test, size	12.250		18.110		324.000	18.989		6.250																						
	<i>P</i> > 0.05		<i>P</i> > 0.05		<i>P</i> < 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05																						
<i>t</i> -test, size	0.687	33.163			1.941	5.354		3.528																						
	<i>P</i> > 0.05	<i>P</i> < 0.01			<i>P</i> > 0.05	<i>P</i> < 0.01		<i>P</i> > 0.05																						
<i>F</i> -test, sex	7.215	10.667			1.000	1.500		1.000																						
	<i>P</i> > 0.05	<i>P</i> > 0.05			<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05																						
<i>t</i> -test, sex	0.655	13.170			1.937	11.314		0.244																						
	<i>P</i> > 0.05	<i>P</i> < 0.01			<i>P</i> > 0.05	<i>P</i> > 0.05		<i>P</i> > 0.05																						

F-test and *t*-test values are cited for the estimation of level of significance of temperature influence on the call parameters (*T*), the sex and size of animals is equal; size influence (size), the sex and temperature of animals is equal; and sex influence (sex) — size and temperature of animals is equal. For other abbreviations see Tables 1 – 3.

small males, a decrease of pulse group duration, pulse duration, interval between pulses; and the number of pulse groups per call increases.

However, the large animals do not demonstrate such regularity. Perhaps, it can be explained by the fact, that in laboratory conditions frogs did not have time to cool off completely to 8°C in allocated time.

Frequencies maxima change chaotically, the third frequencies maxima can disappear with the increase of temperature. The small females do not demonstrate *F3*.

The small frogs (we compared frogs of similar sex, at the same temperature) have higher number of pulses per group, but the pulses are shorter, than pulses of the large ones. Values of the frequencies maxima of small frogs are higher, that of large ones, *F3* can disappear.

There are some differences in voice parameters of small and large frogs, depending on their sexual appartaining.

Large females have longer call duration than large males, and intervals between the pulse groups are shorter, and there is a contrary situation with small frogs.

The first frequencies maximum of females is higher than the males' one, independently of the size.

Large males of *Rana lessonae* have longer call duration, than large females, and vice versa, with small frogs. In other words, situation with *Rana lessonae* is reverse to the one with *Rana ridibunda*.

Females demonstrate the longer interval between pulse groups and the greater number of pulses per pulse group, males demonstrate longer interval between pulses, than females.

First and second frequencies maxima are observed in all groups, and *F3*, only in a group of small males.

The small frogs of *Rana ridibunda* utter the shorter call with less number of pulses per call.

REFERENCES

- Egiasarian E. M. and Schneider H.** (1990), "Advertisement call of tree frogs in Armenia (Anura, Hylidae)," *Zool. Anz.*, **225**(3/4), 113 – 122.
- Green D. M.** (1982), "Mating call characteristics of hybrid toads at Long Point, Ontario," *Can. J. Zool.*, **60**, 12.
- Malmos K. B., Sullivan B. K., and Trip Lamb** (2001), "Calling behavior and directional hybridization between two toads (*Bufo microscaphus* × *B. woodhousii*) in Arizona," *Evolution*, **55**(3), 626 – 630.
- Nevo E. and Schneider H.** (1983), "Structure and variation of *Rana ridibunda* mating call in Israel (Amphibia, Anura)," *Israel J. Zool.*, **32**, 40 – 60.
- Radwan N. M. M. and Schneider H.** (1988), "Social behaviour, call repertory and variation in the calls of the pool frog, *Rana lessonae* (Anura: Ranidae)," *Amphibia-Reptilia*, **9**, 329 – 351.
- Schneider H. and Sofianidou T. S.** (1985), "The mating call of *Rana ridibunda* (Amphibia, Anura) in Northern Greece as compared with those of Yugoslavian and Israeli population: proposal of a new subspecies," *Zool. Anz.*, **214**(5/6), 309 – 319.
- Schneider H., Sofianidou T. S., and Kyriakopoulou-Sklavounou P.** (1988), "Calling behavior and calls of *Rana dalmanina* (Anura, Ranidae) in Greece," *Zool. Jb. Physiol.*, **92**, 231 – 243.

**PRELIMINARY DATA ON BODY SIZE DIFFERENCES
IN ADULTS OF *Testudo hermanni hermanni* GMELIN, 1789:
COMPARISON BETWEEN TWO WESTERN MEDITERRANEAN
INSULAR POPULATIONS AND THE CONTINENTAL
POPULATION OF SOUTHERN TUSCANY**

C. Corti,¹ M. A. L. Zuffi,² L. Bassu,³ C. Fresi,⁴ and M. G. Satta³

Body size differences have been found between the continental population of southern Tuscany and the Sardinian insular populations of *Testudo hermanni hermanni*. The insular populations are clearly bigger than the continental ones.

Keywords: *Testudo hermanni*, body size differences, western Mediterranean.

INTRODUCTION

The Hermann's tortoise is distributed in the Mediterranean coastal regions from Catalonia to Turkey, through the Italian Peninsula and the Balkans. In Italy *T. hermanni* is mainly distributed along the Tyrrhenian coastal zones and on the main islands of Sardinia and Sicily. The Hermann's tortoise is also present on the Sardinian satellite islands of Asinara, Piana dell'Asinara, Santa Maria, Maddalena, S. Stefano, Caprera, and Tavolara (Poggesi et al., 1995).

Our study has been carried out on the following Italian populations: Continental Tuscany, Sardinia (main island), and Asinara Island. The aim of our study was to detect if size differences are present within the island populations and, between the latter and the mainland population. Data on size of western Mediterranean populations of *T. hermanni* are reported by Cheylan (2001) in the *Handbuch der Reptilien und Amphibien Europas*. But detailed comparisons within the Sardinian populations and between the latter and those of continental Italy are still lacking.

MATERIAL AND METHODS

Tortoises have been captured in spring, summer and autumn, measured and released. The following morphological characters have been measured to the nearest 0.1 mm adopting the method by Zuffi and Gariboldi (1995) in 140 alive females and 94 males: carapace length; carapace width; plastron length; plastron width; carapace height and tail length; each specimen has been weighed before release.

The analysis of variance and covariance on males and females has been carried out separately because of the clear sexual dimorphism known for the Mediterranean tortoises (Willemsen and Hailey, 2003; and reference therein). Covariance analysis has been performed using carapace length as a constant to normalise all plotted data. According to a *t*-test analysis on all the selected parameters, males differed significantly from females for all the characters (*P* ranging from 0.010 to 0.0001). After each ANOVA analysis we performed a post-hoc lesser significance test in order to better discriminate between localities. Correlation analysis has been performed on all parameters separately for each considered area in order to detect how the selected characters are correlated. Because of the presence of markedly smoothed carapaces of the Asinara specimens we have reconstructed the hypothetical carapace height of this population using a simple regression taken from the Sardinian sample.

Histograms of shell measurements have been used to see the distribution of size classes within and between the studied populations. The statistical package used is SPSS 8.0.

RESULTS

Size differences are particularly evident in both males and females between the insular and the continental populations even if more marked in females, but the Asinara specimens are in average bigger than those ones of the main island population for most of the studied characters. Tails of the Tuscan population are significantly shorter than those of the insular populations (males, ANCOVA_{tail length} $F = 40.794$, $P < 0.0001$, $df = 3$; locality = 0.943, $P > 0.05$; females, ANCOVA_{tail length} $F = 65.614$, $P < 0.0001$, $df = 3$; locality = 2.106, $P > 0.05$). Body mass of the insular populations is significantly bigger than in the Tuscan population. Carapaces of the

¹ Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, I-50125 Florence, Italy; E-mail: claudia.corti@unifi.it.

² Museo di Storia Naturale e del Territorio, Università di Pisa, Calci (Pisa), Italy; E-mail: marcoz@museo.unipi.it.

³ Consorzio Mediterraneo, Arborea, Oristano, Italy; E-mail: arbolab@lt.arborea.it.

⁴ CEEA, Centro Esperienza per l'Educazione Ambientale, Baratz (Sassari) Italy.

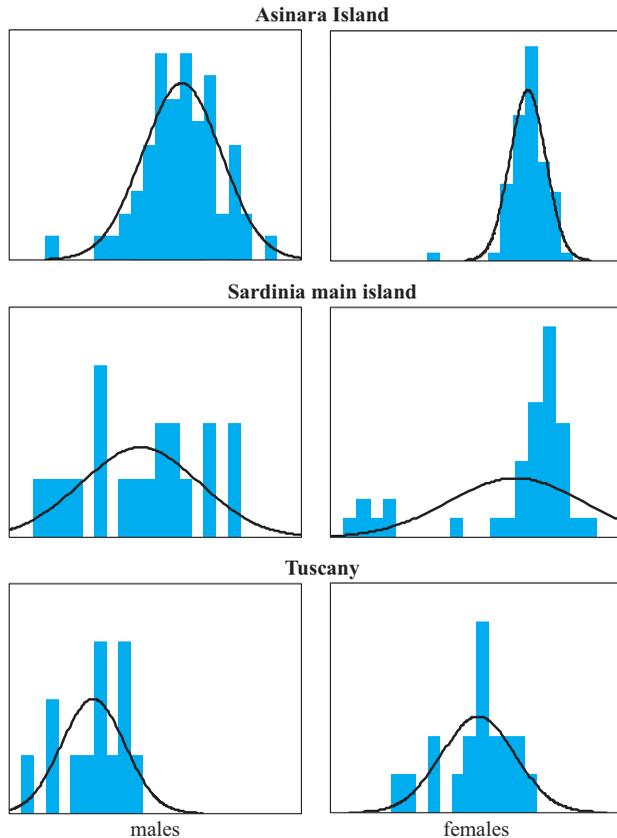


Fig. 1. Carapace length in males and females of *Testudo hermanni*. Abscissa range in males is 110 – 180 mm carapace length, in females is 80 – 210 mm carapace length. Normal curve is shown.

Asinara Island population are in average the highest, particularly evident in females (males, ANCOVA_{carapace height} $F = 12294.1$, $P < 0.0001$, $df = 1$; locality = 1.675, $P = 0.193$; females, ANCOVA_{carapace height} $F = 5026.23$, $P < 0.0001$, $df = 1$; locality = 4.510, $P = 0.086$).

Between the islands and continental population we find significant differences in most of the analysed shell morphological characters (Bonferroni, post-hoc test in males and females, $P < 0.05$).

Histograms of shell measurements show, both in males and females, that in the Tuscan and main island populations the size classes are differently distributed while in the Asinara Island population these classes are symmetrically and normally distributed towards the groups of highest values (Fig. 1).

CONCLUSIONS

The insular populations are clearly bigger than those ones of continental Tuscany. This supports previous find-

ings in which island populations of reptiles show a larger size (Mertens, 1934; Palkovacs, 2003; references therein). Within the Asinara Island population it seems that there is a trend to be bigger than on the main island population, as indicated by the average values.

In a more careful analysis of the obtained results, it seems that a difference between the two islands populations is mostly secondary to a peculiar distribution of samples collected in the Asinara Island. In particular the narrow distribution of carapace size, mainly restricted to the highest size classes, might indicate a low frequency of “young” adults rather than a peculiarity of this area. This phenomenon is particularly evident in the female population; the remarkable size of Asinara sampling enforces the power of this analysis. On the main island, even within a relatively smaller sample, most of the size classes are more homogeneously represented. A possible explanation of this finding could be represented by the onset of environmental events influencing either the survival or the development of this population, particularly within the female gender.

Acknowledgments. We acknowledge the “Parco Nazionale dell’Asinara” for permission to enter the Island and lodging facilities. We are particularly grateful to the “Corpo Forestale della Regione Autonoma Sardegna — Comando dell’Asinara” for the assistance on the Island during field work and in particular we thank Antonio Adolfi, Bernardo Brau, Roberto Mura and Gavino Spanu.

We thank Paola Zingarelli which provide some data regarding the population of southern Tuscany. Research has been partially sponsored by the INTERREG II. A project (University of Pisa).

REFERENCES

- Cheylan M.** (2001), “*Testudo hermanni* Gmelin, 1789 — Griechische Landschildkröte,” in: Fritz U. (ed.), *Handbuch der Reptilien und Amphibien Europas. Schildkröten (Testudines)*. I, Aula Verlag, Germany.
- Mertens R.** (1934), “Die Insel Reptilien,” *Zoologica*, **84**, 1 – 205.
- Palkovacs E. P.** (2003), “Explaining adaptive shifts in body size on islands: a life history approach,” *Oikos*, **103**, 37 – 44.
- Poggesi M., Agnelli P., Borri M., Corti C., Finotello P. L., Lanza B., and Tosini G.** (1995), “Erpetologia delle isole circumsarde,” *Biogeographica*, **18**[1994], 583 – 618.
- Willemsen R. E. and Hailey A.** (2003), “Sexual dimorphism of body size and shell shape in European tortoises,” *J. Zool. (Lond.)*, **60**, 353 – 365.
- Zuffi M. A. L. and Gariboldi A.** (1995), “Sexual dimorphism in Italian populations of the European pond terrapin, *Emys orbicularis*,” in: Llorente G. A., Montori A., Santos X., and Carretero M. A. (eds.), *Scientia Herpetologica*, Agal, Barcelona, pp. 124 – 129.

TAXONOMIC VALUE OF THE PHOLIDOSIS AND PATTERN CHARACTERS IN SYSTEMATIC AND PHYLOGENY OF GENUS *Eirenis*

I. B. Dotsenko¹

Keywords: Colubridae, *Eirenis*, character, pholidosis, pattern, phylogeny.

INTRODUCTION

The taxonomic value of characters is inversely dependent on their variability. Quantitative characters, for example as the number of scales and their proportions, are acceptable for determination of affinities between species and species groups in snakes; low values of variation coefficient (CV) may serve as a criterion for taxonomic value by statistical treatment. Characters of pigmentation pattern are less suitable for taxonomic purposes than those of scalation because of high variability of the former. They are even more rarely applied as a basis for phylogenetic considerations and conclusions. This study is an attempt to analyze the most general trends in formation of patterns among some genera of colubrid snakes.

MATERIAL AND METHODS

The specimens of genera *Coluber*, *Eirenis*, *Elaphe*, *Natrix* (169 specimens of 26 species) stored in National Scientific Museum of Nature (NMNH, Kiev, Ukraine) were examined to determine the basic types of patterns. Photos of *Eirenis* species from J. F. Schmidtler publications and photos from Internet were also used. The method “Multivariations test” (Schmidtler, 1986, 1993) was applied to conduct the similarity analysis of the characters of scalation of *Eirenis* species. 32 specimens of *E. medus* and 28 specimens of *E. punctatolineatus* were examined by 15 values (7 MVTn and 8 MVTr). Data about other *Eirenis* species (Schmidtler, 1993: Table 3) also were involved. All these data were shown graphically in coordinates axis (x, MVTn; y, MVTr).

RESULTS

Several simple types of patterns and their combinations can be distinguished within Colubrinae (in *Coluber* and *Elaphe*), as well as in Natricinae. These types occur in

different genera and even subfamilies; they can be treated as basic (initial) patterns. One of them is characterized by absence of any spots on the head and body (as in some specimens of *E. hakkariensis*, *E. thospitis*, *C. caspius*, *C. schmidtii*, and *E. longissima*); the other one consists of small blackish spots grouped in longitudinal-transverse rows (other specimens of *E. hakkariensis* and *E. thospitis*, juveniles of *C. caspius* and *C. schmidtii*, some specimens of *Natrix tessellata*).

One may suggest, that the head and (or) neck patterns formed by transverse stripes of different configuration are more young in their evolutionary development in some species of the genus *Eirenis*. Similar head and (or) neck patterns, in combination with the body pattern or without the latter, are known also in other genera of Colubridae (for example, in *Coluber*: *C. ventromaculatus*, some specimens of *C. viridiflavus*, *C. ravergeri*, *C. rubriceps*, and *C. laurenti*, or *Elaphe*). Schmidtler (1993) has correctly shown similarity between the specific patterns in *Eirenis* and *Coluber*, treating these similarities as convergent.

The other question still lacks a solution is the origin of the pattern consisting of small irregular spots on head and neck and narrow postero-lateral lining of temporal shields laterally of the short middle longitudinal line on the upper side of the neck. This pattern can be treated as an independent evolutionary gain, or a reduction of the pronounced dark pattern formed by transverse stripes (as, for example, in *E. modestus*). The latter explanation was suggested by Schmidtler (1993), who treated the patterns of *E. thospitis* and *E. hakkariensis* as being derived from that of *E. modestus semimaculatus*. This explanation seems not very convincing because, firstly, the pattern characterizing *E. modestus* is always more intensively pigmented and clear in juveniles, being usually lined by a light (yellowish) contour. Secondly, the totally different (from that of *E. modestus*) pattern is known, alongside *E. thospitis* and *E. hakkariensis* (including juveniles). In addition to *E. medus* this type is rather common in juvenile *Coluber caspius* and occurs in some juveniles of *C. laurenti* (= *C. gemonensis*). Therefore, this type of pigmentation may represent a plesiomorphic state of head and neck pattern (in compari-

¹ Zoological Museum, National Scientific Museum of Nature, National Academy of Sciences, Kiev, Ukraine; E-mail: Dotsenko@svitonline.com

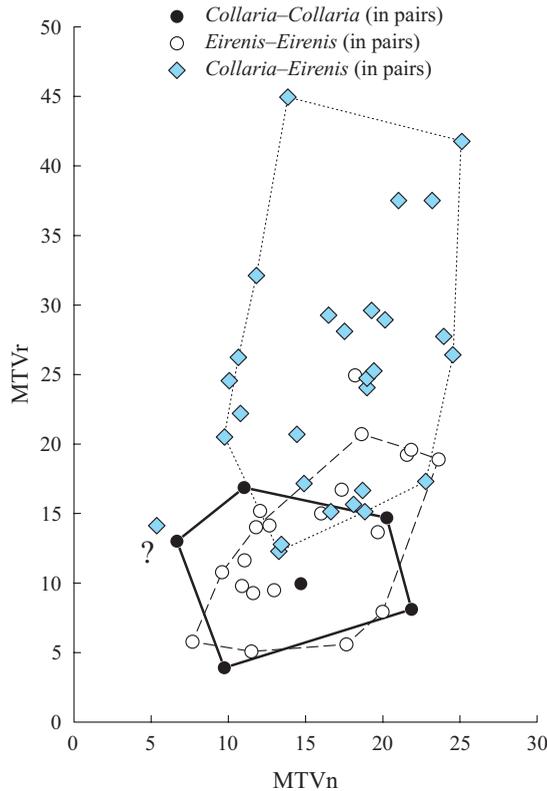


Fig. 1. Plotting of the MVTr and MVTn values (Schmidtler, 1993: Table 3) obtained by intrasubgeneric and intersubgeneric comparisons.



Fig. 2. The juveniles *Eirenis modestus* collected in Tbilisi Botanical Garden (ZM NSMN 1032/2630–2637). Photo by E.M.Pisanets.

son with that of *E. modestus*), or an independent development, but not a derivative of *E. modestus* pattern. Pigmentation of *C. laurenti* juveniles may look as the faded adult *E. modestus* pattern, but is never bright and clear.

Thus, the described types of patterns and their combinations observed in species of the genus *Eirenis* might appear independently and discretely according to homological variation. Potential for such a variation may be determined by the genotype. It is suggested, that one pattern can hardly be treated as a reduction of any other one. In particular, the neck pattern of *E. hakkariensis* – *E. thospitis* type cannot be treated as a reduction of the neck stripe of *E. modestus*, as suggested by Schmidtler (1993). Similar trend can be shown also among species of the genus *Coluber*. The situation in *Elaphe* is much more complicated: in some species (for example, in *E. quatuorlineata* and *E. scalaris*); different subspecies and aberrations have either longitudinal or transverse stripes on the body, in the latter case in combination with a characteristic head pattern (different from that of *E. modestus*) or without any pattern on pileus and neck. All these patterns are, however, very diverse, variable and peculiar; they cannot be treated as modifications of any initial types. Also the state “absence of patterns on the head, neck and body” can be found among species of the latter genus (e.g., in *E. longissima*). It is necessary to mention, that coloration and patterns have more adaptive value for the climbing species of *Elaphe* (e.g., *E. situla*), than for the cryptic snakes, hence more diversity and complexity in the former taxa.

Thus, similar expression of different combinations of head and body patterns can be traced in different genera of colubrid snakes. The most similarity in this aspect is between genera *Eirenis* and *Coluber*.

Scalation patterns in species of the genus *Coluber*, in particular *Coluber caspius* and *C. rhodorhachis*, are also the closest to *Eirenis* in the most stable characters with low values of CV (number of scales around the body, L/Lcd, ventralia, subcaudalia, ventralia/subcaudalia, lorealia, supralabialia, and temporalia).

DISCUSSION

An interesting method of measuring of morphological distance between species by index of reduction of the pholidosis components (Reduktions index RI, Multivariations tests MVTr and MVTn) suggested by Schmidtler (1993) was applied by him for species, preliminarily classified according to the characters of pigmentation. The species group *Eirenis modestus*-complex characterized by the similar pattern of pileus and neck was distinguished on this base. Species of the mentioned complex have 15 or 17 rows of scales and were earlier divided between the nominative subgenus and the subgenus *Collaria* (Dotsenko, 1989). It was already noted (Dotsenko, 2001) that all spec-

imens of the nominative subspecies, having either the pattern of *E. modestus* type or other type of pigmentation, are distinguished from *Collaria* by higher values of RI. Plotting of the MVTr and MVTn values obtained by intrasubgeneric (within *Eirenis* s. str. and *Collaria*, respectively) and intersubgeneric (*Eirenis* s. str. – *Collaria*) comparisons. It was shown, that the latter values are as a rule higher than the former (Fig. 1). The only exception is the pair *E. (Collaria) thospitis* – *E. (Eirenis) hakkariensis*. However, both mentioned species have the pattern different from that in *Eirenis modestus*-complex, and therefore cannot be discussed in this context. Thus, the analysis of original and Schmidtler's (1993) data confirmed the existence of two *Eirenis* subgenus, coming in the contradiction with Schmidtler's concept of *E. modestus*-complex, defined on the basis of the pattern.

The high level of individual variability in *E. modestus* is also noteworthy. The juveniles collected within the limited territory of Tbilisi Botanical Garden (Fig. 2) have demonstrated the patterns characteristic not only for the nominative subspecies, but also similar to *E. modestus ci-*

licius, and even to *E. aurolineatus*, as described by Schmidtler (1993). Thus the more reliable diagnostic characters of pholidosis should support validity of the latter taxa.

REFERENCES

- Dotsenko I. B.** (1989), "A review of the genus *Eirenis* (Reptilia, Colubridae). 2. The structure of the genus *Eirenis*," *Vestnik Zool.*, **5**, 23 – 29 [in Russian].
- Dotsenko I. B.** (2001), "The significance of pattern's features for the taxonomy and phylogenicschemes in Colubrid snakes: an example of the genus *Eirenis*," in: *Proc. of the 1th Meet. Nikolsky Herpetol. Soc. "The Problems of Herpetology*," Pushchino – Moscow, pp. 83 – 86 [in Russian].
- Schmidtler J. F.** (1986) "Orientalische Smaragdeideschen: 1. Zur Systematik und Verbreitung von *Lacerta viridis* in der Turkei (Sauria, Lacert.)," *Salamandra*, **22**, 29 – 46.
- Schmidtler J. F.** (1993) "Zur Systematik und Phylogenie des *Eirenis-modestus*-Komplex in Süd-Anatolien," *Spixiana*, **16**(1), 79 – 96.

VARIATION OF *Hyla savignyi*: A COLOR PATTERN OF CYPRIOTE AND MAINLAND POPULATIONS

V. Gvoždík¹ and J. Moravec²

Keywords: Hylidae, *Hyla savignyi*, variation, color pattern, Cyprus.

INTRODUCTION

Audouin (1809) distinguished *Hyla savignyi* from *Hyla arborea* (Linnaeus, 1758) on the basis of its different color pattern (absence of upward loop on the lateral dark stripe). Later, Boulenger (1882) mentioned the former taxon as a variety of *H. arborea* whereas Nikolsky (1918) gave it a subspecific rank. More recently, Schneider and Nevo (1972) referred to differences in the mating calls of the both taxa and proposed to elevate the former one to the specific level. This approach was followed by number of other authors and today the name *H. savignyi* is widely used for the tree frog populations distributed in southern and eastern Turkey, Transcaucasia, western Iran, Iraq, Levant, north-eastern part of Sinai, Cyprus, and south-western part of Arabian Peninsula. Nevertheless, except of the description of the general coloration of *H. savignyi*, till now there are no available data dealing more thoroughly with the color pattern of this species and its possible geographical variation.

Examining the morphological variation of *H. savignyi* we noticed remarkably frequent occurrence of spotted to striped dorsal color pattern in Cypriote population. The aim of this brief report is (i) to draw attention to this phenomenon, (ii) to describe the patterned form of dorsal coloration of *H. savignyi*, and (iii) to quantify the geographic distribution of this form.

MATERIAL AND METHODS

The material consisted of 599 museum specimens of *H. savignyi* from its whole distribution area. Additional data were collected directly in the field in Cyprus, Turkey, Syria, Lebanon, Israel and Jordan. The comparison of the color pattern of the Cypriote population with the population from adjacent mainland (Mediterranean zone of Tur-

key, Syria, and Lebanon) was based on 421 specimens (Cyprus: 240, mainland: 181; see Fig. 1 for localities).

Variation in the dorsal color pattern consists in presence, shape and arrangement of dark dorsal spots, which, according to our observation on living specimens, can undergo on the background color independent color changes. We defined two basic groups of color pattern for needs of our study: (i) pattern-less and (ii) patterned. The latter was subdivided into two subgroups: (ii-a) spotted, individuals bearing irregularly distributed spots; (ii-b) striped, individuals with more or less complete longitudinal stripes formed by oblong spots. Pattern of dark permanent little dots, which occurred in *H. arborea* too, was omitted within our investigation of the dorsal color pattern.

RESULTS AND DISCUSSION

The examination of the studied material revealed that the frequency of the patterned specimens is higher in the Cypriote population than in the populations from the other parts of the range of *H. savignyi*. The comparison proper of the samples from Cyprus and the adjacent mainland approved a significantly higher occurrence of patterned specimens in the island population (45.4%, 109/240 vs. 23.2%, 42/181, $\chi^2 = 22.13$, $df = 1$, $p < 0.0001$; Fig. 2). There was also a significantly higher occurrence of striped

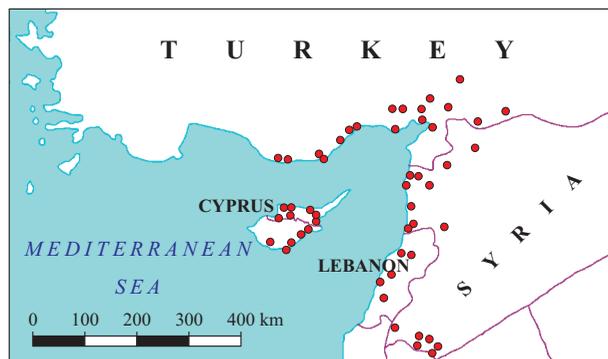


Fig. 1. Schematic map of localities of the material examined.

¹ Department of Zoology, Charles University, Viničná 7, CZ-128 44 Praha 2, Czech Republic; E-mail: vgvozdik@email.cz.

² Department of Zoology, National Museum (Natural History), Václavské náměstí 68, CZ-115 79 Praha 1, Czech Republic; E-mail: jiri.moravec@nm.cz.

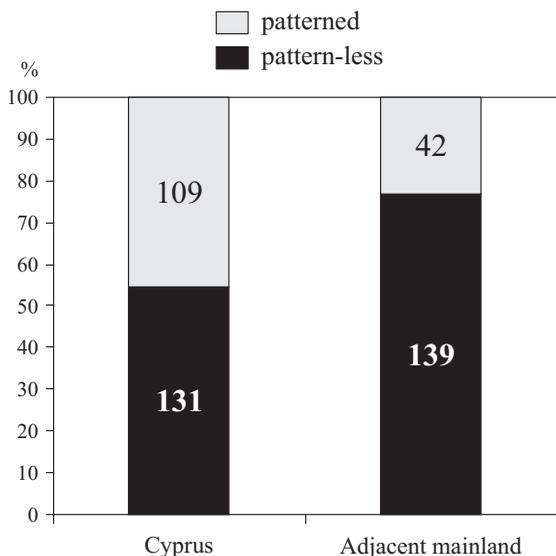


Fig. 2. Comparison of ratios of patterned specimens.

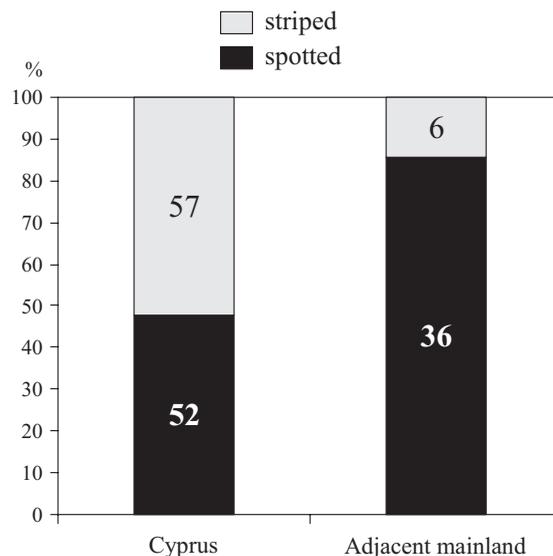


Fig. 3. Comparison of ratios of striped specimens within patterned specimens.

individuals within the patterned part of the Cypriote population (52.3%, 57/109 vs. 14.3%, 6/42, $\chi^2 = 18.01$, $df = 1$, $p < 0.0001$; Fig. 3).

The dorsal color pattern can be more or less obvious in dependence of the current physiological conditions. According to our observations from the field and captivity, the intensity of the dorsal color pattern changes in dependence on daily cycle and on activity of frog. The color pattern is more visible at nights, when the spots/stripes turn from green (different tone from background color) to dark brown or black. Nevertheless, it is obvious during daytime too. Interesting finding is that already Boulenger (1898) mentioned the striped or spotted pattern in *H. savignyi* (at that time as *H. arborea* var. *savignyi*) on page 251: “Some specimens (Cyprus) have four stripes or series of spots in addition to the lateral.” He also supplied this record by figure of Cypriote specimen on plate XV (see Fig. 4).

Our findings indicate that Cypriote population of *H. savignyi* has undergone a certain degree of differentiation from the mainland populations. These findings are also supported by our other morphological and bioacoustic data. The most obvious difference is in body size. Tree frogs of Cypriote population are significantly smaller than tree frogs from adjacent mainland (Gvoždik et al., in preparation). Already Schmidtler (1984) gave notice of this phenomenon. He considered Cypriote tree frogs as a “dwarf form” of supposedly subspecific status. On the other hand Böhme and Wiedl (1994) discussed weak insular endemism of the herpetofauna of Cyprus. One of the possibilities how to answer this problem is the insufficient knowledge of amphibians and reptiles biodiversity in the region

of eastern Mediterranean. The study pointing to specific status of Cypriote water frogs could be used as an example (Plötner et al., 2001). Tarkhishvili and Gokhelasvili (1999) wrote about tree frogs from Cyprus that they “are morphologically similar to *H. savignyi*, although nowadays they are assumed to represent different species.” This information is very interesting but certainly incorrect, be-



Fig. 4. Illustration of a Cypriote specimen of *H. savignyi* published by Boulenger (1898: Plate XV, Figure 3).

cause no particular investigations of variation of *H. savignyi* have been accomplished neither from Cyprus nor from other parts of the distribution range so far.

Therefore, taxonomic status of Cypriote tree frogs needs further verification on the basis of particular morphological, bioacoustic and genetic studies.

Acknowledgments. We thank W. Böhme (Bonn, Germany), R. Danielsson (Lund, Sweden), U. Fritz (Dresden, Germany), R. Günther (Berlin, Germany), J. Hallermann (Hamburg, Germany), U. Kaya (Bornova-Izmir, Turkey), P. Lymberakis (Irakleio, Greece), J. Mariaux (Geneve, Switzerland), J. B. Rasmussen (Copenhagen, Denmark), F. Tiedemann (Vienna, Austria), J. V. Vindum (San Francisco, CA, USA) for loans of preserved specimens or access to them *in situ*. First author would like to thank the foundation “Nadáni Josefa, Marie a Zdeňky Hlávkových” for the financial support of the expedition to Turkey and Cyprus. The work of the second author was supported by the Czech Ministry of Culture (projects Nos. RK01P03OMG006 and MK0CEZ99F0201).

REFERENCES

- Audouin V.** (1809), “Explication sommaire des planches de reptiles (supplément),” in: *Description de l’Égypte. Histoire naturelle. Vol. 1. Imprimerie Impériale*, Paris, pp. 161 – 184.
- Böhme W. and Wiedl H.** (1994), “Status and zoogeography of the herpetofauna of Cyprus, with taxonomic and natural history notes on selected species (genera *Rana*, *Coluber*, *Natrix*, *Vipera*)”, *Zool. Middle East*, **10**, 31 – 51.
- Boulenger G. A.** (1882), *Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum. 2nd Edition*, Reprinted 1966 by Wheldon & Wesley, Verlag J. Cramer, London.
- Boulenger G. A.** (1898), *The Tailless Batrachians of Europe. Part II. Ray Sociandy*. London, p. 251, pl. XV.
- Nikolsky A. M.** (1918), *Fauna Rossii i Sopredel’nykh Stran. Zemnovidnye (Amphibia)* [Fauna of Russia and Adjacent Countries. Amphibians (Amphibia)], Izd. RAN, Petrograd.
- Plötner J., Ohst T., Böhme W., and Schreiber R.** (2001), “Divergence in mitochondrial DNA of Near Eastern water frogs with special reference to the systematic status of Cypriote and Anatolian populations (Anura, Ranidae)”, *Amphibia-Reptilia*, **22**, 397 – 412.
- Schmidtler J. F.** (1984), “Zur Bestandsituation der Amphibien und hydrophilen Reptilien auf der Insel Zypern”, *Salamandra*, **20**(1), 43 – 49.
- Schneider H. and Nevo E.** (1972), “Bio-acoustic study of the yellow-lemon treefrog, *Hyla arborea savignyi* Audouin”, *Zool. Jb. Physiol.*, **76**, 497 – 506.
- Tarkhnishvili D. N. and Gokhleshvili R. K.** (1999), “The amphibians of the Caucasus”, *Adv. Amphibian Res. Former USSR*, **4**, 1 – 239.

SIBLING SPECIES, ADVERTISEMENT CALLS, AND REPRODUCTIVE ISOLATION IN FROGS OF THE *Leptodactylus pentadactylus* SPECIES CLUSTER (AMPHIBIA, LEPTODACTYLIDAE)

W. R. Heyer,¹ R. O. de Sá,² and A. Rettig²

Keywords: *Leptodactylus pentadactylus* species cluster, advertisement calls, reproductive isolation, speciation.

INTRODUCTION

A recent re-evaluation of morphological and advertisement call variation in the large species of frogs of the *Leptodactylus pentadactylus* cluster discovered more examples of sibling species as defined by Ernst Mayr in his influential book *Animal Species and Evolution*. All previously documented instances of sibling species in frogs demonstrated advertisement call differentiation consistent with the calls serving as pre-mating isolating mechanisms. However, we find one instance of two species with non-distinguishable adult morphologies as well as non-distinguishable advertisement calls. Presumably, the new instances of sibling species reflect retention of ancestral adult morphologies and advertisement calls. Larval and habitat differentiation appear to be important factors in the speciation processes in this group of frogs.

Ernst Mayr defined sibling species as, “morphologically similar or identical natural populations that are reproductively isolated,” in his influential book *Animal Species and Evolution* (Mayr, 1963 : 34). He considered the phenomenon to be important to understanding the complexity and scope of speciation processes in animals.

Mayr’s concept of sibling species has been documented in the frog genus *Leptodactylus* (Barrio, 1973; Heyer et al., 1996). In these examples, reproductive isolation among sibling species was documented through analysis of advertisement calls. That is, advertisement calls are very different from each other among species whose adults are morphologically identical. Females use the calls to distinguish and select males of their own species to mate with in any mixed chorus where sibling species co-occur.

In the process of re-evaluating variation in the *Leptodactylus pentadactylus* species cluster, we discovered instances of sibling species that do not demonstrate reproductive isolation on the basis of advertisement calls.

Herein we summarize the nature of the differentiation involved and discuss the evolutionary implications of our findings.

MATERIAL AND METHODS

Features for several color patterns, adult morphological characters, and measurements were analyzed in terms of geographic variation for the species currently known as *Leptodactylus knudseni*, *labyrinthicus*, and *pentadactylus* (Heyer et al., 2005). These data were used to postulate species boundaries for the taxa studied.

Molecular sequence data were obtained for 1807 base pairs of 12S and 16S mitochondrial rDNA genes using standard techniques (see Heyer et al., in press). The sequence data have GenBank accession numbers AY947855-82. Voucher specimen data are presented in the *Appendix*. The sequence data were analyzed with maximum likelihood and parsimony methods using PAUP* (Swofford, 1998).

RESULTS AND DISCUSSION

Taxonomic Novelties

In order to discuss the biological implications of our results it is necessary to give a brief synopsis of some of our taxonomic findings. A set of specimens from the State of Pará, Brasil identified in collections either as *L. knudseni* or *L. labyrinthicus* represent a new species. This new species will be referred to as the Pará species for purposes of this paper. The taxon currently recognized as *L. labyrinthicus* contains three species: *L. labyrinthicus*, *L. vastus*, and a new species from coastal Venezuela. Herein we use the available name *L. vastus* for the taxon occupying northeastern Brazil (justification in Heyer, in press). The taxon currently recognized as *L. pentadactylus* consists of four species: *L. pentadactylus* and three new species re-

¹ Amphibians and Reptiles, MRC 162, PO Box 37012, Smithsonian Institution, Washington, DC 20013-7012, USA; E-mail: heyerr@si.edu.

² Department of Biology, University of Richmond, Richmond, VA 23173, USA; E-mail: rdesa@richmond.edu.

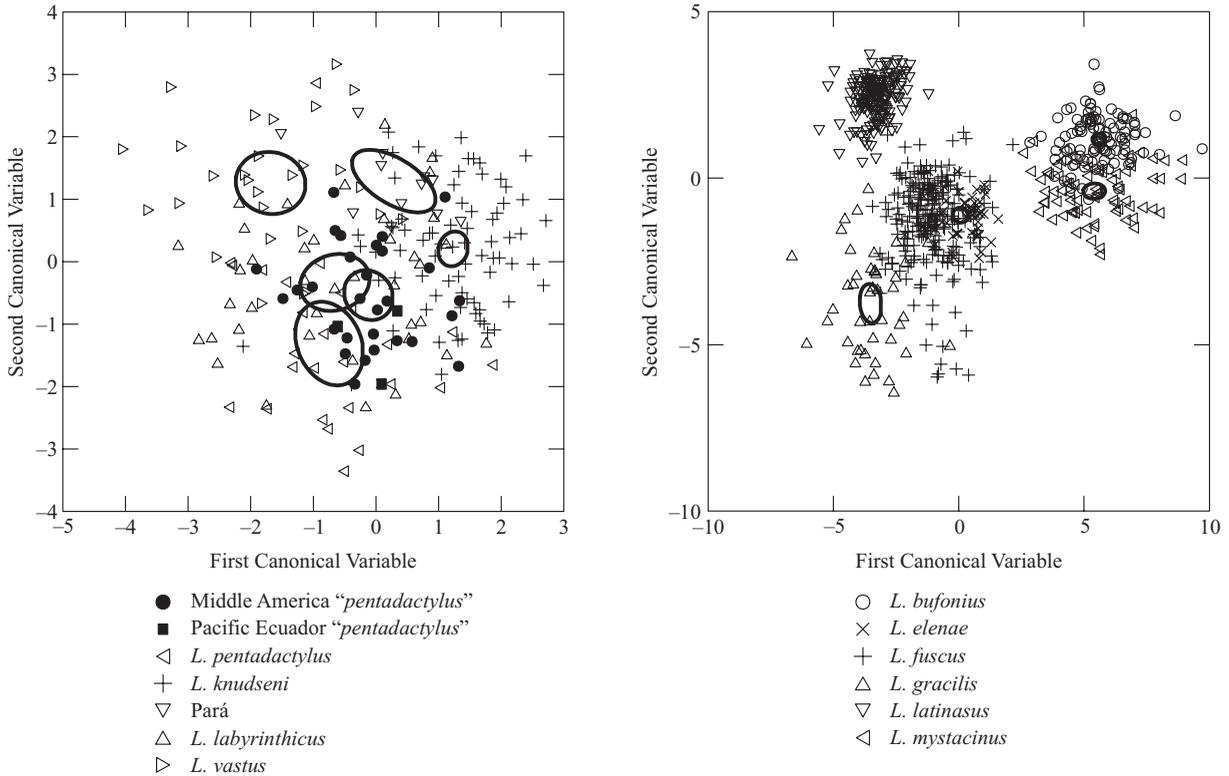


Fig. 1. Discriminant function analyses of male measurement data for comparable sets of species of the *Leptodactylus pentadactylus* species cluster (left) and the *Leptodactylus fuscus* group (right). The *L. fuscus* group species are those that occur together in the Gran Chaco of South America. Note that the data on the right are more structured than those on the left, for example, there is only a single cloud of points on the left and at least three clouds of points on the right and the sample confidence ellipses ($p = 0.683$) are much smaller on the right than the left. There are 6 confidence ellipses on the left (none for Pacific Ecuador “*pentadactylus*” with $N = 3$) and 6 on the right (the ellipses for *L. fuscus* and *L. latinus* are not visible due to small sizes of ellipses and density of data points).

ferred to herein as Middle American “*pentadactylus*,” Pacific Colombia “*pentadactylus*,” and Pacific Ecuador “*pentadactylus*.” The status of *L. knudseni* is unchanged. We analyzed DNA from all these species except for the coastal Venezuela and Pacific Colombia “*pentadactylus*” species.

Lack of Adult Morphological and Advertisement Call Differentiation

There is much less morphological differentiation among members of the *L. pentadactylus* cluster than found in other major clades of *Leptodactylus* studied to date. The measurement data illustrate the sort of differentiation exhibited by all the adult morphological data (Fig. 1). In particular, adult morphologies can not distinguish *L. knudseni* from Middle American “*pentadactylus*” nor *L. labyrinthicus*, *L. vastus*, and the northern Venezuela species from each other (Heyer, in press).

The advertisement calls of *L. knudseni* can not be consistently differentiated from the calls of Middle American “*pentadactylus*” (Fig. 2). The calls of these two species are

so similar to those of *L. labyrinthicus* that it is likely that females of any of these species would select males of the wrong species in a mixed chorus should they occur sympatrically.

Although there is no adult morphological or advertisement call differentiation among certain of the species, there are other features that are diagnostic. For example, *L. knudseni* and Middle American “*pentadactylus*” differ from each other in several larval internal buccal cavity characters as well as juvenile color patterns (Heyer, in press).

Genetic Relationships

The results of the mitochondrial rDNA gene analyses are best illustrated by the maximum likelihood bootstrap tree (Fig. 3) and the comparison of sequence differences among the samples analyzed (Table 1).

Contrary to expectations based on the morphological and advertisement call data, neither *L. knudseni*/Middle American “*pentadactylus*” nor *L. labyrinthicus*/*vastus* are

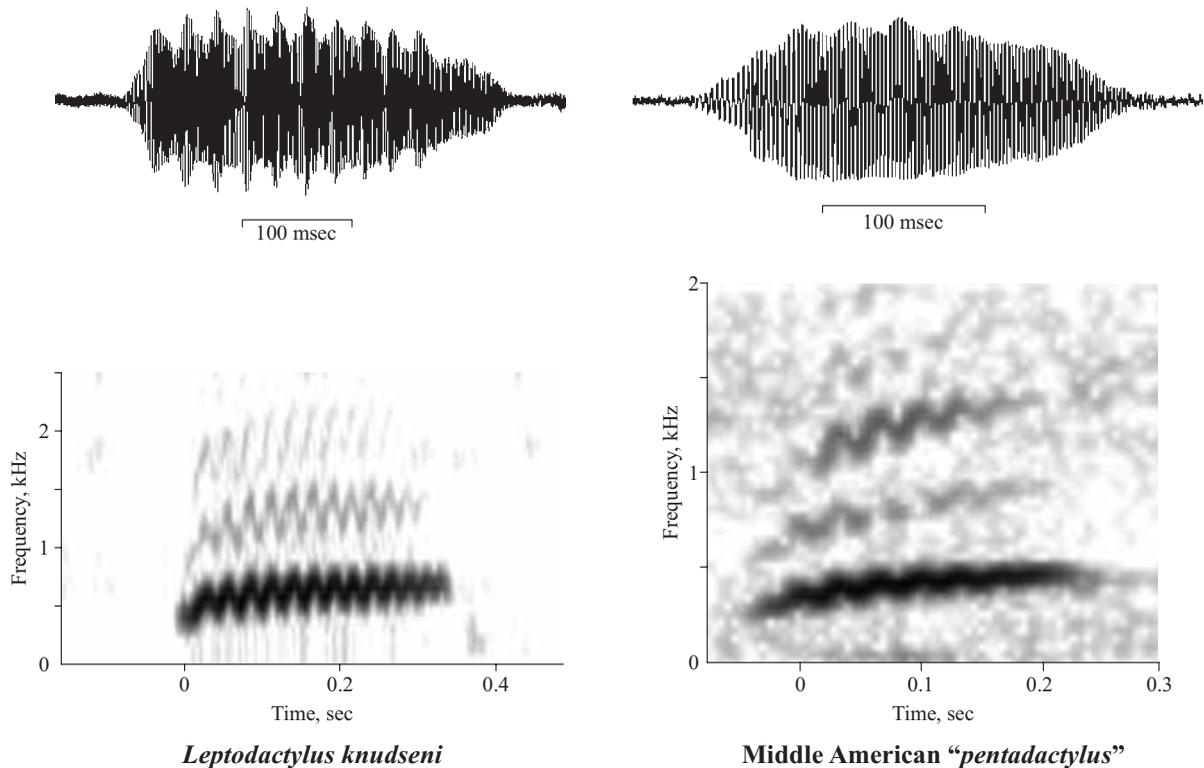


Fig. 2. Wave forms and audiospectrograms of *Leptodactylus knudseni* (USNM recording 228, cut 1) (left) and Middle American "pentadactylus" (USNM recording 106, cut 1) (right). The calls have the same duration, structure, and overlapping broadcast frequencies.

sister-species in the molecular cladogram (Fig. 3) that reflects their genetic relationships.

Sibling Species in Frogs Revisited

Our study demonstrates additional examples of sibling species as defined by Mayr (1963) in frogs of the genus *Leptodactylus*. Although sibling species in frogs are not common, they are not unexpected because they have been documented in several families of frogs. We did expect to find that advertisement calls of sibling species in frogs would serve as a reproductive isolating mechanism. Finding sibling species that do not differ in advertisement calls but demonstrate extensive molecular differentiation has not been previously reported in frogs that we are aware of. There are two implications of our findings.

1) Ancestral adult morphologies and advertisement calls are retained in several species of the *L. pentadactylus* cluster. The fact that advertisement calls do not differ among some species of the *L. pentadactylus* cluster requires rethinking of the role of advertisement calls in the speciation processes of frogs.

2) Other factors than adult morphology and advertisement calls play an important role in speciation within the *L. pentadactylus* cluster. There is much more larval varia-

tion within the *L. pentadactylus* cluster than observed in other major *Leptodactylus* clades. The variation includes differentiation in internal buccal cavity morphology, oral disk morphology, and aquatic versus terrestrial larvae (Heyer, in press). Habitat differentiation is pronounced among the species of the *L. pentadactylus* cluster in general and especially in those species lacking differences in adult morphology or advertisement calls (Heyer, in press). For example, *L. labyrinthicus* is closely associated with the Cerrado Morphoclimatic Domain, *L. vastus* with the Caatinga Morphoclimatic Domain, whereas the morphologically similar Pará species occurs only within the rain forests of the Amazonian Equatorial Morphoclimatic Domain as defined by Ab'Sáber (1977).

Our study indicates that the role of advertisement calls in frog speciation processes requires re-evaluation. We have been able to identify this finding because we analyzed three different data sets for the same taxa of frogs: morphological, advertisement call, and genetic. We suspect that additional examples of sibling species lacking differentiation in advertisement calls will be found in the future with analyses of variation in morphology, advertisement calls, and molecules simultaneously on the same taxa.

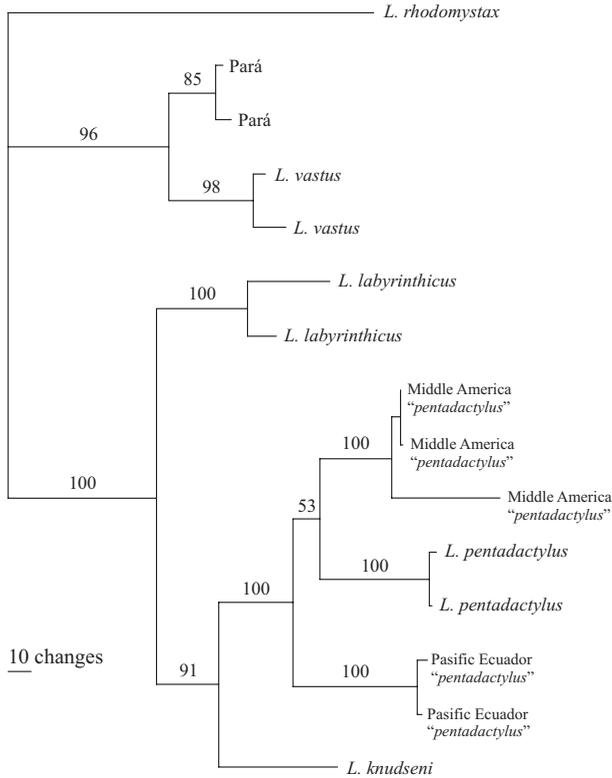


Fig. 3. Maximum likelihood bootstrap analysis of combined 12S and 16S mitochondrial rDNA data. Numbers are bootstrap values greater than 50% support.

Acknowledgments. We thank our colleagues Ulisses Caramaschi, Andrew Chek, Luis A. Coloma, Ronald I. Crombie, Jeremy Jacobs, and Miguel T. Rodrigues for allowing us to utilize tissue samples they collected.

George R. Zug (USNM) kindly reviewed the manuscript.

Financial support was provided by the Neotropical Lowlands Research Program, Smithsonian Institution (Richard P. Vari, Principal Investigator) and the National Science Foundation (awards # 9815787 and #0342918 and subsequent REU amendments) to RdS and WRH.

REFERENCES

Ab’Sáber A. N. (1977), “Os domínios morfoclimáticos na América do Sul. Primeira aproximação,” *Geomorfologia (São Paulo)*, No. 52, 1 – 22 + map.

Barrio A. (1973), “*Leptodactylus geminus* una nueva especie del grupo *fuscus* (Anura, Leptodactylidae),” *Physis*, **32**, 199 – 206.

Heyer W. R. (in press), “Variation and taxonomic clarification of the large species of the *Leptodactylus pentadactylus* species group (Amphibia: Leptodactylidae) from Middle America, northern South America, and Amazonia,” *Arquivos de Zoologia*.

Heyer W. R., de Sá R. O., and Muller S. (2005), “On the enigmatic distribution of the Honduran endemic *Leptodactylus silvanimbus* (Amphibia: Anura: Leptodactylidae),” in: Donnelly M. A., Crother B. I., Guyer C., Wake M. H., and White M. E. (eds.), *Ecology and Evolution in the Tropics: A Herpetological Perspective*, Univ. of Chicago Press, Chicago, pp. 81 – 101.

TABLE 1. General Time-Reversible Distance Matrix

	Lr	Lk	P1	P2	Lv1	Lv2	Ll1	Ll2	MAp1	MAp2	MAp3	PE1	PE2	Lp1	Lp2
Lr	–														
Lk	0.30	–													
P1	0.30	0.14	–												
P2	0.31	0.14	0.01	–											
Lv1	0.31	0.17	0.04	0.05	–										
Lv2	0.32	0.15	0.05	0.05	0.01	–									
Ll1	0.31	0.13	0.18	0.18	0.19	0.18	–								
Ll2	0.30	0.10	0.15	0.16	0.20	0.19	0.03	–							
MAp1	0.34	0.09	0.15	0.15	0.19	0.18	0.12	0.10	–						
MAp2	0.34	0.09	0.15	0.15	0.19	0.18	0.12	0.10	0.00	–					
MAp3	0.36	0.12	0.18	0.18	0.19	0.18	0.08	0.14	0.03	0.03	–				
PE1	0.34	0.12	0.18	0.18	0.21	0.19	0.16	0.12	0.07	0.07	0.12	–			
PE2	0.34	0.12	0.18	0.18	0.21	0.19	0.16	0.13	0.07	0.07	0.11	0.00	–		
Lp1	0.35	0.11	0.21	0.22	0.25	0.23	0.16	0.13	0.06	0.06	0.10	0.09	0.09	–	
Lp2	0.34	0.10	0.20	0.21	0.24	0.22	0.16	0.13	0.06	0.06	0.10	0.09	0.09	0.00	–

Lr, *Leptodactylus rhodomystax*, MZUSP 940333; Lk, *Leptodactylus knudseni*, QCAZ 13077; P1, *Pará* species, MZUSP 70023; P2, *Pará* species, MZUSP 70075; Lv1, *Leptodactylus vastus*, MTR 976629 (MZUSP ???); Lv2, *Leptodactylus vastus*, USNM 284552; Ll1, *Leptodactylus labyrinthicus*, USNM 303175; Ll2, *Leptodactylus labyrinthicus*, UC 233; MAp1, Middle American “*pentadactylus*” species, USNM 347153; MAp2, Middle American “*pentadactylus*” species, USNM 298079; MAp3, Middle American “*pentadactylus*” species, USNM 534219; PE1, Pacific Ecuador “*pentadactylus*” species, QCAZ 17056; PE2, Pacific Ecuador “*pentadactylus*” species, QCAZ 19859; Lp1, *Leptodactylus pentadactylus*, USNM 303466; Lp2, *Leptodactylus pentadactylus*, MZUSP 70917.

- Heyer W. R., García-Lopez J. M., and Cardoso A. J.** (1996), “Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species,” *Amphibia-Reptilia*, **17**, 7 – 31.
- Leviton A. E., Gibbs Jr. R. H., Heal E., and Dawson C. E.** (1985), “Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology,” *Copeia*, **1985**, 802 – 832.
- Mayr E.** (1963), *Animal Species and Evolution*, The Belknap Press of Harvard Univ. Press, Cambridge (MA), pp. xiv + 1 – 797.
- Swofford D. L.** (1998), *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4*, Sinauer Assoc. Sunderland (MS).

Appendix

Voucher specimen data used in molecular analyses. Museum designations follow Leviton et al. (1985) with the addition of Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito (QCAZ).

***Leptodactylus knudseni*.** QCAZ 13077 — Ecuador, Francisco Orellana, Parque Nacional Yasuní.

***Leptodactylus labyrinthicus*.** Ulisses Caramaschi field number 233 — Brasil, São Paulo, Piraju. USNM 303175 — Brasil, São Paulo, Fazenda Jataí, 5 km S of Luis Antonio.

Middle American “*pentadactylus*” species. USNM 298079, 347153 — Panama, Bocas del Toro, Isla Popa. USNM 534219 — Honduras, Colon, Quebrada Machin.

Pacific Ecuador “*pentadactylus*” species. QCAZ 17056 — Ecuador, Esmeraldas, Alto Tambo. QCAZ 19859 — Ecuador, Esmeraldas, Bosque Protector La Perla.

Pará species. MZUSP 70023 — Brasil, Pará, Aldeia A-Ukre. MZUSP 70075 — Brasil, Pará, Rio Vermelho.

***Leptodactylus pentadactylus*.** MZUSP 70917 — Brasil, Pará, Serra de Kukoinhokren. USNM 303466 — Brasil, Pará, near Cachoeira do Espelho, ca. 50 km (airline) S of Altamira.

***Leptodactylus rhodomystax* (outgroup taxon).** MZUSP 70375 — Brazil, Pará, Serra de Kukoinhokren.

***Leptodactylus vastus*.** MTR 976629 (to be deposited in MZUSP) — Brasil, Mato Grosso, Gaúcha do Norte. USNM 284552 — Brasil, Pernambuco, near Caruaru.

DNA POLYMORPHISM AND GENETIC DIFFERENTIATION OF *Testudo graeca* L.

A. Korsunenکو,^{1,2} V. Vasilyev,¹ S. Pereshkolnik,³ L. Mazanaeva,⁴
R. Lapid,⁵ A. Bannikova,² and S. Semyenova¹

Keywords: DNA, RAPD-PCR, *Testudo*, genetic differentiation.

INTRODUCTION

The main object of this investigation is the wide spread spur-thighed tortoise *Testudo graeca* L. which mostly inhabit northern Africa but is also present in south-eastern Europe and has been introduced at several other locations including Greece and southern Spain. The species *T. graeca* includes 7 subspecies (Ananyeva et al., 1998): *T. g. graeca* Linnaeus, 1758; *T. g. terrestris* Forskal, 1775; *T. g. ibera* Pallas, 1814; *T. g. zarudnyi* Nikolsky, 1896; *T. g. nikolskii* Chkhikvadze et Tuniyev, 1986; *T. g. armeniaca* Chkhikvadze et Bakradze, 1991; *T. g. pallasi* Chkhikvadze, 1989.

Two subspecies of *T. graeca* are found on the territory of Russia: *T. g. pallasi* (western Caspian Sea coast) and *T. g. nikolskii* (northeastern Black Sea coast). There are many facts concerning the morphological polymorphism (number of claws on the forelegs, colour of claws, number and sizes of spurs, structure of carapace scutes) found in the populations of these subspecies (Kostina and Galichenko, 1998; Leontyeva et al., 1998) but there is no confirming evidence for molecular analysis of these subspecies. Now we have study the genetic variability and relationships of three *Testudo* species (*T. graeca*, *T. marginata*, *T. kleinmanni*) using RAPD PCR (Williams et al., 1990). This method has been widely used to determine the levels of genetic variation within and among populations and to describe relationships among species.

MATERIAL AND METHODS

Blood samples (100 ml) were taken from 32 individuals of 3 species (*T. graeca*, *T. marginata* from northern Greece, *T. kleinmanni* from Cairo) and 4 subspecies (*T. g. pallasi* from different regions of Daghestan, *T. g. nikolskii* from Novorossiisk region — the northeastern

Black Sea coast, *T. g. terrestris* from Central Israel, *T. g. ibera* from Turkey) of the genus *Testudo* and from 5 tortoises of *Agrionemys horsfieldii* (different regions of Central Asia). Five primers have been used for amplification: OPA-17 (5'-GACCGCTTGT-3'), R-45 (5'-GCCGTC-CGAG-3'), R-65 (5'-GGGACGTCTC-3'), SB-2 (5'-GACGGCCAGTATT-3'), OPT-14 (5'-AATGCCGCAG-3'). For each RAPD-pattern a 1/0 (the presence/absence of the amplified bands) matrix was constructed and analyzed. Genetic distances between individuals were estimated with the coefficient

$$GD_{xy} = (N_x + N_y) : (N_x + N_y + N_{xy}),$$

where N_x and N_y is the number of bands present in x or y individuals, and N_{xy} is the number of bands shared by x and y individuals. Statistical support for the branching pattern of the UPGMA-trees was obtained with the bootstrap method (1000 replicates) and performed by the program TREECON FOR WINDOWS (Van de Peer and De Wachter, 1994).

RESULTS AND DISCUSSION

To analyze intrapopulation differentiation of tortoises from Daghestan DNA samples of 21 *T. g. pallasi* were examined. Five primers revealed 180 scorable markers ranging in size from 250 to 1000 bp (Fig. 1). Five specimens of *A. horsfieldii* from different regions of Central Asia were analyzed to compare their genetic polymorphism with that of discovered in Daghestan population of *T. g. pallasi*. There are two separated clusters on the dendrogram of genetic distances with bootstrap values (data are not shown). The specimens of *T. g. pallasi* form one cluster which includes tortoises from different regions of Daghestan while the specimens of *A. horsfieldii* are united in the other cluster. The minimum and maximum of genetic distances for the pair of *T. g. pallasi* are approximately 0.25 and 0.65, respectively, while the minimum and maximum of genetic distances for the pair of *A. horsfieldii* are approximately 0.25 and 0.45, respectively. It indicates

¹ Institute of Gene Biology Russian Academy of Sciences, Vavilova str., 34/5, 119334 Moscow, Russia; E-mail: korsanna@mail.ru.

² Moscow State University, Department of Vertebrate Zoology, Moscow, Russia.

³ Moscow Zoo, Moscow, Russia.

⁴ Daghestan State University, Makhachkala, Russia.

⁵ Hebrew University of Jerusalem, Rehovot, Israel.

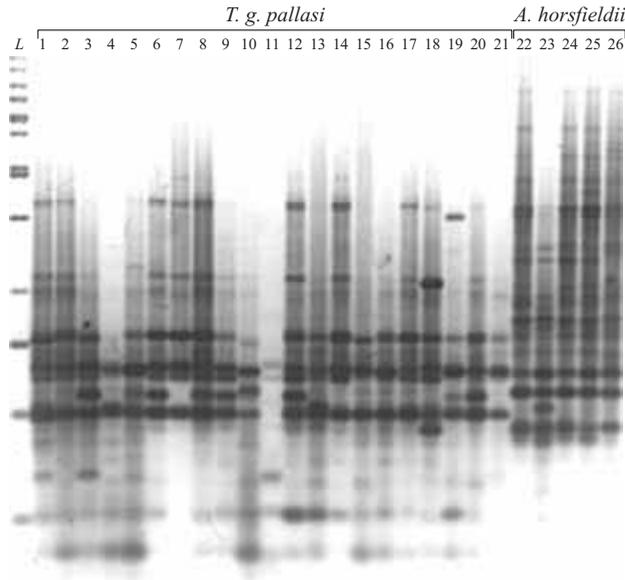


Fig. 1. RAPD-patterns of *Testudo graeca pallasii* (1 – 21) and *Agrionemys horsfieldii* (22 – 26) individuals revealed by primer R-45. L, marker of molecular weights (1 kb Ladder).

the high level of genetic differentiation for *T. g. pallasii* population.

19 DNA samples of *T. graeca* (13 *T. g. pallasii*, 3 *T. g. nikolskii*, 2 *T. g. ibera*, 1 *T. g. terrestris*), 1 sample of *T. marginata* and 1 of *T. kleinmanni* have been used to test the genetic relationships between these species. Three primers (OPA-17, R-65, R-45) revealed 174 markers ranging in size from 250 to 1500 bp. Two main clusters are represented in Fig. 2. One of them includes *T. marginata* and *T. kleinmanni* (patterns 20, 21). Previously similar results have been obtained with mt DNA polymorphic markers (Van der Kuyl et al., 2002). The second cluster consists of 4 subclusters including 4 subspecies: *T. g. terrestris*, *T. g. nikolskii*, *T. g. ibera*, *T. g. pallasii*. All subclusters have a significant bootstrap support (68%-99%). It is interesting that specimens of *T. g. nikolskii* form one cluster together with geographically isolated specimens of *T. g. ibera* but not with samples from a less remote population of *T. g. pallasii*.

Thus we found close genetic relationships between *T. g. nikolskii* and *T. g. ibera* subspecies that may be explained by their originating from a common ancestor group. These subspecies lived in Pliocene on one single land consisted of the western Black Sea coast, southern Crimea, eastern Balkans and Middle East. These parts were united in land Pontida. That is why present herpetofauna of these geographic regions is rather similar. *T. g. pallasii* originated from the other ancestor group in-

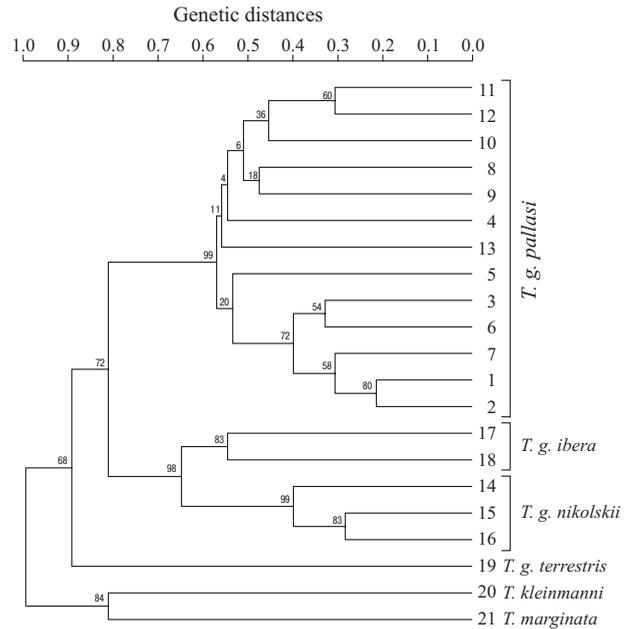


Fig. 2. UPGMA-tree of genetic distances between specimens of three *Testudo* species based on variability of 174 RAPD-markers.

habited Iran's territory (Inozemtsev and Pereshkolnik, 1987).

CONCLUSIONS

1. The high level of genetic variability in *T. g. pallasii* population were revealed using RAPD PCR analysis.
2. The genetic differentiation of the three studied groups (*T. g. pallasii*, *T. g. nikolskii*, *T. g. ibera*) based on RAPD markers does not contradict with the earlier recognition of the three subspecies. It was found that *T. g. nikolskii* is more closely genetically related to *T. g. ibera* than to *T. g. pallasii*.
3. Two species, *T. marginata* and *T. kleinmanni*, were found to be more closely related to each other than to *T. graeca*. These RAPD PCR data are in a good agreement with the differentiation of these species determined by the mtDNA polymorphic markers (Van der Kuyl et al., 2002).

This research was partly supported by the Russian Foundation for Basic Research (grant No. 02-04-48516) and State Programm of Integration (grant No. CH0064/885).

REFERENCES

- Ananjeva N. B., Borkin L. Y., Darevsky I. S., and Orlov N. L. (1998), *Encyclopedia of Russian Nature. Amphibians and Reptiles*, ABF, Moscow, pp. 195 – 203 [in Russian].

- Inozemtsev A. A. and Pereshkolnik S. L.** (1987), "To retain the part of Pontida," *Priroda*, **8**, 38 – 49 [in Russian].
- Kostina G. N. and Galichenko M. V.** (1998), "Some morphological peculiarities of *Testudo graeca* L. from South Daghestan," in: *The Influence of Anthropogenic Factors on the Structure and Functioning of Biocenoses and Their Separate Components*, Moscow, pp. 80 – 84 [in Russian].
- Leontyeva O. A. et al.** (1998), "Comparative ecological and morphological characteristics of *Testudo graeca nikolskii* and *T. g. ibera* in the Caucasus," in: Miaud C. and Guyétant R. (eds.), *Current Studies in Herpetology*, Soc. Eur. Herpetol., Le Bourget du Lac, pp. 263 – 268.
- Van de Peer Y. and De Wachter R.** (1994), "TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment," *Comput. Applic. Biosci.*, **10**, 569 – 570.
- Van der Kuyl A. et al.** (2002), "Phylogenetic relationships among the species of genus *Testudo* (Testudines: Testudiniidae) inferred from mitochondrial 12S rRNA gene sequences," *Mol. Phylogenet. Evol.*, **22**, 174 – 183.
- Williams J. G. K. et al.** (1990), "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucl. Acids Res.*, **18**, 6531–6535.

FIRST DATA ON THE GEOGRAPHIC VARIATION OF *Emys orbicularis* IN UKRAINE: mtDNA HAPLOTYPES, COLORATION, AND SIZE

T. Kotenko,¹ O. Zinenko,² D. Guicking,³ H. Sauer-Gürth,³ M. Wink,³ and U. Fritz⁴

Keywords: *Emys orbicularis*, geographic variation, molecular phylogeography, Ukraine.

INTRODUCTION

Morphological differences between European pond turtles, *Emys orbicularis* (Linnaeus, 1758), from the Crimea and of other parts of Ukraine were noted for the first time by Szczerbak (1966). He stated that Crimean turtles are reaching a maximum carapacial length of only 160.5 mm and are, therefore, distinctly smaller than in other regions. Szczerbak (1966) believed that this low maximum shell length is due to a poorer food supply and the smaller size of Crimean water bodies compared with pond turtle habitats in other parts of the range. However, according to Fritz (1992, 1994), this size difference reflects a taxonomic differentiation. Fritz (1992, 1994) attributed the small and light colored pond turtles from the southern Crimea to the eastern Mediterranean subspecies *Emys orbicularis hellenica* (Valenciennes, 1832), whereas the large, dark specimens from the northern Crimea and the mainland of Ukraine were thought by him to represent *Emys orbicularis orbicularis* (Linnaeus, 1758). Later, investigations on the mitochondrial phylogeography of *E. orbicularis* supported that pond turtles from the Ukrainian mainland represent the nominotypical subspecies. These specimens share their mtDNA haplotype with *E. o. orbicularis* from most other northern parts of the species' range (Lenk et al., 1999). However, until now no mtDNA data have been available for Crimean *E. orbicularis*. Moreover, Fritz's (1992, 1994) taxonomic allocation of Crimean pond turtles was based on only few specimens as Ukrainian *E. orbicularis* are rare in Central European museum collections. Thus, also additional morphological data are in dire need. Here we present new data on size, coloration, and the molecular phylogeography of *E. orbi-*

cularis in Ukraine and focus on the status of Crimean populations.

MATERIAL AND METHODS

Turtles for this study were collected during our field trips in Ukraine and its Autonomous Republic of Crimea in 1974, 1979, and 2000 – 2003. Additional specimens were studied from the following museum collections: National Museum of Natural History (National Academy of Sciences of Ukraine, Kiev, Ukraine), Museum of Nature (Kharkov National Karazin University, Kharkov, Ukraine), and Museum für Tierkunde (Dresden, Germany). Among these museum specimens are pond turtles from the Dnepr Delta, Luchyste, Sovet'skyi, and the Kerch Peninsula which were collected in the early 1960's, mainly by N. N. Szczerbak and V. A. Sedov. 152 adult and 51 juvenile turtles from 15 localities, representing 10 populations, were measured with a caliper to the nearest 0.1 mm and their color and pattern were recorded (Table 1, Fig. 1). During field work, blood samples for genetic investigations were obtained by coccygeal vein puncture (Haskell and Pokras, 1994). Samples were stored as described in Arctander (1988). Total genomic DNA was extracted following standard proteinase K and phenol-chloroform protocols (Sambrook et al., 1989). PCR and sequencing are explained in detail in Lenk et al. (1999). We define haplotypes according to individual mtDNA sequences (Lenk et al., 1999). Haplotype and haplotype lineage nomenclature follows Lenk et al. (1999) and Fritz et al. (in press).

We sequenced a 1031 bp portion of the mitochondrial cytochrome b gene for 33 specimens as given in Table 1. Of the 1031 aligned sites, 69 are variable. 63 substitutions are transitions, and six are transversions; 41 sites are parsimony informative. 13 sites are variable at the first, eight at the second, and 48 at the third codon position. For each sequence, variable sites were checked individually to prevent errors from wrong sequencer output. We calculated a minimum spanning network with the program Arlequin (Schneider et al., 2000), in which all *Emys orbicularis*

¹ Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Vul. B. Khmelnyts'kogo, 15, Kyiv-30, MSP 01601, Ukraine; E-mail: kotenko@iz.freenet.kiev.ua.

² Museum of Nature, Kharkiv National Karazin University, Vul. Trinklera, 8, Kharkiv 61022, Ukraine; E-mail: zinenkoa@yahoo.com.

³ Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, INF 364, D-69120 Heidelberg, Germany; E-mail: wink@uni-hd.de.

⁴ Museum für Tierkunde, Staatliche Naturhistorische Sammlungen Dresden, A.-B.-Meyer-Bau, Königsbrücker Landstr. 159, D-01109 Dresden, Germany; E-mail: uwe.fritz@snsd.smwk.sachsen.de.

TABLE 1. Studied *Emys orbicularis* Populations, Straight Line Carapacial Lengths (SCL, only adults) and mtDNA Haplotypes

Population	Sex	n	SCL, mm			mtDNA haplotypes
			mean	minimum	maximum	
Desna River (A, Litochky, 2001)	Females	2	–	176.0	182.5	Ia (n = 2)
Merla River (B, Kolontayiv, 2001; C, Volodymyrivs'ke Forestry, 1975, and Gorodne, 2001)	Males	2	–	145.5	154.5	Ia (n = 2, Kolontayiv);
	Females	7	168.2	155.0	178.0	Ia, Ih (n = 2, Gorodne)
Mzha River (D, Merefa, 2001, 2003)	Females	9	170.0	156.0	188.5	Ia (n = 1)
Siverskii Donets River (E, Gaidary, Koropovo, Gomol'sha, and Cherkas'kyi Byshkyn, 2002; F, Balakleya, 2003, and Sezonna, 1969; G, Izyum, 2001)	Males	1	–	175.0		Ia (n = 1, Izyum)
	Females	11	175.9	145.5	195.0	
Siverskii Donets River (H, Levkivka, Lysogirka, and Chervonyi Donets', 1990 – 1993) (Lyamzin, 1993)	Males	44	160.0	126.0	171.0	–
	Females	43	175.0	160.0	200.0	
Siverskii Donets River, total (localities E – H)	Males	45	160.3	126.0	175.0	Ia (n = 1, Izyum)
	Females	54	175.2	145.5	200.0	
Dnepr Delta (J, Gerois'ke, 1960's, 1979, 2000; K, Rybal'che and Vynogradne, 1974, 1979 and 2000; L, Gola Prystan', 1974)	Males	10	145.9	112.9	173.5	Ia (n = 15, Gerois'ke);
	Females	33	166.4	143.5	189.5	Ia (n = 1, Vynogradne)
Northern Crimea (M, Dzhankoi, 2000)	Males	1	–	165.6		Ie (n = 1)
East Sivash Region, Crimea (N, Sovet'skyi, 1961, 1962)	Males	20	127.3	113.4	137.0	–
	Females	9	138.5	122.5	153.6	
Crimean Mountains (O, Luchyste, 1961, 2000, 2001)	Males	16	127.9	112.9	137.1	Ic (n = 4)
	Females	15	131.9	113.4	154.2	
Kerch Peninsula, Crimea (P, Él'tigen, 2001)	Males	6	138.6	135.3	140.7	Ic (n = 1);
	Females	6	158.5	147.5	172.5	Ii (n = 3)
Kerch Peninsula, Crimea (Q, Kerch, 1961)	Females	4	141.0	134.5	149.3	–
Crimea (mainly Luchyste, Sovet'skyi, and Kerch) (Szczerbak, 1966)	Both sexes	80	–	–	160.5	–

Collection dates (years) are given for all localities; specimens collected prior to 2000 are mainly museum vouchers, others have been released after study. Letters preceding localities refer to Fig. 1. For the Siverskii Donets population (locality H) and the Crimea, literature data are added.

mtDNA haplotypes identified until now have been included (Fritz et al., in press) to demonstrate how the Ukrainian haplotypes are related.

RESULTS

Crimean *Emys orbicularis* (East Sivash Region, Crimean Mountains, Kerch Peninsula) are on average smaller than European pond turtles from the Dnepr Delta and more northerly Ukrainian localities. However, a male from Dzhankoi (northern Crimea) and some females from Él'tigen (Kerch Peninsula) are of medium to large size (Table 1). They exceed the previously recorded maximum size of 160.5 mm for Crimean *E. orbicularis* (Szczerbak, 1966). All specimens from Luchyste, Sovet'skyi, and Kerch are distinctly lighter colored than the turtles from all other Ukrainian localities. These light colored turtles possess mainly yellow plastra and throats. Moreover, the primary carapacial color of many specimens from Sovet'skyi and of some turtles from Luchyste and Kerch is yellowish brown or smoky brown instead of black. The iris coloration of an adult male from Luchyste is yellow (the other males from Luchyste, Sovet'skyi, and Kerch are museum

specimens so that the iris coloration could not be studied). Similar coloration characters are also known to occur in *E. o. hellenica* from the Balkans, which is also similar in size (Fritz, 2003).

Remarkably, pond turtles from Kerch Peninsula show considerable variation. Specimens from Kerch (Fig. 1, locality Q) in the holdings of the National Museum of Natural History (Kiev, Ukraine) and the Museum für Tierkunde (Dresden, Germany), collected in 1961, resemble in coloration and size turtles from Luchyste and Sovet'skyi. However, turtles captured in 2001 near Kerch, in Él'tigen (Fig. 1, locality P), are larger and darker colored. Their shell and soft part coloration resembles Dnepr Delta turtles or other specimens of *E. o. orbicularis*; the iris coloration of adult males is brick-red, orange, or red-brown, as characteristic for *E. o. orbicularis*.

At the remote locality Luchyste in the Crimean Mountains we found in 2000 and 2001 light colored, small turtles, which are morphologically in good agreement with the old museum specimens from there. Unfortunately, we are not sure about the current situation at Sovet'skyi. All studied turtles from Sovet'skyi are museum specimens collected in 1961 and 1962. The only male from Dzhankoi, studied in 2000, is very dark colored, has a reddish iris



Fig. 1. Localities and mtDNA haplotypes ($n = 33$) for *Emys orbicularis* in Ukraine. Localities represented by asterisks were not studied genetically. Letters of localities refer to Table 1, numbers by the letters or symbols refer to the number of specimens bearing this haplotype.

and is indistinguishable from *E. o. orbicularis* from more northerly regions.

Regarding genetics, we identified five different mtDNA haplotypes in Ukraine (Fig. 1). All haplotypes belong to lineage I of Lenk et al. (1999). In the Crimean Mountains and on the Crimean Kerch Peninsula the most differentiated haplotypes were detected (Ic, Ii). They differ by three to five mutation steps from the haplotypes found in the northern steppe part of the Crimea and on the Ukrainian mainland (Fig. 2).

DISCUSSION

In this paper we confirm that turtles from some Crimean localities resemble *Emys orbicularis hellenica* from the Balkans morphologically. However, in no case mtDNA haplotypes of lineage IV have been recorded in the Crimea. This lineage is characteristic for *E. o. hellenica* (Lenk et al., 1999; Fritz, 2001, 2003). Although we cannot

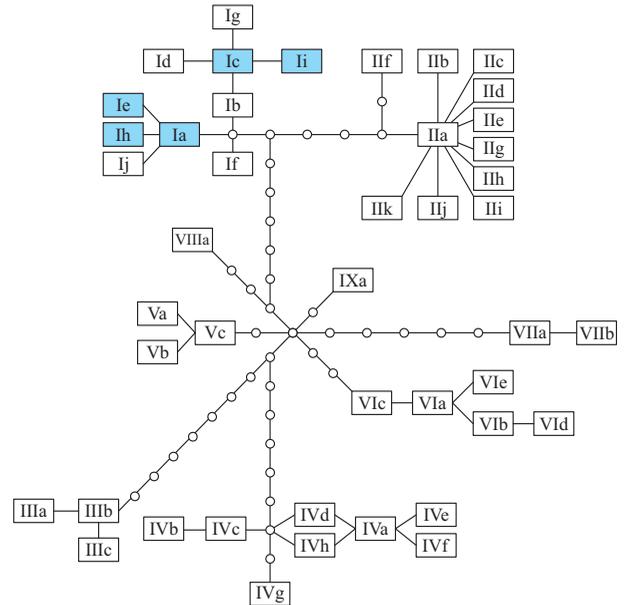


Fig. 2. Minimum spanning network of all 44 known *Emys orbicularis* mtDNA haplotypes (modified from Fritz et al., in press). Each line represents a single mutation step, missing haplotypes are presented by open circles. Gray boxes indicate haplotypes found in Ukrainian samples.

exclude that this finding is due to a loss of lineage IV haplotypes during a former genetic bottleneck, the current data set argues rather for an independently acquired morphological similarity of Crimean and Balkanic pond turtles.

In the south of Ukraine a much higher mtDNA haplotype diversity occurs than in the north. This reflects surely a rapid postglacial range expansion of *E. orbicularis*. A similar situation is found in many other taxa displaying the same long distance dispersal pattern (e.g., Hewitt, 1996, 2001; Taberlet et al., 1998; Cruzan and Templeton, 2000).

However, we are not sure that a glacial refugium for haplotype Ia turtles was located on the Crimea. Today, haplotype Ia is known from the Ukrainian mainland, Poland, Lithuania, northern Russia, Kazakhstan, the southeastern Balkans, and Turkey (Fritz et al., in press). It corresponds mainly to large, dark pond turtles. The distribution of haplotype Ia agrees well with the range of the nominotypical subspecies *E. o. orbicularis* (Lenk et al., 1999; Fritz, 2003). The mtDNA haplotypes (Ic, Ii) of the Crimean Mountains and the Kerch Peninsula differ from the haplotypes found in the Dnepr Delta (Ia) and from more northerly localities in Ukraine (Ia, Ih; Fig. 2). This differentiation is also paralleled by a morphological gap in size and coloration of turtles from the Dnepr Delta vs. the Crimean Mountains (Luchyste) plus old museum specimens from the Crimean localities Sovet'skiy and Kerch.

Haplotype Ia and its rare variant Ih have been not recorded in the Crimea until now, but the closely related haplotype Ie. However, the turtle bearing haplotype Ie originated from a locality in the northern steppe zone of the Crimea (Dzhankoi). This area has been connected with the Dnepr by the construction of the North Crimean Canal in the late 1960's. The museum specimens from the Kerch Peninsula, collected in 1961, differ morphologically from the turtles studied by us forty years later there. The North Crimean Canal was extended to the Kerch Peninsula around 1975. Thus, it might be that northern *E. o. orbicularis* bearing haplotype Ie (and probably Ia) are recent invaders, which perhaps already genetically impacted the Kerch Peninsula population. If this hypothesis is correct, the survival of native Crimean turtles is seriously threatened by the current immigration of the Dnepr turtles.

It is obvious that we need further research to understand the diversity of Ukrainian *E. orbicularis* better. This will be the prerequisite for developing any effective conservation measures.

REFERENCES

- Arctander P.** (1988), "Comparative studies of Avian DNA by restriction fragment polymorphism analysis," *J. Ornithol.*, **129**, 205 – 216.
- Cruzan M. B. and Templeton A. R.** (2000), "Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record," *TREE*, **15**, 491 – 496.
- Fritz U.** (1992), "Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 2. Variabilität in Osteuropa und Redefinition von *Emys orbicularis orbicularis* (Linnaeus, 1758) und *E. o. hellenica* (Valenciennes, 1832)," *Zool. Abh. Mus. Tierkd. Dresden*, **47**(5), 37 – 77.
- Fritz U.** (1994), "Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 4. Variabilität und Zoogeographie im pontokaspischen Gebiet mit Beschreibung von drei neuen Unterarten," *Zool. Abh. Mus. Tierkd. Dresden*, **48**(4), 53 – 93.
- Fritz U.** (2001), "*Emys orbicularis* (Linnaeus, 1758) — Europäische Sumpfschildkröte," in: U. Fritz (ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/III A. Schildkröten (Testudines). I. (Bataguridae, Testudinidae, Emydidae)*, AULA-Verlag, Wiebelsheim, pp. 343 – 515.
- Fritz U.** (2003), *Die Europäische Sumpfschildkröte*, Laurenti, Bielefeld.
- Fritz U., Guicking D., Lenk P., Joger U., and Wink M.** (in press), "When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain," *Biologia*.
- Haskell A. and Pokras M. A.** (1994), "Nonlethal blood and muscle tissue collection from redbelly turtles for genetic studies," *Herpetol. Rev.*, **25**, 11 – 12.
- Hewitt G. M.** (1996), "Some genetic consequences of ice ages, and their role in divergence and speciation," *Biol. J. Linn. Soc.*, **58**, 247 – 276.
- Hewitt G. M.** (2001), "Speciation, hybrid zones and phylogeography — or seeing genes in space and time," *Mol. Ecol.*, **10**, 537 – 549.
- Lenk P., Fritz U., Joger U., and Wink M.** (1999), "Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758)," *Mol. Ecol.*, **8**, 1911 – 1922.
- Lyamzin I. I.** (1993), *Peculiarities of the Ecology and Morphological Variation of the European Pond Turtle Emys orbicularis in the Middle Course of the Siverskii Donets River. Graduate Thesis*, Kharkov University [in Russian].
- Sambrook J., Fritsch E. F., and Maniatis T.** (1989), *Molecular Cloning: a Laboratory Manual. 2nd Edition*, Cold Spring Harbor Lab. Press, Cold Spring Harbor.
- Schneider S., Roessli D., and Excoffier L.** (2000), *Arlequin ver. 2.000. A Software for Population Genetics Data Analysis*, Genetics and Biometry Laboratory, University of Geneva, Geneva.
- Szczerbak N. N.** (1966), *Amphibians and Reptiles of the Crimea. Herpetologia Taurica*, Naukova dumka, Kiev [in Russian].
- Taberlet P., Fumagalli L., Wust-Saucy A.-G., and Cosson J.-F.** (1998), "Comparative phylogeography and postglacial colonization routes in Europe," *Mol. Ecol.*, **7**, 453 – 464.

CHROMOSOMAL CHANGES AND FORM-FORMATION, SUBSPECIATION IN THE WIDERANGED EUROASIAN SPECIES *Zootoca vivipara* (EVOLUTION, BIOGEOGRAPHY)

L. Kupriyanova,¹ G. Odierna,² T. Capriglione,² E. Olmo,³ and G. Aprea²

Keywords: *Zootoca vivipara*, chromosomal rearrangements, sex chromosomes, karyotype variations, evolution, form-formation, speciation, biogeography.

INTRODUCTION

The wideranged Euroasian species *Zootoca vivipara* (family Lacertidae) is a rare species, possessing transpale-arctic distribution from the Pyrenees up to the archipelago of the Sea of Japan. A further very important feature is that *Zootoca vivipara* has two reproductive modes in different populations. Primitive oviparous populations inhabit western Europe (the Pyrenees region and the south-eastern central Europe), whereas advanced viviparous populations occur in the other part of the distribution range.

Karyological investigations of *Zootoca vivipara* from many geographically distant populations have shown that the species is characterized by differences in the diploid number [$2n = 36/36$ in both sexes or 36 (male)/ 35 (female)]; in the system of sex chromosomes (ZW or $Z_1 Z_2 W$) and in the types and structure of W sex chromosome (Kupriyanova, 1990, 1997; Odierna et al., 1993).

Morphological differentiation of the populations is poorly pronounced because only four subspecies are recognized: *Z. v. vivipara*, *Z. v. carniolica*, *Z. v. sakhalinensis* (*nomen nudum*), and *Z. v. pannonica*.

Modern cytogenetical studies have revealed five structures of karyotypes among ovi- and viviparous populations of *Z. vivipara* from different geographic ranges.

KARYOTYPE VARIATIONS

One type is the karyotype for specimens from primitive oviparous populations of new genetically described subspecies *Z. v. carniolica* (Mayer et al., 2000) from Slovenia. $2n = 36A$ (acrocentric) (male)/ $36A$ female, system

of sex chromosomes is ZW, where W is fully heterochromatic micro chromosome (w). All autosomes have tiny centromeric heterochromatic C-bands. W-sex chromosome arose as a result of deletion of a primitive acrocentric macrochromosome W (Odierna et al., 2001). Thus, karyological data obtained have confirmed the status of new subspecies. The karyotype structure is sharply different from that of other oviparous and viviparous populations of *Z. vivipara*.

The next two cytotypes are for specimens from oviparous populations of *Z. v. vivipara* from the Pyrenees region (Kupriyanova and Böhme, 1997; Odierna et al., 1998). $2n = 36A$ (male)/ $35A$ (female), system of sex chromosomes is $Z_1 Z_2 W$. Autosomes and acrocentric macro- W_{1A} or subtelocentric W_{1B} -sex chromosomes have tiny heterochromatic C-bands. Acrocentric macro- W_{1A} chromosome has arisen as a result of tandem fusion of auto- and macro W-sex chromosome ("Pyrenean" form). Its karyotype characteristics in sex- and autosomes suggest a higher rank of this form (Odierna et al., 1998).

Two other karyotype structures were found in specimens of advanced viviparous forms of *Z. v. vivipara* from different localities, the first one from central and eastern Europe and Asia and the second from central and western Europe. The former have the same karyotype structure like that of the "Pyrenean" form. $2n = 36A$ (male)/ $35A$ (female) with $Z_1 Z_2 W$ system of sex chromosomes and acrocentric/subtelocentric type of W_2 -sex chromosome (Kupriyanova, 1990). However, unlike the "Pyrenean" form most of chromosomes, including W_2 -sex chromosome of these specimens possess considerable heterochromatic C-bands ("Russian/eastern" form). Therefore the mechanism of chromosome changes is heterochromatinization events.

One more karyotype was discovered for specimens of advanced viviparous forms of *Z. v. vivipara* from central and western Europe. $2n = 36A$ (male)/ $35A$ (female) with $Z_1 Z_2 W$ system of sex chromosomes and meta-/subtelocentric type of W_3 -sex chromosome (Chevalier et al.,

¹ Department of Karyotaxonomy, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: Vladimir@VZ8122.spb.edu.

² Dipartimento di Biologia evolutiva e comparata, Università di Napoli "Federico II," I-80100 Napoli, Italy; E-mail: Gaetano.Odierna@unina.it.

³ Istituto di Biologia e Genetica, Università Politecnica delle Marche, Via Brecce Bianche, I-60131 Ancona, Italy.

1979; Kupriyanova, 1990; Odierna et al., 1993). Metacentric W_3 -sex chromosome arose as a result of pericentric inversion of acrocentric sex chromosome. This karyotype has also intensive heterochromatin C-bands in sex chromosome and autosomes (“western” form).

The mechanisms and steps of chromosomal changes of sex chromosomes in the evolution of *Zootoca vivipara* are as follows: deletion, tandem fusion, heterochromatinization event, and pericentric inversion.

Viviparous specimens of *Z. vivipara* from Sakhalin Island, geographically belonging to *Z. v. sakhalinensis* (*nomen nudum*), differed neither by the karyotype (Kupriyanova and Böhme, 1997) nor by haplotype (Mayer and Böhme, 2000) from the “Russian/eastern” form of *Z. v. vivipara*. Therefore these specimens should be considered as *Z. v. vivipara*.

The karyotype of viviparous specimens of *Z. v. pannonica* from Austria did not also differ from that of the “Russian/eastern” form of *Z. v. vivipara* (Kupriyanova and Böhme, 1997). Recent cytogenetical investigations have revealed one other karyotype structure in the subspecies (Odierna et al., 2004). Therefore the question about validity of *Z. v. pannonica* should be revised.

Sympatry, hybrid or contact zones between different chromosomal forms and subspecies have not been found. From the karyological data all of them appear to have distinct distribution range. However it becomes clear that karyologically *Z. vivipara* constitutes a mosaic of populations.

As a result of cytogenetical research three chromosomal forms of *Z. v. vivipara* have been described. Recently one more new chromosomal form has been discovered (Odierna and Kupriyanova, in press). Thus, *Z. vivipara* represents karyologically a complex, including subspecies *Z. v. carniolica* and several chromosomal forms of *Z. v. vivipara*. These studies have shown that the forms have their own distinct distribution ranges; both primitive oviparous “Pyrenean” form *Z. v. vivipara* and *Z. v. carniolica* are characterized by narrow ranges, whereas both advanced viviparous forms of *Z. v. vivipara* possess wide ranges.

GENETIC VARIATIONS

Analyses for 12S rRNA and cytochrome b genes of *Z. vivipara* have shown geographic variation in their haplotypes (Surget-Groba et al., 2001). Five clusters mainly correlating with the karyotype’s groups have been observed.

From the allozyme analysis a mean genetic distance, e.g., between oviparous and viviparous populations from the Pyrenees region and between the former and those from the Balkanic region are short. Nei’s index between the populations are 0.12 (Bea et al., 1990) and 0.102 (Guillaume et al., 1997). These values do not appear to reach the species rank. The laboratory cross experiments between oviparous and viviparous specimens produced some vital hatchlings and demonstrated incomplete reproductive isolation (Heulin et al., 1989; Arrayaco et al., 1996).

EVOLUTION IN THE COMPLEX

All the characteristics listed give a rare possibility to use the species as a model for studying some general evolutionary and biogeographic questions.

Evidently karyological differentiation in the complex is high. Chromosomal rearrangements accompany the active form-formation and subspeciation processes. Steps and mechanisms of these changes in the evolution of the species have been suggested (Kupriyanova, 1990; 1997; Odierna et al 1993; 1998; 2001; Surget-Groba et al., 2001). These are deletion, tandem fusion, heterochromatinization events, and pericentric inversion. It becomes clear that karyotype features may serve as a good marker for the identification of different populations of *Z. vivipara* in the complex.

All these facts argue the significance of cytogenetical data for the understanding of the evolution, phylogeny and biogeography in the complex. Investigations of new markers of W-sex and autosomes of the species may provide more detailed information on their structure. Karyological and different comparative staining analyses of C-banding/CMA₃/DAPI may elucidate in detail the evolutionary steps and a possible role of chromosomal changes in the process of form-formation and subspeciation.

Therefore this paper presents for the first results of karyotype and cytogenetical analyses of specimens of *Z. vivipara* from three earlier unstudied geographically distant populations. In the paper we discuss the biogeography and evolutionary problems and possible modes of form-formation and subspeciation in the complex.

MATERIAL AND METHODS

Nine lizards of *Z. vivipara* (8 females and 1 male) from the upper part of the Eastern Carpathian Great Ridge (Transcarpathian region, Ukraine) and eleven lizards of *Z. vivipara* from Leningrad (4 females and 1 male) and

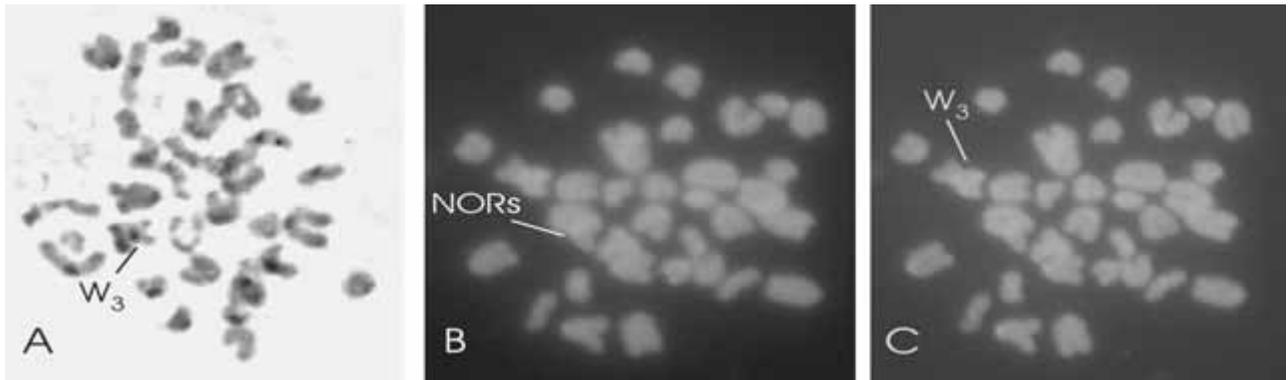


Fig. 1. Metaphases of blood cells of females *Zootoca vivipara*. $2n = 35$ (population No. 1). **A**, C-banded karyotype with submetacentric (SV) W_3 -sex chromosome showing three (one centromeric and two telomeric) heterochromatic C-bands, “western” form of *Zootoca vivipara vivipara*; **B**, Chromomycin A_3 stained karyotype showing weak telomeric C-bands of NO regions of two chromosomes; **C**, DAPI stained karyotype, showing centromeric and one telomeric C-bands of W_3 -sex chromosome and centromeric C-bands of some autosomes of *Zootoca v. vivipara*.

Pskov (5 females and 1 male) regions, Russia were collected. For clarifying mode of reproduction some females were kept in terrarium up to hatching of offspring.

C-banding was carried out according to Sumner’s method (Sumner, 1972), fluorochrome staining (chromomycin A_3 and DAPI according to Schmid and Guttenbach method (Schmid and Guttenbach, 1988); digestions with endonucleases AluI (Mezzanotte et al., 1983).

RESULTS AND DISCUSSION

Males of *Z. vivipara* from these populations had 36 acrocentric chromosomes.

Observations in a terrarium have shown that all specimens belong to advanced viviparous forms.

Karyotype Structure and Identification of Populations

Females of *Z. vivipara* from the Carpathian region (population No. 1) had 35 chromosomes with Z Z W system of sex chromosomes and biarmed meta (V)/submetacentric (SV) W_3 -sex chromosome.

Most of autosomes and W_3 chromosome possessed conspicuous centromeric C-bands. Autosomes had thin telomeric C-bands whereas W_3 chromosome displayed two intensive telomeric C-bands (Fig. 1A).

Telomeric C-bands of the NORs bearing chromosomes were weakly stained with GC-specific fluorochrome chromomycin A_3 (Fig. 1B). Centromeric and one telomeric C-bands of the sex chromosome were weakly stained with AT specific fluorochrome DAPI (Fig. 1C). After treatment with endonuclease AluI, only the centro-

meric bands of the autosomes and a single band of W_3 chromosome persisted.

From these chromosome markers the specimens of *Z. vivipara* from population No. 1 appeared to be similar to those of specimens belonging to the “western” form of *Z. v. vivipara* from the Trento Alps (Odierna et al., 1998). Therefore examined viviparous lizards from the Carpathian region belong to the “western” form of subspecies *Z. v. vivipara*.

The karyotype of females of *Z. vivipara* from north-western region of Russia (populations Nos. 2 and 3) had $2n = 35A$, with Z Z W system of sex chromosomes. W sex chromosome was uniarmed acrocentric(A)/subtelocentric (ST) W_2 . Most of autosomes and W_2 chromosome possessed conspicuous centromeric and telomeric C-bands. Additionally W_2 sex chromosome has interstitial C-band (Fig. 2A). From these chromosome markers these specimens of *Z. vivipara* from populations Nos. 2 and 3 belong to the “Russian/eastern” form of *Z. v. vivipara*.

It follows that the cytogenetical data are good markers for identification of populations of *Z. vivipara* throughout the distribution range.

Telomeric C-bands of the NORs bearing chromosomes were intensively stained with GC-specific fluorochrome chromomycin A_3 (Fig. 2B). Centromeric C-bands of some autosomes, centromeric and interstitial C-bands of the W_2 sex chromosome were intensively stained with AT specific fluorochrome DAPI (Fig. 2C). Unlike W_3 -sex chromosome of “western” form after treatment with endonuclease AluI, the centromeric and interstitial bands of W_2 chromosome were resistant.

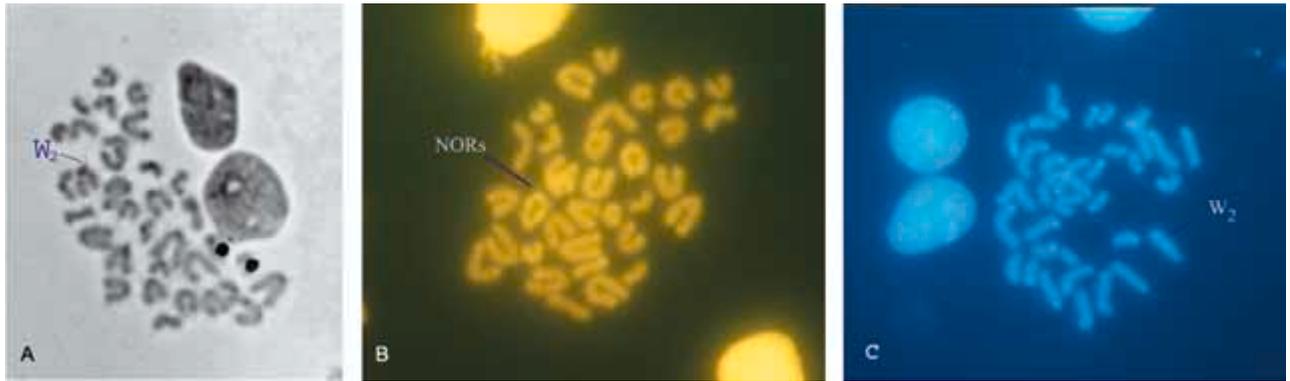


Fig. 2. Metaphases of blood cells of females *Zootoca vivipara*. $2n = 35$ (populations Nos. 2 and 3). **A**, C-banded karyotype with acrocentric (A) W_2 -sex chromosome showing three (centromeric, telomeric and interstitial) heterochromatic C-bands (“Russian/eastern” form of *Zootoca vivipara vivipara*); **B**, Chromomycin A_3 stained karyotype showing intensive telomeric C-bands of NOR regions; **C**, DAPI stained karyotype showing centromeric and interstitial C-bands of W_2 .

Chromosomal Reorganization in the Evolution of *Z. vivipara*

Comparative analyses have revealed that two cytogenetic characteristics of the “Russian/eastern” form are the same as those found by Odierna and his coauthors (Odierna et al., 1998) in the “pyrenean” form. They are: 1. intensively stained with GC-specific fluorochrome chromomycin A_3 telomeric C-bands of the NORs bearing chromosomes; 2. intensively stained with AT specific fluorochrome DAPI centromeric C-bands of some autosomes, centromeric and interstitial C-bands of the W_2 -sex chromosome.

By contrast, specimens of the “western” form displayed other cytogenetical markers.

1. Weakly stained with chromomycin A_3 telomeric C-bands of the NORs bearing chromosomes.

2. Weakly stained with DAPI centromeric and telomeric C-bands of W_3 chromosome.

Thus, cytogenetical data obtained again argue that intensive karyotype reorganization accompany active form-formation and subspeciation in the evolution of *Z. vivipara*. As has been mentioned above, the karyotypes of both primitive “Pyrenean” form and subspecies *Z. v. carniolica* are characterized by low amount of heterochromatine and by narrow range. In contrast, both advanced the “Russian/eastern” and “western” forms of *Z. v. vivipara* with wide range possess a considerable amount of heterochromatine in their karyotype. These data suggest that the latter karyotype is evolutionarily plastic (Kupriyanova and Odierna, 2002). Cytogenetical results obtained seem to be inconsistent with the hypothesis (Heulin et al., 1993) for arising of advanced viviparous form in some region of eastern Europe because primitive oviparous forms have

been observed neither in this region nor in south-eastern populations of Russia yet. Advanced viviparous “Russian/eastern” form of *Z. v. vivipara* inhabits this region (Kupriyanova et al., 2003), whereas “western” form of *Z. v. vivipara* lives in central and western Europe. However “western” form has recently been karyologically found in north-western region of Russia. Both these forms and oviparous *Z. v. carniolica* differing in karyotype structure live in central Europe.

Our data again confirm the assumption (Kupriyanova and Böhme, 1997; Surget-Groba et al., 2001) that the Carpathian basin may be considered as a center of form-formation and subspeciation of *Z. vivipara*. The Baltic basin is a zone of a secondary contact of two forms (Kupriyanova, 1997, 2004). Karyologically *Z. vivipara* constitutes a mosaic of populations inhabiting different European and Asian countries. Conservation of some of these populations is needed (Odierna et al., 2004; Kupriyanova, 2004).

Chromosomes and Modes of Form-Formation and Subspeciation

In connection with the facts established a question of possible modes form-formation and subspeciation in the evolution of this wideranged Euroasian species arises.

These several morphologically no diagnostic criptic forms of *Z. v. vivipara* have some serious karyotype’s and to a lesser extent haplotype’s differences.

The model of allopatric differentiation is associated with climatic changes. The Pleistocene glaciation could have caused the separation of the original population into two (or more) groups. All populations examined are allopatric or parapatric. No sympatry, hybrid or contact zones have been found.

Modern cytogenetical data show that rearrangement of chromosomes may represent a powerful mechanism for reproductive isolation. For instance, karyological variations in the complex *Lacerta kulzeri* support the King's model of chromosomal primary allopatry (in den Bosch et al., 2003).

Alterations in morphology and/or heterochromatin content of sex chromosomes are known to have a negative impact on hybrid fertility in some rodents (Lyapunova et al., 1990).

We found two the same molecular markers of chromosomes of the primitive "Pyrenean" form and of the advanced "Russian/eastern" form of *Z. v. vivipara*. Interestingly, the shape and heterochromatin distribution of W chromosome of these two forms are very similar (Odierna et al., 1998, 2001) and furthermore the adaptive value of viviparity has been showed in this lizard (Odierna et al., 2004). These data obtained allow us to consider another scenario. They may suggest that chromosomal reorganization could have accompanied colonization and adaptive radiation events.

The next tasks to be investigated are:

Summarizing we would like to emphasize that now we have different information about *Z. vivipara* complex but it is still not enough for understanding the situation. Further international cytogenetical researches of this wide-ranged Euroasian species should attempt to clarify and to test several aspects of the process of form-formation and subspeciation and biogeography in order to

1. karyologically to identify a larger number of populations to determine the quantity of different forms and subspecies and to clarify their distribution range;
2. to protect or to conservate some rare populations or those of them with narrow range;
3. to find new molecular markers of chromosomes of these cryptic forms and subspecies using modern techniques;
4. to precise *in situ* localization of specific (sex linked) genes;
5. to resolve the questions about taxonomic status and phylogenetic relationships of discovered chromosomal forms of *Z. v. vivipara*.

Acknowledgments. Funds were obtained from the Russian Foundation for Basic Science (grant No. 02-04-48611); from Presidium St. Petersburg's Scientific Centre of Russian Academy of Sciences; Min. Nauka Scien. School (grant No. 1647.2003.4); from the program of Presidium RAN "Dynamics of Genopool," from University of Napoly "Federico II."

REFERENCES

- Arrayago M., Bea A., and Heulin B. (1996), "Hybridization experiments between Oviparous and viviparous straits on *Lacerta vivipara*: a new insight into the evolution of viviparity in reptiles," *Herpetologica*, **52**(3), 333 – 342.
- Bea A., Guillame C., Arrayago M., Heulin B., and Paster G. (1990), "Phenotypes enzymatiques du lézard vivipare (*Lacerta (Zootoca) vivipara* Jacq.): premières données comparatives entre populations ovipares et vivipares," *C. R. Acad. Sci. Paris*, **310**, 237 – 243.
- Bosch H. A. J. in den, Odierna G., Aprea G., Barucca M., Canapa A., Capriglione T., and Olmo E. (2003), "Karyological and genetic variation in Middle Eastern lacertid lizards, *Lacerta laevis* and the *Lacerta kulzeri* complex: a case of chromosomal allopatric speciation," *Chromosome Res.*, **11**, 165 – 178.
- Chevalier M., Dufaure J., Lecher P. (1979), "Cytogenetic study of *Lacerta* (Lacertidae, Reptilia) with particular reference to sex chromosomes," *Genetica*, **50**, 11 – 18.
- Heulin B., Arrayago M., and Bea A. (1989), "Expérience d'hybridation entre les ovipare et vivipare du lézard *Lacerta vivipara*," *C. R. Acad. Sci. Paris. Ser. III*, **308**, 341 – 346.
- Heulin B., Guillame C., Bea A., and Arrayago M. (1993), "Interpretation biogéographique de la bimodalité de reproduction du lézard *Lacerta vivipara* Jacquin (Sauria; Lacertidae): Un modèle pour l'étude de l'évolution de la viviparité," *Biogéographica*, **69**, 3 – 13.
- Kupriyanova L. (1990), "Cytogenetic studies in lacertid lizard," in: *Cytogenetics of Amphibians and Reptiles*, Birkhäuser Verlag, Basel, pp. 242 – 245.
- Kupriyanova L. (1997), "Is the Baltic Sea basin a zone of secondary contact between different chromosomal forms of *Zootoca vivipara*?" *Mem. Soc. Fauna Flora Fenn.*, **73**, 115 – 117.
- Kupriyanova L. and Böhme W. (1997), "New data on the distribution of different forms of *Zootoca vivipara* in eastern and western Europe: chromosomal analysis," *Herpetologia Bonnensis*, **13**, 199 – 205.
- Kupriyanova L. and Odierna G. (2002), "Perspective approach to the problem of stability of karyotype: chromosome structure of bisexual and unisexual lizard species of the Lacertidae (allo-/sympatric and hybridogeneous speciations)," in: *Proc. of the Second Int. Conf. "Problems of Species and Speciation."* *Evolutionary Biology. Vol. 2*, Tomsk State University, Tomsk, pp. 238 – 254 [in Russian].
- Kupriyanova L., Zavyalov E., and Tabachischin V. (2003), "Viviparous lizard *Zootoca vivipara* (Lacertidae) from the north of Low Volga region: cytogenetical analysis and ecology," in: *Proc. of the 3th Conf. of Herpetologists of Volga Region*, Institute of Ecol. of Volga region, Tol'yatti, pp. 36 – 39 [in Russian].
- Kupriyanova L. (2004, in press), "Cytogenetical approaches to the problem of form-formation and subspeciation in the complex *Lacerta vivipara*," *Tsitologia* [in Russian].
- Lyapunova E., Baklushinskaya I., Kolomiets O., and Mazurova O. (1990), "Analysis of fertility of hybrids between different chromosomal forms of *Ellobius tancrei*, differing on a

- one pair of robertsonian metacentrics,” *Dokl. Akad. Nauk USSR*, **310**(3), 721 – 723 [in Russian].
- Mayer W. and Böhme W.** (2000), “A note on the validity and distribution of *Zootoca vivipara sachalinensis*. Rada prirodovedna,” *Casopis Narod. Muzea*, **169**(1–4), 123 – 124.
- Mayer W., Böhme W., Tiedemann F., and Bischoff W.** (2000), “On oviparous population of *Zootoca vivipara* (Jacquin 1787) in south-eastern Central Europe and their phylogenetic relationship to neighbouring viviparous and South-west European oviparous populations (Squamata: Sauria: Lacertidae),” *Herpetozoa*, **13**, 59 – 69.
- Mezzanotte R., Bianchi U., Vanni R., and Ferrucci L.** (1983), “Chromatin organization and restriction nuclease activity on human metaphase chromosomes,” *Cytogenet. Cell Genet.*, **36**, 562 – 566.
- Odierna G., Kupriyanova L., Capriglione T., and Olmo E.** (1993), “Further data on sex chromosome of Lacertidae and a hypothesis on their evolutionary trend,” *Amphibia-Reptilia*, **14**, 1 – 11.
- Odierna G., Aprea G., Capriglione T., Arribas O., Kupriyanova L., and Olmo E.** (1998), “Progressive differentiation of the W sex-chromosome between oviparous and viviparous populations of *Zootoca vivipara* (Reptilia, Lacertidae),” *Ital. J. Zool.*, **65**, 295 – 303.
- Odierna G., Heulin B., Guillaume C., Vogrin N., Aprea G., Capriglione T., Surget-Groba Y., and Kupriyanova L.** (2001), “Evolutionary and biogeographical implications of the karyological variations in the oviparous and viviparous forms of the lizard *Lacerta (Zootoca) vivipara*,” *Ecography*, **24**, 332 – 340.
- Odierna G., Aprea G., Capriglione T., and Puky M.** (2004, in press), “Chromosomal evidence for the double origin of viviparity in the European common lizards *Lacerta (Zootoca) vivipara*,” *Herpetol. J.*, **14**.
- Schmid M. and Guttenbach M.** (1988), “Evolutionary diversity of reverse fluorescent chromosome bands in vertebrates,” *Chromosome*, **97**, 101 – 114.
- Sumner A. T.** (1972), “A simple technique for demonstrating centromeric heterochromatin,” *Exp. Cell. Res.*, **75**, 304 – 306.
- Surget-Groba Y., Heulin B., Guillaume C., Thorpe R., Kupriyanova L., Vogrin N., Maslak R., Mazzotti S., Venczel M., Ghira I., Odierna G., Leontyeva O., Monney J., and Smith N.** (2001), “Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity,” *Mol. Phylogen. Evol.*, **18**, 449 – 459.

MORPHOLOGICAL VARIATION IN TWO CRYPTIC FORMS OF THE COMMON SPADEFOOT TOAD (*Pelobates fuscus*) FROM EASTERN EUROPE

G. A. Lada,¹ L. J. Borkin,¹ and S. N. Litvinchuk²

Keywords: *Pelobates fuscus*, Anura, cryptic speciation, morphometrics, coloration.

INTRODUCTION

According to the nuclear DNA content measured by means of flow cytometry, two forms of *Pelobates fuscus* in eastern Europe were recognized: the “western” form with smaller genome size and the “eastern” form with larger genome size (Barabanov et al., 1998; Borkin et al., 2001a, 2001b, 2003a, 2003b). The existence of the two forms was confirmed by allozyme analysis (Borkin et al., 2001a, 2003b; Khalturin et al., 2003). Apart from biochemical characters, we studied morphological variation in the western and the eastern forms of *P. fuscus*.

MATERIAL AND METHODS

Three hundred and nineteen adult specimens (178 males and 141 females) from 68 localities in Russia (47), Ukraine (14), Belarus’ (4), Moldova (2), and Latvia (1) were used in the study. 228 specimens were allocated to the western or the eastern form by DNA flow cytometry (Borkin et al., 2001b). The remaining spadefoot toads were assigned to either forms on the basis of their localities. Samples from the same physical geographical regions were united together (Fig. 1).

Thirteen standard morphometric measurements (Terentjev and Chernov, 1949; Terentjev, 1950) were taken on each specimen’s right side using a digital caliper (to the nearest 0.1 mm). Nineteen ratios expressed various body proportions were calculated and analyzed.

Dorsal coloration patterns of 307 specimens of *P. fuscus* were examined (we failed to identify coloration patterns in others specimens because of inadequate fixation). Frequencies of various kinds of five obvious elements of dorsal patterns were analyzed. These were the light medial stripe, light lateral stripes, the connection between medial

and lateral stripes, the dark stripe between eyes, and its connection with dark dorsal zones (Fig. 2).

Canonical discriminant analysis and standard statistical parameters (mean, SD, min – max, Kolmogorov – Smirnov’s test) were used to treat obtained data.

RESULTS

Linear parameters. 294 specimens grouping in 35 samples were studied. Minimum sample size contained three specimens. Canonical discriminant analysis was applied (Fig. 3). Samples of the western and the eastern types formed two groups in terms of external morphology.

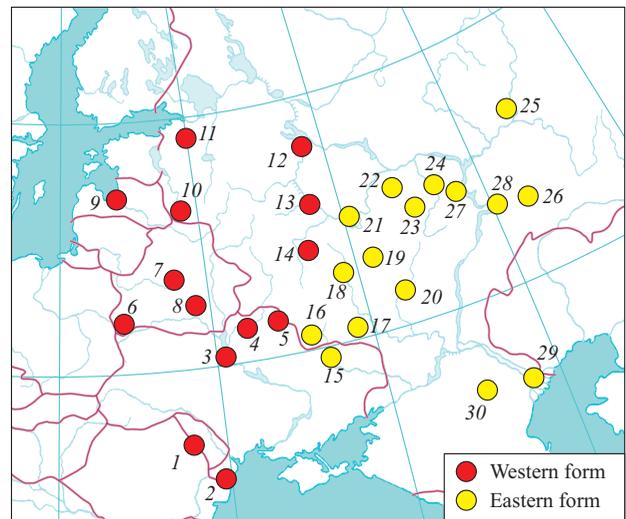


Fig. 1. Locality numbers of samples of *Pelobates fuscus*: 1, Moldova; 2, Odessa Oblast’; 3, Kiev Oblast’; 4, Chernigov Oblast’; 5, Sumy Oblast’; 6, Brest Oblast’; 7, Minsk Oblast’; 8, Gomel’ Oblast’; 9, Latvia; 10, Pskov Oblast’; 11, Leningradskaya Oblast’; 12, Yaroslavl’ Oblast’; 13, Moscow Oblast’; 14, Tula Oblast’; 15, Kharkov Oblast’; 16, Belgorod Oblast’; 17, Voronezh Oblast’; 18, Lipetsk Oblast’; 19, Tambov Oblast’; 20, Saratov Oblast’; 21, Ryazan Oblast’; 22, Nizhegorodskaya Oblast’; 23, Mordovia; 24, Chuvashia; 25, Udmurtia; 26, Bashkortostan; 27, Ul’yanovsk Oblast’; 28, Samara Oblast’; 29, Astrakhan Oblast’; 30, Kalmykia.

¹ Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, 199034 St. Petersburg, Russia; E-mail: esculenta@mail.ru, borkin@spass-sci.spb.ru.

² Institute of Cytology, Russian Academy of Sciences, 194064 St. Petersburg, Russia; E-mail: slitvinchuk@yahoo.com.

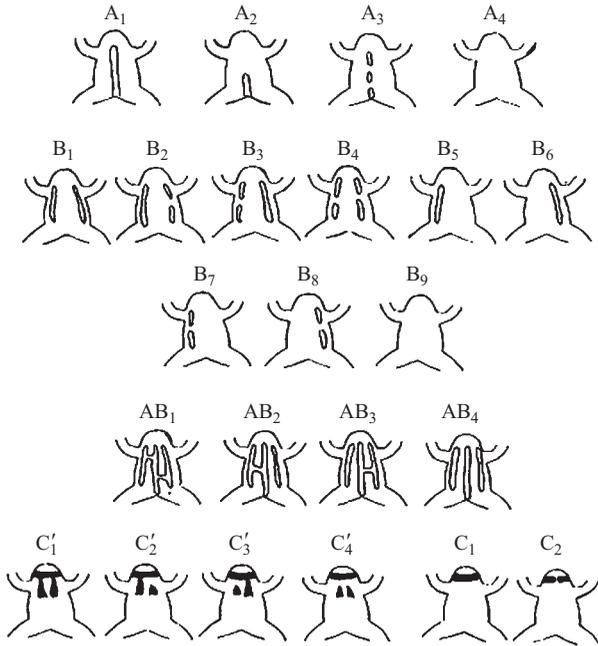


Fig. 2. Variants of 5 elements of the dorsal pattern of *Pelobates fuscus*: A, light medial stripe; B, light lateral stripes; AB, connection between A and B; C, dark stripe between eyes; C', connection between C and dark dorsal zones.

Small overlapping was associated with specimens of the western type from Odessa Oblast' (males), Leningradskaya Oblast' (both sexes), Kiev Oblast' (females), and with specimens of the eastern type from Ul'yanovsk Oblast' (males), Tambov Oblast' (both sexes), and Kalmykia (females). Totally the overlapping included 21% of all specimens under the study. Thus, the majority of samples belonged to either form of *P. fuscus* were separated.

Ratios. The comparison of the western and the eastern forms showed significant differences between means of seven ratios for males and females, respectively, Lt.c./Sp.n., Sp.oc./Sp.n., Sp.p./Sp.n. in particular (Table 1). These three most important ratios included the linear parameter Sp.n. (the distance between nostrils). According

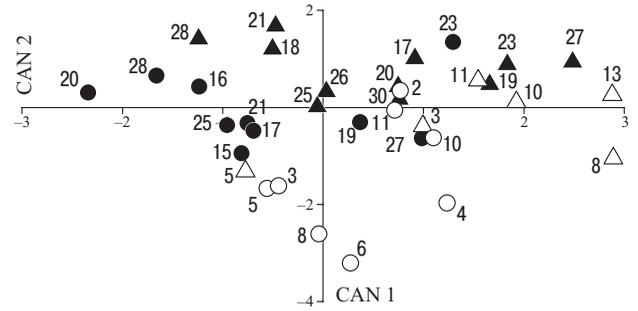


Fig. 3. Plot of populations centroids for males and females of two cryptic forms of *Pelobates fuscus* in the space of first and second canonical axes (CAN). Sample numbers the same as in Fig. 1. Males of western form males of eastern form; females of western form females of eastern form. ○, Males of western type; △, females of western type; ●, males of eastern type; ▲, females of eastern type.

to Tables 2 – 4, the western form as a whole was characterized by greater distance between nostrils. However, the ranges of three ratios in two forms of *P. fuscus* markedly overlapped. Moreover, significant differences between populations within each form were observed. Moreover, sometimes, differences between two samples of the same type (for instance, between males from Saratov and Ul'yanovsk Oblast's) can reach the level of differences between total samples of these forms.

Sexual dimorphism. Among 20 characters, only three ratios (L./L.c., L./D.r.o., and D.r.o./Sp.n.) showed significant differences between males and females of the western form of *P. fuscus* (Table 5). The sexual dimorphism seems to be more expressed in the eastern form because significant differences were found in eight characters.

Coloration pattern. Significant differences in the frequency of seven variants (especially light lateral stripes and dark stripe between eyes) were found between two cryptic forms of *P. fuscus*. Frequencies of various kinds of light lateral stripes and dark stripe between eyes are demonstrated in Table 6. The largest differences were revealed in variants B₁, B₄ as well as in two variants of C. However,

TABLE 1. The Significant Level of Differences in Morphometric Characters Between Two Forms of *Pelobates fuscus*

Sex	L.	L./T.	L./D.p.	L./L.c.	L./D.r.o.	F./T.	T./D.p.	D.p./C.i.	L.c./Lt.c.	L.c./D.r.o.
Males	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.	n.s.	n.s.	0.001
Females	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.	n.s.	n.s.	0.01
Sex	L.c./L.o.	Lt.c./Sp.oc.	Lt.c./Sp.p.	Lt.c./Sp.n.	Sp.oc./Sp.p.	Sp.oc./Sp.n.	Sp.oc./D.r.o.	Lt.p./Sp.p.	Sp.p./Sp.n.	D.r.o./Sp.n.
Males	n.s.	0.01	n.s.	0.001	n.s.	0.001	0.001	n.s.	0.001	n.s.
Females	n.s.	n.s.	n.s.	0.001	n.s.	0.001	0.01	n.s.	0.001	0.01

Note. n.s., non significant, $p < 0.05$, $p < 0.01$, $p < 0.001$.

TABLE 2. The Ratio Lt.c./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
The “Western” Type								
Sumy	8	4.48	0.33	4.11 – 5.10	7	4.44	0.21	4.17 – 4.74
Gomel’	6	4.60	0.25	4.17 – 4.90	—	—	—	—
Pskov	6	4.10	0.25	3.71 – 4.38	11	4.13	0.25	3.61 – 4.43
Total	44	4.42	0.39	3.27 – 5.29	40	4.31	0.42	3.61 – 6.15
The “Eastern” Type								
Voronezh	19	4.68	0.21	4.36 – 5.29	9	4.51	0.21	4.33 – 5.33
Tambov	19	4.48	0.23	3.98 – 4.97	13	4.51	0.43	3.32 – 5.11
Saratov	15	4.97	0.49	3.72 – 5.75	—	—	—	—
Ryazan	11	4.67	0.26	4.28 – 5.13	—	—	—	—
Mordovia	—	—	—	—	13	4.49	0.17	4.22 – 4.83
Udmurtia	19	4.86	0.33	4.21 – 5.48	16	4.70	0.23	4.42 – 5.09
Ul’yanovsk	7	4.43	0.46	3.45 – 4.76	—	—	—	¼
Samara	27	4.81	0.37	4.24 – 6.11	16	4.75	0.03	4.39 – 5.00
Kalmykya	—	—	—	—	9	4.82	0.30	4.26 – 5.12
Total	134	4.71	0.36	3.45 – 6.11	111	4.69	0.33	3.32 – 5.76

TABLE 3. The Ratio Sp.oc./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
The “Western” Type								
Sumy	8	1.71	0.13	1.54 – 1.90	7	1.80	0.14	1.54 – 2.00
Gomel’	6	1.67	0.08	1.59 – 1.77	—	—	—	—
Pskov	6	1.65	0.10	1.51 – 1.79	11	1.68	0.11	1.48 – 1.89
Total	44	1.69	0.15	1.29 – 2.08	40	1.69	0.16	1.41 – 2.15
The “Eastern” Type								
Voronezh	19	1.73	0.13	1.47 – 2.03	9	1.71	0.16	1.36 – 1.88
Tambov	19	1.78	0.16	1.51 – 2.10	13	1.75	0.20	1.35 – 2.06
Saratov	15	2.14	0.24	1.59 – 2.50	—	—	—	—
Ryazan	11	1.92	0.24	1.53 – 2.16	—	—	—	—
Mordovia	—	—	—	—	13	1.81	0.15	1.53 – 2.03
Udmurtia	19	1.90	0.21	1.49 – 2.17	16	1.86	0.15	1.58 – 2.07
Ul’yanovsk	7	1.70	0.16	1.46 – 1.94	—	—	—	—
Samara	27	1.99	0.15	1.76 – 2.35	16	2.06	0.02	1.82 – 2.30
Kalmykya	—	—	—	—	9	1.83	0.15	1.60 – 2.00
Total	134	1.88	0.23	1.30 – 2.50	111	1.86	0.19	1.35 – 2.30

TABLE 4. The Ratio Sp.p./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*.

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
The “Western” Type								
Sumy	8	1.40	0.09	1.28 – 1.55	7	1.37	0.19	1.19 – 1.55
Gomel’	6	1.27	0.06	1.21 – 1.35	—	—	—	—
Pskov	6	1.33	0.13	1.16 – 1.55	11	1.30	0.13	1.06 – 1.47
Total	44	1.33	0.15	1.02 – 1.94	16	1.30	0.14	1.05 – 1.70
The “Eastern” Type								
Voronezh	19	1.48	0.11	1.33 – 1.76	9	1.50	0.09	1.38 – 1.65
Tambov	19	1.31	0.13	1.14 – 1.60	13	1.35	0.14	1.00 – 1.57
Saratov	15	1.53	0.20	1.18 – 1.85	—	—	—	—
Ryazan	11	1.40	0.13	1.18 – 1.61	—	—	—	—
Mordovia	—	—	—	—	13	1.28	0.13	1.02 – 1.48
Udmurtia	19	1.45	0.16	1.16 – 1.76	16	1.37	0.12	1.07 – 1.54
Ul’yanovsk	7	1.27	0.17	0.93 – 1.47	—	—	—	—
Samara	27	1.54	0.14	1.18 – 1.78	16	1.56	0.02	1.40 – 1.93
Kalmykya	—	—	—	—	9	1.46	0.12	1.31 – 1.75
Total	134	1.43	0.17	0.93 – 1.85	111	1.41	0.16	1.00 – 1.93

TABLE 5. The significant level of differences in morphometric characters between males and females in two forms of *Pelobates fuscus*

<i>P. fuscus</i> form	L.	L./T.	L./D.p.	L./L.c.	L./D.r.o.	F./T.	T./D.p.	D.p./C.i.	L.c./Lt.c.	L.c./D.r.o.
“Western”	n.s.	n.s.	n.s.	0.05	0.01	n.s.	n.s.	n.s.	n.s.	n.s.
“Eastern”	0.001	0.01	0.001	0.001	0.001	n.s.	0.01	0.001	0.05	n.s.

<i>P. fuscus</i> form	L.c./L.o.	Lt.c./Sp.oc.	Lt.c./Sp.p.	Lt.c./Sp.n.	Sp.oc./Sp.p.	Sp.oc./Sp.n.	Sp.oc./D.r.o.	Lt.p./Sp.p.	Sp.p./Sp.n.	D.r.o./Sp.n.
“Western”	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01
“Eastern”	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Note. n.s., non significant; $p < 0.05$, $p < 0.01$, $p < 0.001$.

TABLE 6. Frequencies (per cent) of various kinds of some elements of the dorsal pattern in two forms of *Pelobates fuscus*

<i>P. fuscus</i> form	Light lateral stripes (B)							Dark stripe between eyes (C)	
	B ₁	B ₂	B ₃	B ₄	B ₇	B ₈	B ₉	C ₁	C ₂
“Western”	29.4	2.9	1.5	35.3	0	0	14.7	26.2	73.8
“Eastern”	7.0	2.6	1.7	81.6	0.4	0.4	6.1	66.1	33.9

almost all variants of all elements were observed in the both forms of *P. fuscus*.

CONCLUSION

To summarize, some significant differences between averaged values of morphological characters of two forms of *P. fuscus* were revealed. However, diagnostic characters, which could allow to make reliable identification of each specimen, were not found. This fact confirms that these forms could be recognized cryptic. Such a morphological stasis of *P. fuscus* may be explained by the effect of stabilizing selection which maintains the optimum phenotype as a result of the adaptation to burrowing mode of life of this anuran species (Borkin et al., 2003a; Khalturin et al., 2003).

Acknowledgments. Financial support was provided by the Russian Foundation for Basic Research (grant No. 02-04-49631), by the Russian Federal Programs “Integration” (grants E 0121 and E 3248) as well as by the Russian Government grant “Scientific School” (grant No. NSh 1647.2003.4).

REFERENCES

- Barabanov A. V., Borkin L. J., Litvinchuk S. N., and Rosanov J. M. (1998), “On distribution of *Pelobates fuscus* and *P. syriacus* in Asia,” in: *Abstrs. of the Third Asian Herpetol. Meet.*, Almaty, p. 10.
- Borkin L. J., Litvinchuk S. N., Milto K. D., Rosanov J. M., and Khalturin M. D. (2001a), “Cryptic speciation in *Pelobates fuscus* (Anura, Pelobatidae): cytometrical and biochemical evidences,” *Dokl. RAN*, **376**(5), 707 – 709 [in Russian].
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., and Milto K. D. (2001b), “Cryptic speciation in *Pelobates fuscus* (Anura, Pelobatidae): evidence from DNA flow cytometry,” *Amphibia-Reptilia*, **22**(4), 387 – 396.
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., Khalturin M. D., Lada G. A., Borisovsky A. G., Milto K. D., and Faizulin A. I. (2003a), “Distribution of two cryptic forms of spadefoot toad (*Pelobates fuscus*) in the territory of Volga River Basin,” in: *Proc. of the Third Conf. of Volga Herpetologists*, Tolyatti, pp. 3 – 6 [in Russian].
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., Khalturin M. D., Lada G. A., Borisovsky A. G., Faizulin A. I., Kotserzhinskaya I. M., Novitsky R. V., and Ruchin A. B. (2003b), “New data on the distribution of two cryptic forms of the common spadefoot toad (*Pelobates fuscus*) in Eastern Europe,” *Russ. J. Herpetol.*, **10**(2), 111 – 118.
- Khalturin M. D., Litvinchuk S. N., Borkin L. J., Rosanov J. M., and Milto K. D. (2003), “Genetic variation in two cryptic forms, with different genome size, of the common spadefoot toad, *Pelobates fuscus* (Pelobatidae, Anura, Amphibia),” *Tsitologiya*, **45**(3), 308 – 323 [in Russian].
- Terentjev P. V. (1950), *The Frog*, Sovetskaya Nauka, Moscow [in Russian].
- Terentjev P. V. and Chernov S. A. (1949), *Opredelitel' Presmykayushchikhsya i Zemnovodnykh*, Sovetskaya Nauka, Moscow [in Russian]; (1965), *Key to Amphibians and Reptiles. English version*, Smithsonian Institution, Washington.

GEOGRAPHIC DIFFERENTIATION IN NEWTS (*Triturus*) OF EASTERN EUROPE: GENOME SIZE, ALLOZYMES, AND MORPHOLOGY

S. N. Litvinchuk,¹ L. J. Borkin,² J. M. Rosanov,¹
D. V. Skorinov,² M. D. Khalturin,¹ G. Džukić,³
M. L. Kalezić,⁴ and L. F. Mazanaeva⁵

Keywords: eastern Europe, *Triturus*, systematics, genome size, allozymes, morphology.

Eight species of the genus *Triturus* inhabit eastern Europe. The geographic variation in salamanders of this vast territory was poorly studied. The goal of present paper was to evaluate such variation, evidenced by DNA flow cytometry, allozymes and standard morphological treatments.

The amount of DNA per nucleus (genome size) was measured by flow cytometry (Borkin et al., 2001; Litvinchuk et al., 2004). The details of the allozyme and morphological techniques have been published previously (Litvinchuk et al., 1994; Litvinchuk and Borkin, 2000, 2003).

The *Triturus cristatus* superspecies is a large group of European salamandrids consisting of seven species and subspecies (Arntzen, 2003; Table 1). The study of allozymes, genome size and morphology allowed us (Litvinchuk et al., 1997, 1999; Litvinchuk, 1998) to reveal quite narrow hybrid zone between *T. cristatus* and *T. dobrogicus* in the Ukrainian Transcarpathians (Fig. 1). Therefore, our data supported that these former subspecies of *T. cristatus* should be elevated to full distinct species in the framework of the *T. cristatus* superspecies (Arntzen, 2003). Currently we recognized two subspecies of the Danube newt (Litvinchuk and Borkin, 2000). Most part of the species range, including lowland of Ukrainian Transcarpathians, is inhabited by *T. dobrogicus macrosomus*, as well as Danube and Dnepr river deltas — by *T. d. dobrogicus* (Litvinchuk and Borkin, 2002). Two other members of the *T. cristatus* superspecies were also splitted in two subspecies, namely *T. carnifex carnifex* and *T. c. macedonicus* (Kalezić et al.,

1997), as well as *T. karelinii karelinii* and *T. k. arntzeni* (Litvinchuk et al., 1999).

The genome size variation in the *Triturus cristatus* superspecies ($n = 836$) was polymodal (Table 1). Differences in the nuclear DNA content between *T. cristatus* and *T. dobrogicus* (both subspecies included) were small. Another group was formed by two subspecies of *T. carnifex* and the Balkan subspecies *T. karelinii arntzeni* (Litvinchuk et al., 1999). The group with the largest genome size contained *T. k. karelinii* only. According to genome size data, in the Caucasus, the subspecies consists of two geographically separate groups of populations. These are the western group (the northwestern Caucasus) and the eastern group (Dagestan, Georgia, and Azerbaijan).

The allozyme analysis ($n = 265$; Litvinchuk et al., 1994, 1999; Litvinchuk, 1998) showed obvious between-

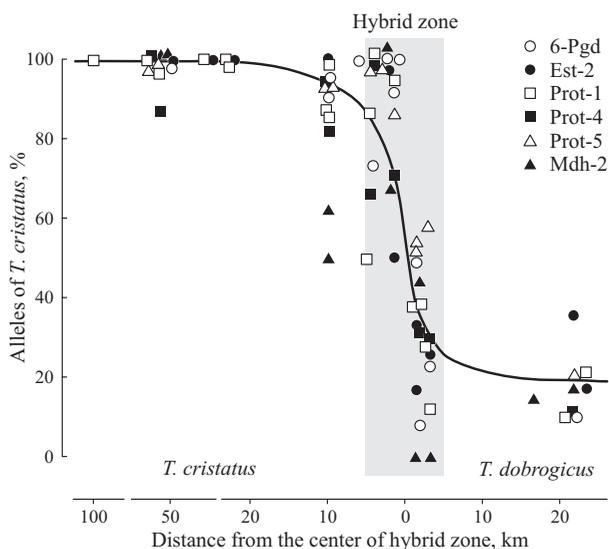


Fig. 1. The distribution of *T. cristatus* alleles in samples from the hybrid zone between *T. cristatus* and *T. dobrogicus* in the Ukrainian Transcarpathians.

¹ Institute of Cytology Russian Academy of Sciences, Tikhoretsky pr., 4, 194064 St. Petersburg, Russia; E-mail: slitvinchuk@yahoo.com.

² Department of Herpetology and Ornithology, Zoological Institute Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: borkin@spass-sci.spb.ru

³ Institute for Biological Research "Sinića Stanković," Belgrade, Serbia and Montenegro.

⁴ Institute of Zoology, Belgrade, Serbia and Montenegro.

⁵ Dagestan State University, Makhachkala, Russia.

TABLE 1. Genome Size Variation (in picograms) in East European Newts of the genus *Triturus*

Taxon	Locality	N_{samples}	N_{spec}	Mean \pm SD	Range
<i>Triturus alpestris</i>					
<i>alpestris alpestris</i>	Maloe Opol'e Eminence (Lvov Oblast', Ukraine)	2	16	59.7 \pm 0.5	59.0 – 61.1
	Ukrainian Carpathians	2	12	59.8 \pm 0.6	58.7 – 60.7
	Romanian Bihor Mts.	1	1	60.0	
	Montenegro	1	2	60.1	59.9 – 60.3
<i>alpestris montenegrinus</i>	Montenegro	1	36	59.5 \pm 0.4	58.3 – 60.6
<i>alpestris serdarus</i>	Montenegro	1	6	59.5 \pm 0.1	59.4 – 59.6
<i>Triturus cristatus</i> superspecies					
<i>carnifex carnifex</i>	Northeastern Italy	2	3	58.2 \pm 0.0	58.2 – 58.3
<i>carnifex macedonicus</i>	Montenegro	2	11	58.3 \pm 0.6	57.5 – 59.3
<i>cristatus</i>	Eastern Europe	51	423	55.5 \pm 0.5	54.2 – 57.1
<i>dobrogicus dobrogicus</i>	Danube River Delta	3	78	56.3 \pm 0.5	55.0 – 57.6
	Dnepr River Delta	1	1	56.6	
<i>dobrogicus macrosomus</i>	Danube River Basin	11	177	56.2 \pm 0.4	55.1 – 57.4
<i>karelinii arntzeni</i>	Serbia	2	14	58.8 \pm 0.5	58.2 – 59.7
<i>karelinii karelinii</i>	European Istanbul (Halkali)	1	2	62.6	62.4 – 62.8
	Northwestern Anatolia	1	2	64.0	63.7 – 64.4
	The Crimea	4	68	62.7 \pm 0.5	60.8 – 63.5
	Northwestern Caucasus	6	36	62.4 \pm 0.4	61.6 – 63.1
	Dagestan	1	8	63.4 \pm 0.2	63.3 – 63.8
	Eastern Georgia	1	1	63.6	
	Southeastern Azerbaijan	2	12	63.5 \pm 0.3	63.0 – 63.9
<i>Triturus vulgaris</i> group					
<i>montandoni</i>	Maloe Opol'e Eminence (Lvov Oblast', Ukraine)	3	36	59.7 \pm 0.5	58.6 – 60.6
	Ukrainian and Romanian Carpathians	4	14	59.7 \pm 0.8	58.2 – 60.8
	Gutyi Mts. (Zakarpatskaya Oblast', Ukraine)	1	9	59.2 \pm 0.7	58.5 – 60.1
<i>vulgaris ampelensis</i>	Romanian Transylvania	2	34	58.6 \pm 0.6	57.1 – 60.1
<i>vulgaris ampelensis?</i>	Romanian Bihor Mts. (Cimpeni)	1	10	57.0 \pm 0.2	56.7 – 57.3
<i>vulgaris graecus</i>	Montenegro	1	11	62.3 \pm 1.0	60.7 – 63.5
<i>vulgaris kosswigi?</i>	European Istanbul (Halkali)	1	34	58.1 \pm 0.4	57.1 – 58.8
<i>vulgaris lantzi</i>	Abkhazia	3	15	63.6 \pm 0.5	62.9 – 64.7
	Northwestern Caucasus	7	39	62.6 \pm 0.5	61.8 – 63.6
<i>vulgaris schmidlerorum</i>	Northwestern Anatolia	1	6	59.7 \pm 0.4	59.1 – 60.3
<i>vulgaris vulgaris</i>	Eastern Europe	63	430	56.7 \pm 0.6	54.6 – 59.4
<i>Triturus ophryticus</i>					
<i>ophryticus</i> ssp.	Northwestern Anatolia	1	4	63.1 \pm 0.6	62.5 – 63.7
<i>ophryticus ophryticus</i>	The Caucasus	7	20	67.6 \pm 0.7	66.8 – 68.8

N_{samples} and N_{spec} are numbers of sample locations and specimens studied, respectively.

population differences within two species only: *T. cristatus* and *T. karelinii* (Fig. 2). The treatment of standard morphometrical characters and trunk vertebrae count allowed us to identify all four species of the complex, and even both subspecies of *T. dobrogicus* (Litvinchuk et al., 1999; Litvinchuk and Borkin, 2000).

The second large group of newts in eastern Europe contained two species, namely: *T. vulgaris* with several currently recognized subspecies and *T. montandoni*. The latter two species plus *T. helveticus* comprise the *T. vulgaris* group. The smooth newt has the widest distribution in

eastern Europe. The genome size variation in this species was polymodal ($n = 638$). We recognized four groups composed by various subspecies (Table 1). The smallest amount of the nuclear DNA was characteristic to *T. v. vulgaris* and morphologically intermedian between *T. v. vulgaris* and *T. v. ampelensis* population from Romanian Bihor Mountains. The Transylvanian *T. v. ampelensis* and the sample from European part of Istanbul provided the second group. The latter sample was morphologically intermedian between *T. v. vulgaris* and *T. v. kosswigi*. The Anatolian subspecies *T. v. schmidlerorum* formed the

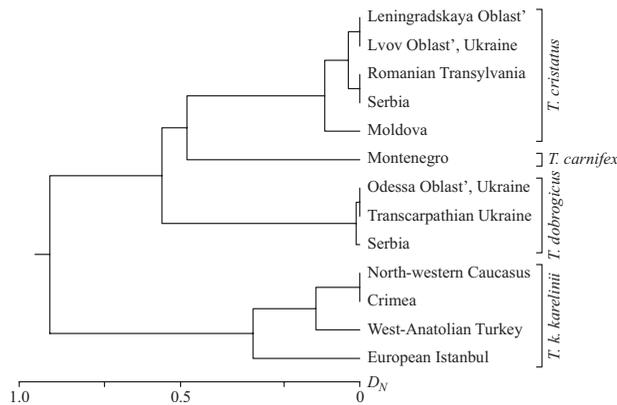


Fig. 2. UPGMA dendrogram for the *Triturus cristatus* superspecies based upon Nei's (1972) genetic distance.

third group. Finally, the Balkan subspecies *T. v. graecus* and the Caucasian newt *T. v. lantzi* had the largest genome size within the superspecies. Abkhazian populations of the latter subspecies had slightly larger amount of nuclear DNA in comparison with newts of the northwestern Caucasus (Krasnodar and Stavropol' Krai's).

The study of allozyme variation in the smooth newt (18 loci, $n = 197$) showed that the levels of differences between three subspecies were quite different (Fig. 3). Two European subspecies *T. v. vulgaris* and *T. v. ampelensis* ($D_{\text{Nei}'72} = 0.030$) were much closer each other than both to the Caucasian *T. v. lantzi* ($D_{\text{Nei}'72} = 0.174$). The analysis of standard morphological characters in the smooth newt ($n = 1412$) showed well differences between most subspecies, in the exception of *T. v. vulgaris* and *T. v. lantzi*.

Triturus montandoni is endemic to the Carpathian Mountains. Two small and geographically isolated populations from the main species range are situated in western Ukraine. These are the Maloe Opol'e Eminence in Lvov Oblast' and Gutyi Mountains in the Transcarpathians (Litvinchuk et al., 2003). We failed to find any significant differences between the both isolated populations and newts from the main range of the species.

According to our data, the banded newt is suggested to consist of two species: *T. ophryticus* and *T. vittatus* (Litvinchuk et al., 2005). In the northern banded newt (*T. ophryticus*), significant differences between populations from northwestern Turkey and from northeastern Turkey and the Caucasus were expressed in genome size (Table 1), allozyme data ($D_{\text{Nei}'72} = 0.383$; $n = 30$), and trunk vertebrae count (the modal number 12 vs. 13; $n = 219$). Therefore, we allocate the western group of *T. ophryticus* to a separate subspecies (Litvinchuk et al., 2005).

We examined the genome size variation in *T. alpestris* ($n = 73$) as well. We failed to reveal any significant differ-

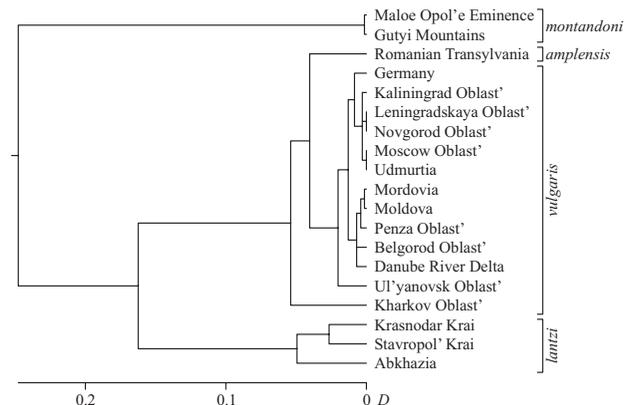


Fig. 3. UPGMA dendrogram for the *Triturus vulgaris* group based upon Nei's (1972) genetic distance.

ences between *T. a. alpestris*, *T. alpestris*, *T. a. montenegrinus*, and *T. a. serdarus* from Montenegro (Table 1). Moreover, the amount of nuclear DNA in four geographically isolated populations of *T. a. alpestris* from the Ukrainian Carpathians, Maloe Opol'e Eminence, Romanian Bihor Mountains, and Montenegro was quite similar.

Thus, we consider that genome size together with other cytogenetic, molecular and morphological characters could be used for analysis of geographical differentiation and speciation in urodelans.

Acknowledgments. The research was supported by the Russian Foundation for Basic Research (grant Nos. 02-04-49631 and 04-04-63165), Russian Federal Program "Integration" (grant Nos. E 0121, E 3248), and by Russian Government grant "Scientific Schools" (No. NSh 1647.2003.4).

REFERENCES

- Arntzen J. W. (2003), "Triturus cristatus Superspezies — Kamolch-Artenkreis," in: Grossenbacher K. and Thiesmeier B. (eds.), *Handbuch der Reptilien und Amphibien Europas. Band 4. Schwanzlurche (Urodela). IIA. Salamandridae II: Triturus I*, AULA-Verlag, Wiebelsheim, pp. 421–514.
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., and Miltov K. D. (2001), "Cryptic speciation in *Pelobates fuscus* (Anura, Pelobatidae): evidence from DNA flow cytometry," *Amphibia-Reptilia*, **22**(4), 387–396.
- Kalezić M. L., Džukić G., Mesáros G., and Crnobrnja-Isailović J. (1997), "The crested newt (*Triturus cristatus* superspecies) in ex-Yugoslavia: morphological structuring and distribution patterns," *Univ. Thought. Nat. Sci. (Priština)*, **4**, 39–46.
- Litvinchuk S. N. (1998), *Systematics and Distribution of Newts of the Triturus cristatus Complex (Salamandridae) in Russia and Adjacent Countries. PhD Thesis*, Zoological Institute, Russian Academy of Sciences, St. Petersburg [in Russian];

full text available in

<http://www.callisto.ru/amphibia/spartak/disser.htm>].

- Litvinchuk S. N. and Borkin L. J.** (2000), “Intraspecific taxonomy and nomenclature of the Danube crested newt, *Triturus dobrogicus*,” *Amphibia–Reptilia*, **21**(4), 419 – 430.
- Litvinchuk S. N. and Borkin L. J.** (2002) “Distribution, ecology and conservation status of the Danube crested newt, *Triturus dobrogicus* (Amphibia, Salamandridae) in Ukraine and Moldova,” *Vestnik Zool. Kiev*, **36**(3), 35 – 44 [in Russian, with English summary].
- Litvinchuk S. N. and Borkin L. J.** (2003), “Variation in numbers of trunk vertebrae and of costal grooves in salamanders of the family Hynobiidae,” *Contrib. Zool.*, **72**(4), 195 – 209.
- Litvinchuk S. N., Borkin L. J., Džukić G., Kalezić M. L., Khalturin M. D., and Rosanov J. M.** (1999), “Taxonomic status of *Triturus karelinii* on the Balkans, with some comments about other crested newt taxa,” *Russ. J. Herpetol.*, **6**(2), 153 – 163.
- Litvinchuk S. N., Borkin L. J., and Rosanov J. M.** (2003), “On distribution of and hybridization between the newts *Triturus vulgaris* and *T. montandoni* in western Ukraine,” *Alytes*, **20**(3 – 4), 161 – 168.
- Litvinchuk S. N., Borkin L. J., and Rosanov J. M.** (2004), “Intra- and interspecific genome size variation in hynobiid salamanders of Russia and Kazakhstan: determination by flow cytometry,” *Asiatic Herpetol. Res.*, **10**, 282 – 294.
- Litvinchuk S. N., Rosanov J. M., and Borkin L. J.** (1997), “A contact zones between the newts *Triturus cristatus* and *Triturus dobrogicus* in the Ukrainian Transcarpathians: distribution and genome size variation,” in: Böhme W., Bischoff W., and Ziegler T. (eds.), *Herpetologia Bonnensis*, Bonn, pp. 229 – 235.
- Litvinchuk, S. N., Rosanov J. M., Borkin L. J., Khalturin M. D., Timofeev B. I., Džukić G., and Kalezić M. L.** (2001). “Genome size and some problems of urodelan systematics (Salamandridae and Hynobiidae),” in: Ananjeva N. B. et al. (eds.), *Voprosy Gerpelogii [Problems of Herpetology]*, Moscow State Univ., Puzshino – Moscow, pp. 168 – 170 [in Russian, with English summary].
- Litvinchuk S. N., Sokolova T. M., and Borkin L. J.** (1994), “Biochemical differentiation of the crested newt (*Triturus cristatus* group) in the territory of the former USSR,” *Abh. Ber. Naturk. Magdeburg*, **17**(Sonderband), 67 – 74.
- Litvinchuk S. N., Zuiderwijk A., Borkin L. J., and Rosanov J. M.** (2005), “Taxonomic status of *Triturus vittatus* (Amphibia: Salamandridae) in western Turkey: trunk vertebrae count, genome size and allozyme data,” *Amphibia–Reptilia*, **26**(1), in press.

CASES OF MIXOPLIIDY IN BROWN FROGS OF UKRAINE

V. V. Manilo¹**Keywords:** chromosomes, polyploidization, karyotype, mitosis, meiosis.

INTRODUCTION

Polyploid cells were detected for the first time in testicle preparations of *Rana arvalis* from surroundings of Zhitomir city (Ukraine) in 1998 (Manilo, 2000). This observation encouraged the further, more detailed cytogenetic investigation of the genus *Rana* in Zhitomir Oblast', as well as in other regions of Ukraine. This paper is focused on the description of karyotypes in brown frogs, but now we have also similar results of the cytogenetic investigation in other species.

MATERIAL AND METHODS

The material investigated is shown in Table 1. Chromosome preparations were obtained by the dripping (pi-

¹ National Museum of Natural History, National Academy of Sciences of Ukraine, B. Khmelnytsky St., 15, 01030 Kiev, Ukraine;
E-mail: manilo@zoomus.freenet.kiev.ua

petting) method from blood cells, marrow and testicles of the colchycinized animals according to the routine methods (Ford and Hamerton, 1956; MacGregor and Warley, 1986). In order to increase mitotic activity, most of animals were treated with phytohemagglutinine M (Difco Laboratories) according to the method described earlier (Manilo, 1986).

RESULTS

Principal morphological features of chromosomes in karyotypes of *Rana arvalis arvalis* and *R. temporaria* are presented in Table 1. Below we describe those characters, which are not shown in Table 1, but are considered important.

***Rana arvalis arvalis*.** Metaphase plates in blood and marrow cells of specimens from all studied populations had the standard karyotype and were identical in their ex-

TABLE 1. Karyotypes of the Brown Frogs

No.	Locality, year	Number of studied specimens (males, females)	Chromosome numbers		Rate of polyploid sets and their chromosome numbers	Karyotype	NF	Presence and location of secondary constrictions
			<i>n</i>	<i>2n</i>				
<i>Rana arvalis arvalis</i>								
1	Bogunia, to north near Zhitomir (1998)	4♂ 2♀	12	24	Up to 15% 4 <i>n</i> = 48 5 <i>n</i> = 60 6 <i>n</i> = 72	12 <i>V</i> + 4 <i>sV</i> + 8 <i>sT</i> 24 <i>V</i> + 8 <i>sV</i> + 16 <i>sT</i> 30 <i>V</i> + 10 <i>sV</i> + 20 <i>sT</i> 36 <i>V</i> + 12 <i>sV</i> + 24 <i>sT</i>	48 96 120 144	2 nd pair, short arm 5 th pair, long arm
2	Zhitomir Oblast', Korosten District, near Ushomyr (2000)	2♂ 1 juv	12	24	10 – 20% 4 <i>n</i> = 48 6 <i>n</i> = 72	12 <i>V</i> + 4 <i>sV</i> + 8 <i>sT</i> 24 <i>V</i> + 8 <i>sV</i> + 16 <i>sT</i> 36 <i>V</i> + 12 <i>sV</i> + 24 <i>sT</i>	48 96 144	—
3	Zhitomir Oblast', Chernyakhovsky District, near Andreevka (2001)	2♀	12	24	10 – 45% 4 <i>n</i> = 48 6 <i>n</i> = 72	12 <i>V</i> + 4 <i>sV</i> + 8 <i>sT</i> 24 <i>V</i> + 8 <i>sV</i> + 16 <i>sT</i> 36 <i>V</i> + 12 <i>sV</i> + 24 <i>sT</i>	48 96 144	—
<i>Rana temporaria</i>								
4	Zakarpatskaya Oblast', Rakhovsky District, near Yasenya (2000)	1♀	13	26	Up to 30% 4 <i>n</i> = 52 6 <i>n</i> = 78	6 <i>V</i> + 18 <i>sV</i> + 2 <i>sT</i> 12 <i>V</i> + 36 <i>sV</i> + 4 <i>sT</i> 18 <i>V</i> + 54 <i>sV</i> + 6 <i>sT</i>	52 104 156	10 th pair, long arm
5	Bogunia, to north near Zhitomir (2000, 2001)	3♂ 6♀	13	26	Up to 20% 4 <i>n</i> = 52 6 <i>n</i> = 78	6 <i>V</i> + 18 <i>sV</i> + 2 <i>sT</i> 12 <i>V</i> + 36 <i>sV</i> + 4 <i>sT</i> 18 <i>V</i> + 54 <i>sV</i> + 6 <i>sT</i>	52 104 156	10 th pair, long arm
6	Zhitomir Oblast', Korosten District, near Ushomyr (2000)	1♂ 2♀	13	26	Up to 20% 4 <i>n</i> = 52 6 <i>n</i> = 78	6 <i>V</i> + 18 <i>sV</i> + 2 <i>sT</i> 12 <i>V</i> + 36 <i>sV</i> + 4 <i>sT</i> 18 <i>V</i> + 54 <i>sV</i> + 6 <i>sT</i>	52 104 156	10 th pair, long arm

Abbreviations. *n*, haploid chromosome set; *2n*, diploid set; *NF*, basic chromosome number; *4n*, tetraploid set; *5n*, pentaploid set; *6n*, hexaploid set; *V*, metacentric; *sV*, submetacentric; *sT*, subtelocentric.

ternal chromosome morphology ($2n = 24$, $NF = 48$). At preparations from tikles two types of dividing cells were present: cells of spermatogonial division (metaphase of mitosis II) and gametes (meiotic metaphase I – diakinesis and metaphase II). Metaphase plates of spermatogonial division included 24, 36, 48, 60, and 72 chromosomes. All of them represented multiplied haploid set ($n = 12$) and did not differ in chromosome morphology from the standard karyotype (Fig. 1a–c; Table 1). Furthermore, the part of dividing cells had the incomplete (aneuploid) chromosome set with several chromosomes more or less than in the standard karyotype. The total rate of polyploid or aneuploid cells was 15 and 5% of the investigated cells of spermatogonial division, respectively. Meiotic metaphases I and II in gametes also showed unusual chromosome sets: the number of diakinetid bivalents varied from $n = 12$ to $4n = 48$ (Fig. 1d–h). Secondary constrictions were observed in most of the metaphase plates from Bogunia on the long arm of the 5th chromosome pair, and on the short

arm of the second pair. Sex chromosomes were not identified.

***Rana temporaria*.** Individuals of this species have been examined from three localities (Bogunia and Ushomyr villages, Zhitomir Oblast') and vicinities of Yasenya village [Urdu-Flavanchuk ridge (alt. 800 m) in Zakarpatskaya Oblast' (Table 1)]. All studied cells of blood and marrow had the standard karyotype ($2n = 26$, see Table 1). Karyological features of testicle cells were similar to those in *R. arvalis*: mixoploidy was recorded in gametes ($n = 13$, $2n = 26$, $3n = 39$) as well as by spermatogonial division ($2n = 26$, $3n = 39$, $4n = 52$, etc., see Fig. 2). Numbers of dividing cells in some microscopic slides exceeded 100, making their counting difficult. The total rate of polyploid cells (in relation to all studied cells) varied between 10 and 30%, aneuploid cells were markedly less frequent — about 5%. As in the first species, no sex chromosomes were identified.

DISCUSSION

The results of our cytogenetic investigations of Zhitomir and Zakarpatskaya Oblast' populations of *R. arvalis* and *R. temporaria* are unexpected and unusual for Ranidae. Since this group is rather ancient and conservative, any hypotheses presuming active evolutionary processes or speciation seem improbable. More likely, the observed multiplied chromosome sets were caused by aberrations of meiosis, while polyploid cells appeared, because the chromosomes did not separate properly during cell division. Furthermore, we suggest that the described aberrations in

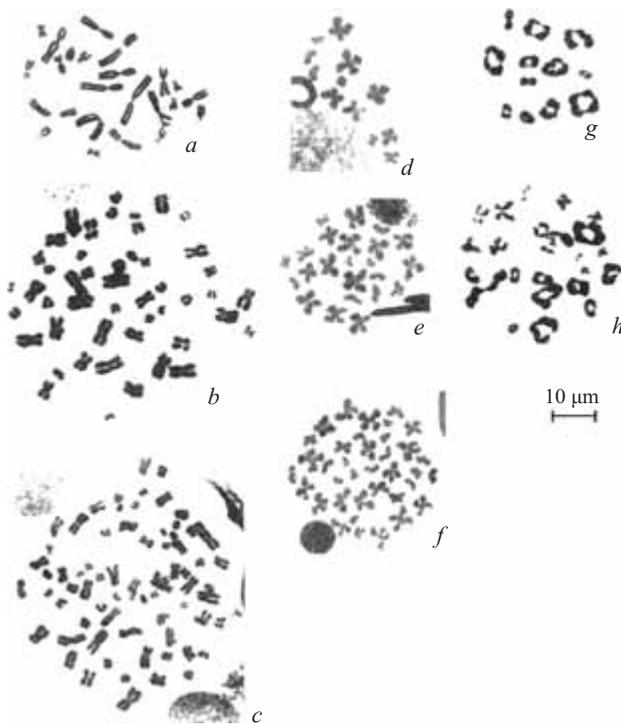


Fig. 1. Metaphase plates of *Rana arvalis*: a, metaphase plate of the normal karyotype ($2n = 24$), spermatogonial cell; b, metaphase plate of a tetraploid ($4n = 48$), spermatogonial cell from Bogunia near Zhitomir; c, metaphase plate of a hexaploid ($6n = 72$), spermatogonial cell from Ushomir; d, metaphase of meiosis II ($n = 12$), haploid gamete of a male from Andreevka; e, metaphase of meiosis II ($2n = 24$), diploid gamete of a male from Andreevka; f, metaphase of meiosis II ($3n = 36$), triploid gamete of a male from Andreevka; g, metaphase of meiosis I, diakinesis ($n = 12$), haploid gamete of a male from Bogunia near Zhitomir; h, metaphase of meiosis I, diakinesis ($2n = 24$), diploid gamete of a male from Bogunia near Zhitomir.

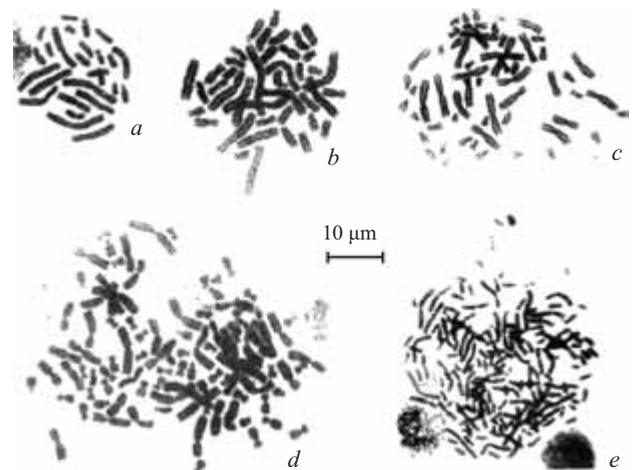


Fig. 2. Metaphase plates of *Rana temporaria*: a, diploid metaphase plate $2n = 26$, spermatogonial cell from Yasenya, Zakarpatskaya Oblast'; b, metaphase plate of a tetraploid ($4n = 52$), spermatogonial cell from Bogunia near Zhitomir; c, d, incomplete (aneuploid) metaphase plate $Xn = 60$; $Xn = 101$ (?) from Yasenya, Zakarpatskaya Oblast'; e, hexaploid metaphase plate $6n = 78$ from Ushomir, Zhitomir Oblast'.

testicles where determined by ecological factors (chemical and radio nuclide pollution of the environment). Indeed, such factors affect mainly germ cells (Mitrochenko et al., 1999). It is known, that amphibians belong to animal groups, which are sensitive to environmental factors, in particular to geochemical and radioactive influences, and may serve as very good markers of such influences (Petrov and Sharygin, 1981; Israel, 1984; Ilyenko and Krapivko, 1989). In addition, biological test systems are often more sensitive than chemical, physical and radiometrical methods (Brusick, 1987). Being a consequence of the negative influence of different mutation agents on somatic and germ cells, development of aneuploid and polyploid cells severely disturbs genetic balance and suppresses viability and reproductive ability of animals. Thus, the obtained results may be used in future for ecological and genetic monitoring in the studied regions.

REFERENCES

- Brusick D.** (1987), *Principles of Genetic Toxicology. 2nd Edition*, Premium Press, New York – London.
- Ford C. E. and Hamerton J. L.** (1956), “A colchicine hipotonic citrate squash sequence for mammal’s chromosomes,” *Staining Technol.*, **31**, 247 – 251
- Ilyenko A. I. and Krapivko T. P.** (1989), *Animal Elology in the Biogeocenosis under Radiation Impact*, Mir, Moscow [in Russian].
- Israel Ju. A.** (1986), *Ecology and Environment Control*, Hidrometeoizdat, Leningrad [in Russian].
- MacGregor H. C. and Varley J. M.** (1986), *Working with Animal Chromosomes*, John Wiley and Sons, Chichester.
- Manilo V. V.** (1986), “Karyotypes of the gecko genera *Alsophylax* and *Crossobamon*,” *Vestnik Zool. Kiev*, **5**, 46 – 54 [in Russian with English summary].
- Manilo V. V.** (2000), “Polyploidy — an ecological signal?” *Visnyk NAN Ukr.*, **5**, 52 – 53 [in Russian with English summary].
- Mitrochenko V. V., Kirichenko O. I., and Kuchma M. D.** (1999), “Influence of radiation on forest plantations,” in: *Fundamentals of the Forest Radioecology*, Kyiv, pp. 52 – 74 [in Ukrainian].
- Petrov V. S. and Sharygin S. A.** (1981), “On the possibility of the application of amphibians and reptilians for the indication of environment pollution,” in: *Terrestrial and Aquatic Ecosystems. Vol. 4*, Izd. GGU, Gorky, pp. 41 – 48 [in Russian].

DISTRIBUTION AND MORPHOLOGICAL VARIABILITY OF *Vipera berus* IN EASTERN EUROPE

K. D. Milto¹ and O. I. Zinenko²

Keywords: Nikolsky's viper, common adder, Viperidae, *Vipera berus*, *Vipera nikolskii*, systematics, distribution, morphological analysis, hemipenes structure, Eastern Europe.

INTRODUCTION

The first the black viper inhabiting Southern Russia was described in 1771 by P. S. Pallas. This morph was cited under different names (*Coluber melanis*, *Vipera melanis*, *Coluber scytha*, *Vipera scytha*, *Vipera melaenis*, *Vipera melaenis* var. *scytha*, *Vipera berus* var. *prester*, *Vipera prester*, *Pelias prester*, *Coluber berus* morpha *prester*, *Pelias berus* var. *nigra*) for many years. Situation changed when V. N. Grubant, A. V. Rudaeva and V. I. Vedmederja (1973) proposed to distinguish the black forest-steppe adder as a full species *Vipera prester*. These authors listed morphological and ecological differences between this species and *Vipera berus*. In 1986 the same authors changed the proposed name and described a new taxon *Vipera nikolskii*. The specific status of *Vipera nikolskii* was accepted by most specialists and included in subsequent herpetological accounts (Golay et al., 1993; Nilson and Andrén, 1997; Ananjeva et al., 1998; Bozhansky, 2001). Nevertheless, this point of view was subjected to criticism (Bakiev et al. 1999; 2000; Joger et al., 1997).

The black viper, *Vipera nikolskii*, from southern parts of the European Russia and Ukraine currently has unclear taxonomical status and distribution. The status of this viper varied from color morph to full species during last 230 years. This work is aimed to determine the diagnostic characters and status of this species. It is based on analysis of external morphology and data on distribution.

MATERIAL AND METHODS

About 1000 specimens from Russia, Ukraine, Moldova, and Belarus' stored in the collections of Zoological Institute Russian Academy of Science, St. Petersburg (ZISP), Museum of Nature of Kharkov National Univer-

sity (MN KNU), National Museum of Natural History Ukrainian Academy of Sciences (NNHM NASU), Zoological Museum of Moscow State University (ZMMU) as well as alive snakes in the nature were examined in this work. We recorded 10 morphological characters traditionally used in systematic of Viperidae (Vedmederja, 1989) and some others, which are useful in *Vipera berus-nikolskii* determination. For each specimen the following characters were used in multivariate analysis: number of ventral scales (ventralia, Ventr.); number subcaudal scales (subcaudalia, S.cd.); number of scales around midbody (squamae dorsalis, Sq.) except of ventral shields; number of supralabial shields (labialia, Lab.); number of sublabial shields (sublabialia, S.lab.); number of scales around the eye (circumocularia, C.oc.); number of subocularia (subocularia, S.oc.) rows; number of the loreal scales between canthal, circumocular, nasal and supralabial shields (lorealia, Lor.); number of scales between apical, canthal and frontal shields (intercanthalia, Ic.); number of shields between supraocular, frontal and parietal shields (parafrontalia, Pf.).

The following features were checked: shape and proportions of the frontal shield (frontale), number of rows of the postocular shields (postocularia, p.o.), type of the dorsal coloration pattern in adults — totally black and black with light elements (visible zigzag, light spots on supralabials and ventrals, reddish throat), venom fluid coloration (colorless in *nikolskii* and yellow in *berus*) and albumin composition (electrophoresis data). Patterns of geographical variation were studied by means of principal component analysis (PCA) using Statistica 6.0 Software Package. Samples were united according to regional distributional principle. Females and males were studied separately because the sexual dimorphism is well expressed. The arithmetical mean of morphological characters for samples were used in analysis.

Hemipenes were everted in fresh killed snakes and preserved in formalin. Terminology on hemipenial morphology follows Keogh (1999). Coloration was determined on the basis of color standard (Bondartsev, 1954).

¹ Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: lacerta@zin.ru.

² Museum of Nature of the Karazin Kharkiv National University, Trinkler st., 8, 61022 Kharkiv, Ukraine; E-mail: zinenkoa@yahoo.com, zinenko@au.ru.

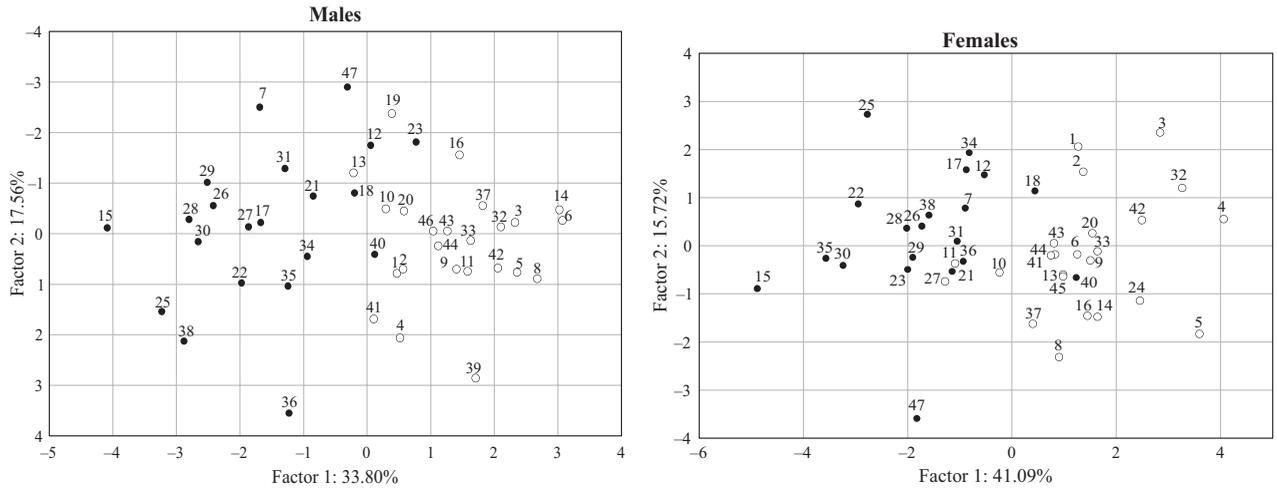


Fig. 1. Ordination of samples along the first two principal components. Numeration of samples is the same as in Table 1. Black dots are populations with predominance of black-colored snakes.

Distributional maps were completed as a result of the collection materials study. Data of geobotanical and geomorphological maps and maps of Glaciations in Europe (Atlas..., 1962, 1984; Markov et al., 1965; Paleogeography of Europe..., 1982; Markova et al., 2002) were used for interpretation and discussion of the viper’s distribution.

RESULTS

In Fig. 1, the ordination of samples along the first two principal components is given, resulting from PC analysis, separately for males and females (samples list see in Appendix). The results were similar but not identical for males and females. The ordination plots demonstrate the existence of two not clearly distinct groups. In both sexes all populations are distributed and in the case of females are divided on two groups along the first PC axis. The first group is completed by populations from northern and north-western part of the Eastern Europe. The second group includes populations from Southern and South-Eastern part of this territory. Also we considered the third group of populations that include individuals with transitional morphological characters between the two previous groups. Groups’ 95% variation interval of samples’ average values of morphological characters, which were calculated for 16 samples of Nikolsky’s viper, 22 samples of males and 23 samples of females of common adder and 11 samples of vipers from mixed populations, are given in Table 1.

We recorded several types of coloration patterns: total melanistic, partially melanistic and normally patterned

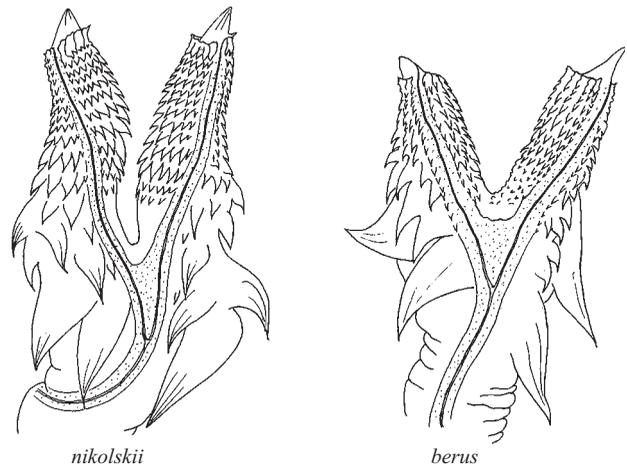


Fig. 2. Hemipenes of *V. b. nikolskii* and *V. b. berus*, sulcate view.

type. Full description of coloration places in chapter “Redescription.” Comparison of hemipenial structure demonstrated good differences in size and structure: hemipenes of common adder are short and compact, while Nikolsky’s viper hemipenes are elongated and deeply forked (Fig. 2). Redetermination of collection materials and analysis of literature data allow the description of the Nikolsky’s viper range (Fig. 3).

DISCUSSION

Some characters that were proposed by Vedmederja et al. in 1986 as diagnostic for Nikolsky’s viper, correlate very well with first principal components (Fig. 4). These

are increased number of ventral, supralabial, sublabial shields and scale rows around midbody. We support the significance of these morphological characters for distinguishing the viper's taxa. An increased number of loreal scales (between preocular, canthal, nasal and supralabial shields), high frequency of registration of two complete or almost complete subocular rows (up to 77.7% of female and 23.3% male specimens have 2 rows), increased number of loreal scales (between preocular, canthal, nasal and supralabial shields) are characters that, according to the results of our analysis, are diagnostic too.

Also shown is a high frequency of two rows of post-ocular shields encountered in Nikolsky's viper. The ratio of the height of second and third supralabials as 0.72 – 1.15 (0.93 ± 0.01) and ratio of height and width of rostral shield as 1.24 – 2.23 (1.65 ± 0.02) were given earlier (Vedmederja et al., 1986).

According to our results Nikolsky's viper has on average a larger full body length.

According to published data, the skull of Nikolsky's viper differs from the common adder skull by 11 measurements and 18 indexes in males and by 5 measurements and 9 indexes in females. They differ in shape of basisphenoid-basioccipital suture: it is clearly W-shaped in both forms, but suture angles are broader, less divided and less extended in *V. nikolskii* (Koldoba, 1983). The venom of Nikolsky's viper is colorless (yellowish in common adder), has less proteolysis activity (Orlov et al., 1990; Murzajeva et al., 1995) and has differences in albumen composition (Davljatov, 1985; Starkov and Utkin, 2001).

Our data on hemipenial morphology (Fig. 2) contradict those of Joger et al. (1997), which may be the consequence of imperfect methodology.

Nikolsky's viper is characterized by some peculiarities of coloration pattern in non melanistic specimens. It is worth mentioning that black coloration can appear in *V. berus berus* populations independently (Forsman, 1993; Monney et al., 1995; Völkl and Thiesmeier, 2002). Inter-

TABLE 1. Variation of Selected Morphological Characters in Populations of *Vipera berus nikolskii* (16 samples), *Vipera berus berus* (22 male and 23 female samples), and *Vipera berus* ↔ *nikolskii* (11 samples), Given as 95% Section of Mean Variability

	<i>V. b. nikolskii</i>		<i>V. berus berus</i>		<i>V. berus berus/V. berus nikolskii</i>	
Ventralia ♀♀	153 – 154.2		147.4 – 149.7		149.7 – 153.3	
Ventralia ♂♂	149.7 – 151.6		143.6 – 145.3		146.5 – 148.5	
Subcaudalia ♀♀	32.0 – 33.3		30.4 – 31.8		31.8 – 32.9	
Subcaudalia ♂♂	40.7 – 42.3		37.8 – 39.9		39.4 – 41.4	
Squamae dorsalis ♀♀	19, 0.5%; 20, 1.5%; 21, 74.0%; 22, 7.2%; 23, 16.8%	21.2–21.7	19, 11.4%; 20, 3.5%; 21, 79.0%; 22, 4.4%; 23, 1.7%	20.6–21.0	19, 4.7%; 21, 81.3%; 22, 6.3%; 23, 7.8%	21.0–21.5
Squamae dorsalis ♂♂	21, 77.3%; 22, 6.7%; 23, 16%	21.3–21.6	19, 17.5%; 20, 2.8%; 21, 71.6%; 22, 3.8%; 23, 4.3%	20.5–20.9	19, 2.2%; 20, 1.1%; 21, 82.3%; 22, 3.3%; 23, 11.1%	21.0–21.5
Labialia ♀♀	9.0 – 9.2		8.6 – 8.8		8.6 – 9.0	
Labialia ♂♂	8.8 – 9.1		8.6 – 8.8		8.7 – 8.9	
Sublabialia ♀♀	10.7 – 11.0		9.9 – 10.3		10.3 – 10.8	
Sublabialia ♂♂	10.6 – 10.8		10.1 – 10.4		10.2 – 10.6	
Circumocularia ♀♀	9.5 – 10.0		9.2 – 9.7		8.8 – 9.8	
Circumocularia ♂♂	9.5 – 10.1		9.2 – 9.6		9.0 – 9.7	
Subocularia ♀♀	1, 31.4%; 1–1.5, 14.4%; 1.5, 28.1%; 1.5–2, 11.8%; 2, 14.4%	1.3 – 1.6	1, 83.8%; 1–1.5, 8.1%; 1.5, 6.9%; 1.5–2, 0.6%; 2, 0.6%	1.0 – 1.1	1, 67.2%; 1–1.5, 11.5%; 1.5, 13.1%; 1.5–2, 1.6%; 2, 6.6%	1.1–1.3
Subocularia ♂♂	1, 62%; 1–1.5, 12%; 1.5, 17.2%; 1.5–2, 2.1%; 2, 6.8%	1.1 – 1.3	1, 97.4%; 1–1.5, 1.3%; 1.5, 1.3%	1.0	1, 88.4%; 1–1.5, 7.0%; 1.5–2, 1.2%; 2, 3.5%	1.0–1.1
Intercanthalia ♀♀	8.1 – 9.9		8.7 – 10.4		8.5 – 10.3	
Intercanthalia ♂♂	7.6 – 9.4		8.3 – 10.0		7.4 – 10.9	
Parafrofrontalia ♀♀	7.6 – 8.9		7.6 – 8.5		7.4 – 8.6	
Parafrofrontalia ♂♂	7.0 – 8.4		7.3 – 8.1		6.9 – 7.7	
Lorealia ♀♀	4.1 – 4.7		2.9 – 3.2		3.4 – 4.1	
Lorealia ♂♂	3.3 – 3.7		2.5 – 2.9		2.5 – 3.3	
Lfr/Ltfr	0.77 – 1.87		0.87 – 1.66		0.45 – 1.78	

For Sq. and S.oc. given frequencies of different states of the character have been pooled for all samples.

estingly, it is absence of typical sexual dichromatism in dorsum pattern.

An important result of the analysis is a strong heterogeneity within both groups of vipers. Well distinguished populations and populations exhibiting a tendency to similarity with another taxon are represented. Some populations have intermediate positions and can not be attributed to any taxa. In our opinion, this situation results from introgressive hybridization. A successful hybridization in captivity was shown earlier (Zinenko, 2003; Kurilenko, 2003). In some cases a sharp transition between two forms was observed in the contact zone. In Cherkassy Oblast', near Kanev, a *berus*-like adder inhabits the left bank of the Dnepr River and Nikolsky's viper inhabits the right bank. In Sumy Oblast', Putivl District both forms live on the same bank of the Seym river, but in different landscapes. However, traces of introgression are present in both cases (Zinenko and Ruzhilenko, 2003; our data). The broad intergradational zone between these taxons covers territory from the middle part of Dnepr in Ukraine to the Volga basin in Russia and includes samples from a third group (see *Appendix*). The intermediate position of vipers from this area has been reported by other authors (Sokolov, 1979; Murzaeva et al., 1995; Bakiev et al., 2000; Peskov et al., 2003; Starkov and Utkin, 2001, 2003; Zinenko and Ruzhilenko, 2003).

The most morphologically specific vipers were recorded far from contact zone with *V. berus berus*. Among samples of vipers studied by us, snakes from Khopyor River in Saratov Oblast' (Russia) and Kirovograd Oblast'

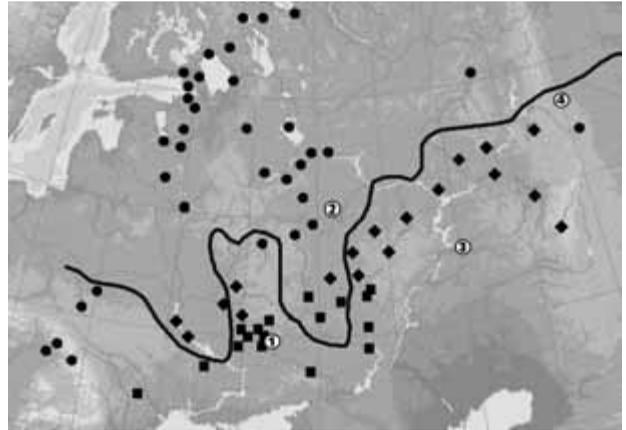


Fig. 3. Distribution *V. b. nikolskii* and *V. b. berus* in the Eastern Europe. ●, localities of *V. b. berus*; ■, *V. b. nikolskii*; ◆, populations with mixed morphological characters. Black solid line indicates southern border of the glacial landscapes (maximal distribution of the Dnepr Glaciation). 1, Ukraine, Kharkov vicinities, between Bezludovka and Vasitschevo, Udy river, terra typica of *Vipera nikolskii* Vedmederja, Grubant et Rudaeva, 1986; 2, Russia, Vladimir Oblast', near Gusevo, Cherseevo village, terra typica of *Vipera berus* var. *sphagnosa* Krassawzeff, 1932; 3, Russia, Samara, terra typica of *Coluber melanis* Pallas, 1771; 4, Russia, Sverdlovskaya Oblast', near Blagodot settl., Kushva st., terra typica of *Coluber scytha* Pallas, 1773.

(Ukraine) have very well expressed morphological characters of Nikolsky's viper. In contrast, clear populations of the common adder inhabit the northwestern part of the East European Plain. The big rivers like Dnepr, Seym, and Volga crossing the Russian Plain in a direction from north to south serve as dispersal routes for vipers. Generally,

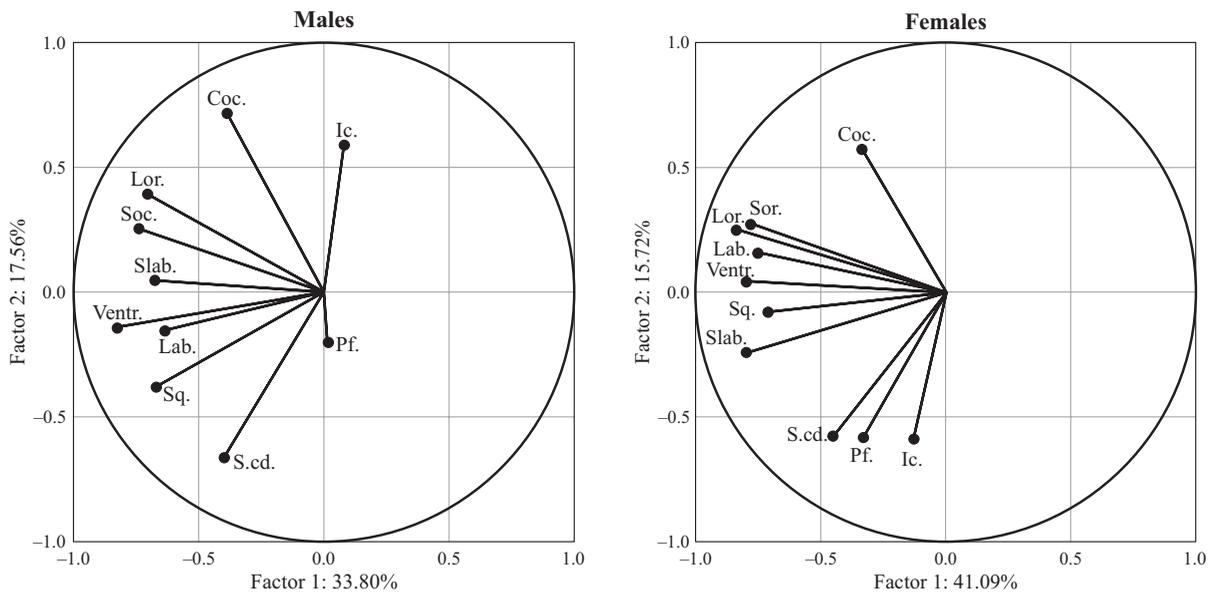


Fig. 4. Projection of characters on the first two factor's plane. Characters abbreviations are the same as in the text.

basins of these rivers are inhabited by vipers with mixed morphological characters of both forms.

A broad zone of intergradation of two vipers is evidence of unhampered interbreeding of these taxa. A genetic similarity of these vipers was confirmed by successful hybridization in captivity and mtDNA structure data (Joger et al., 1997). Morphological and genetic similarity and broad zone of intergradation allow consideration of a Nikolsky's viper only as a subspecies of *Vipera berus*.

TAXONOMICAL COMMENTS

The names proposed by Pallas cannot be applied because terra typica of *Coluber melanis* and terra typica of *Coluber scytha* are located outside the main distribution of Nikolsky's viper, in the zone inhabited by vipers with mixed morphological features. In 1870, K. Pengo used two names. He described a black-colored female (*Pelias berus* var. *nigra*) and it is normally colored new-borns (*Pelias berus* var. *varia*) and concluded that both black and typical vipers are color morphs and belong to one species. The name *nigra* is the primary junior homonym in the genus *Vipera* Laurenti and was preoccupied by *Vipera aspis* var. *nigra* Bonaparte, 1834 (recently, junior synonym of *Vipera aspis aspis*) (Mertens, Wermuth, 1960). An analogous situation with *varia*-name is that used in combinations for the designation of different varieties of the common adder. *Vipera berus* var. *sphagnosa* was described by B. A. Krassawzeff from territory (Cherseevo village, near Gusevo town, Ivanovo Oblast' — recent Vladimir Oblast') located far from Nikolsky's viper range. Invalidity of the name *prester* was shown by Vedmederja et al. in 1986. *Pelias berus* var. *ater* cited in the work of A. Andrzejowski (1832) also cannot be applied as a valid name for Nikolsky's viper. In 1820 Merrem used *ater* for the description of coloration of the melanistic common adder, but not as a specific name. A. Andrzejowski in his work mixed all black adders under the name *Vipera prester* (= *Pelias berus* var. *ater*). Moreover, this name is a junior synonym of *Vipera atra* Meisner, 1820 (= *Vipera aspis*) (Mertens and Wermuth, 1960). Therefore we should conserve the name proposed by Vedmederja, Grubant, and Rudaeva in 1986:

Vipera berus nikolskii VEDMEDERJA, GRUBANT ET RUDAEVA, 1986

Pelias berus — Merrem, 1820: 148 (part.); Schreiber, 1875: 202 (part.).

Vipera berus — Czernay, 1851: 279; Strauch, 1868: 287 (part.); Strauch, 1873: 206 (part.); Lindholm, 1902: 49; Schreiber, 1912: 614 (part.); Brauner, 1904: 30 (part.);

Tarashchuk, 1950: 164; Pashchenko, 1955: 132 (part.); Bruno and Maugeri, 1990: 191 (part.).

Vipera prester — Eichwald, 1830: 234 (part.); Andrzejowski, 1832: 337 (part.); Krynicki, 1837: 61; Grubant et al., 1973: 71.

Pelias berus var. *ater* — Andrzejowski, 1832: 337 (part.).

Pelias prester — Dwigubsky, 1832: 29.

Viperus berus (sic!) — Czernay, 1850: 30; Kheruvimov et al., 1977: 44.

Vipera berus var. *prester* — Kessler, 1853: 92.

Pelias berus var. *nigra* — Pengo, 1870: 17.

Pelias berus var. *varia* — Pengo, 1870: 17.

Vipera praester (sic!) — Brauner, 1904: 30.

Vipera berus var. *praester* (sic!) — Brauner, 1906: 7.

Coluber berus berus — Nikolsky, 1916: 227 (part.); Ognev and Worobiev, 1923: 250; Ptushenko, 1934: 49.

Coluber berus morpha prester — Ognev and Worobiev, 1923: 250.

Vipera berus prester — Krassawzeff, 1932: 80; Tarashchuk, 1959: 231.

Vipera berus berus — Kren, 1939: 187; Tsemsh, 1939: 110; Mertens, Müller, 1940: 55; Terentyev and Chernov, 1949: 271 (part.); Mertens and Wermuth, 1960: 198; Bannikov et al., 1971: 294 (part.); Steward, 1971: 164 (part.); Street, 1979: 194 (part.); Welch, 1983: 86; Brodmann, 1987: 101 (part.).

Vipera nikolskii Vedmederja et al., 1986: 84; Golay, 1993: 287; Tabachishin et al., 1996: 60; Nilson and Andrén, 1997: 396; Ananjeva et al., 1998; Bakiev et al., 1999: 4; Franzen and Heckes, 2000: 63; Pavlov, 2000: 48; Bozhansky, 2001: 348.

Vipera barani — Stumpel-Rienks, 1990: 114.

Vipera berus nikolskii — Joger et al., 1997: 193.

Holotype and terra typica. MNKNU. 14703. adult female, near Bezludovka and Vasishchevo, Kharkov vicinities, Udy river, Ukraine, leg: K. Pengo, 1867.

Paratypes. MNKNU 14703.12 juv.,; ZISP 3376.2 juv. (now lost); ZISP 22012.2 juv. All from the same locality.

Diagnosis. Black colored subspecies of *Vipera berus* with increased number of loreal, ventral, and subcaudal shields. One or two rows of the shields behind eye and between supralabials and eye. Deeply forked hemipenes are elongated (Table 2).

Redescription. L._{min-max} ad 440–760, L. ♂♂ (*n* = 56) 440–645 (512 ± 6.8) mm, L. ♀♀ (*n* = 72) 450–760 (576 ± 6.7) mm; L.cd._{min-max} ad 37–105, L.cd. ♂♂ (*n* = 55) 70–105 (86.2 ± 1.3) mm, L.cd. ♀♀ (*n* = 71) 37–99 (74.1 ± 1.1) mm; Sq._{min-max} 19–23, Sq. ♂♂ (*n* = 251) 21–23 (21.40 ± 0.05), Sq. ♀♀ (*n* = 206) 19–23 (21.41 ± 0.06); Ventr._{min-max} 140–160, Ventr. ♂♂ (*n* = 271) 140–160 (150.03 ± 0.18); Ventr. ♀♀ (*n* = 215)

143 – 160 (153.39 ± 0.22); S.cd._{min-max} 26 – 50, S.cd. ♂♂ (n = 262) 33 – 50 (41.21 ± 0.15), S.cd. ♀♀ (n = 203) 26 – 38 (32.82 ± 0.17); Lab._{min-max} 5 – 11, Lab. ♂♂ (n = 251) 5 – 11 (8.96 ± 0.03), Lab. ♀♀ (n = 210) 8 – 11 (9.07 ± 0.03); Slab._{min-max} 7 – 13, Slab. ♂♂ (n = 199) 7 – 13 (10.67 ± 0.05), Slab. ♀♀ (n = 158) 9 – 13 (10.79 ± 0.06); C.oc._{min-max} 7 – 12, C.oc. ♂♂ (n = 187) 7 – 12 (9.73 ± 0.66), C.oc. ♀♀ (n = 147) 7 – 12 (9.61 ± 0.07); S.oc. ♂♂ (n = 188) 1 – 2 (1.21 ± 0.02); S.oc. ♀♀ (n = 151) 1 – 2 (1.39 ± 0.03); Ic._{min-max} 1 – 19, Ic. ♂♂ (n = 188) 1 – 18 (8.96 ± 0.24), Ic. ♀♀ (n = 147) 2 – 19 (8.81 ± 0.25); Pf._{min-max} 0 – 15, Pf. ♂♂ (n = 188) 0 – 15 (7.64 ± 0.17), Pf. ♀♀ (n = 147) 0 – 14 (8.27 ± 0.19); Lor._{min-max} 1 – 7, Lor. ♂♂ (n = 186) 1 – 6 (3.39 ± 0.07), Lor. ♀♀ (n = 143) 2 – 7 (4.25 ± 0.08); Lfr/Ltfr_{min-max} 0.77 – 1.78, Lfr/Ltfr ♂♂ (n = 133) 0.77 – 1.78 (1.39 ± 0.01), Lfr/Ltfr ♀♀ (n = 109); 1.09 – 1.78 (1.40 ± 0.01).

One-two shield rows between supralabials and eye; one-two shield rows behind the eye; head pholidosis pattern, shape, size, number and arrangement of scales is variable. Number of the scales around the eye is increased (Fig. 5). In comparison to *V. b. berus* hemipenes are large, with elongated apical lobes, well expressed spines, spine lines and basal hooks.

Adult coloration is totally black, only the tip of tail is pigmented by yellow, orange or whitish. In some cases the adults, especially females, have light dots and spots on supralabials and ventrals and reddish-brown colored throat. Non melanistic individuals are rarely encountered. Brown-colored specimens with zigzag on the dorsum (weakly expressed sexual dichromatism), monochromatic brown-colored without zigzag and *berus*-like colored specimens are represented in some populations with a frequency of 10%. The coloration of young specimens is gravel, reddish-brown or deep-brown.

Variation. Individual variability in pholidosis characters is very high. Amongst different populations average values of characters vary significantly. Similarity between *V. b. berus* and *V. b. nikolskii* increases from southern-east to northern-west. Snakes from intermediate populations have mixed morphological characters. The venom composition is mixed in some populations (Samara Oblast', Sumy Oblast') (Mursaeva et al., 1995; Starkov and Utkin, 2003).

Differences are known between western (Ukraine) and eastern (Russia, Volga Basin) populations in pholidosis and body size. Small body size in females and a reduced number of ventral shields are typical for the eastern population. A ratio of second and third upper labial heights was used by V. N. Grubant et al. (1973) as diagnostic character. This character shows wide geographical variability. This ratio is 0.73 – 1.00 for vipers from Kharkov and

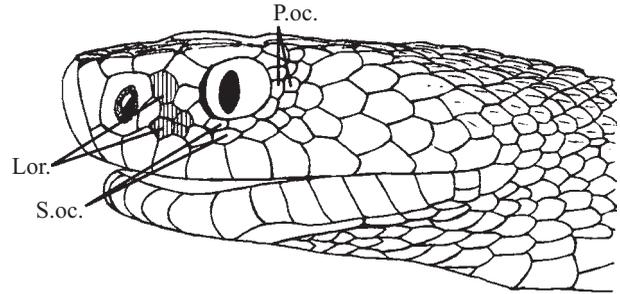


Fig. 5. Polymerized head scalation in *V. b. nikolskii*.

0.80 – 1.16 for individuals from Saratov Oblast' (Vedmederja et al., 1986; Tabachishin et al., 1996).

In the “pure” populations of *V. b. nikolskii* located far from contact zone with *V. b. berus*, all adults are totally black with exception of tail tip. In the hypothetical intergradational zone (Perm Oblast', Samara Oblast', Udmurtia, Tatarstan, northern part of Saratov Oblast', possibly Kursk Oblast'), the black-colored and light-colored individuals with zigzag live together (Ptushenko, 1934; Tarashchuk, 1950; Tabachishin et al., 1996; Al-Zavakhra, 1992; Pavlov, 2000; our data). The normally patterned snakes are not numerous. For example, in Tatarstan the non black coloration is presented in 4.5% (Al-Zavakhra, 1992). The white colored upper labials, reddish throat, light spots on the ventral and other light elements in coloration were registered in such a mixed population (Sokolov, 1979; Zinenko and Ruzhilenko, 2003). Moreover, a monochromatic grayish-beige colored specimen is known (ZISP 21295).

The coloration of young specimens varies from gravel to dark-brown with atro-olivaceus, saturate-fumosus or murinus background and atro-brunneus, atro-olivaceus or atro-cinnamomeus zigzag. Young males are brighter col-

TABLE 2. Comparison of Two Subspecies of *Vipera berus*

<i>Vipera berus berus</i>	<i>Vipera berus nikolskii</i>
0- – 70 % of adults are black, sexual dichromatism in the dorsal pattern is well expressed, juvenile coloration is grayish-brown	90 – 100% of adults are black, sexual dichromatism in the dorsal pattern of coloured specimens is weakly expressed, juvenile coloration is reddish-brown
1 row of scales between supralabial shields and eye	1 – 2 rows of scales between supralabial shields and eye
19 – 21 rows of scales around mid-body	21 – 23 rows of scales around mid-body
Average number of ventral shields is 144 – 149	Average number of ventral shields is 150 – 155
Hemipenes are relatively short, shallowly-forked	Hemipenes are large, deeply forked, with elongated apical lobes
Venom is yellowish	Venom is colorless

ored, with grayish background, females are light-brown. Iris coloration varies from reddish (*rufescens*), chestnut (*spadiceus*) and brown (*rubiginosus*, *argillaceus*, *prunicolor*) in the upper part to *umbrius*, *atro-castaneus*, *nikotianus*, *bistraceus*, *sordide violaceus* and *niger* in the lower part.

In the second year young snakes have cacao-fuscus color with feebly marked zigzag and light dots on the supralabials and ventrals. At the age of 3 – 4 years they get adult coloration.

Sexual dimorphism. Sexual dimorphism at *V. b. nikolskii* in coloration was reported for Tambov Oblast' (Kheruvimov et al., 1977). A chestnut-colored iris is typical in males and from ochre-yellow to reddish in females. The lower part of the tail tip is black in males and terracotta-pale or sepia with terracotta-pale dots in females. In the Kharkov Oblast' and in the vicinities of Kanev the tail tip is bright colored (yellow, orange) in females, and whitish in males. Males with completely black tail are rare. Iris in snakes from Kharkov vicinities is umber, chestnut- and deeply chestnut-colored in males and reddish-brown in females. Like in the Tambov Oblast', males from Kanev have chestnut-colored iris and females have ochre-yellow, reddish and reddish-pale-colored iris.

Non melanistic, normally colored males and females with zigzag may have no differences in dorsal coloration. Hence sexual dimorphism of the coloration pattern in *V. b. nikolskii* is weakly expressed and connected with some details of coloration. Unlike *V. b. berus* (Bruno and Maugeri, 1990; Shine and Madsen, 1994), in *V. b. nikolskii*, the phenomenon of sexual dichromatism is well visible in juveniles only.

Sexual dimorphism is present in pholidosis characters. Like in *V. b. berus*, females of *V. b. nikolskii* have an increased number of ventralia, subcaudalia, intercanthalia, parafrontalia, lorealia, squamae dorsalis, supralabialia, sublabialia, and rows of subocularia.

Range. *V. b. nikolskii* inhabits the south and south-eastern part of the East European Plain, from Podolia Hills in the west to Cis-Volga Hills. Its range includes territories of Moldova, probably Romania (Fuhn and Vancea, 1961), Central Ukraine, and southern part of European Russia. In Ukraine it occurs in Odessa Khmel'nitsky, Vinnitsa, Kirovograd, Cherkassy, Kiev, Poltava, Sumy, Kharkov, Lugansk, and Donetsk Oblast's; in Russia — in Kursk, Belgorod, Voronezh, Tambov, Penza, Saratov, and Volgograd Oblast's.

The northern limit of distribution agrees with description of Vedmederja et al. in 1986. However new records of *V. b. nikolskii*, on the left bank of the Kodyma River in Balta District on the north of Odessa Oblast' (Tabachishin et al., 2003), near Shepetovka in Khmel'nitsky Oblast' of

Ukraine, in Orhei Oblast' of Moldova expand the border of distribution to the west.

The intergradation zone between *V. b. berus* and *V. b. nikolskii* covers the broad territory of eastern Ukraine and Central and Eastern European Russia. Vipers with intermediate morphological characters are recorded for the territory of Cherkassy, Kiev, Chernigov, and Sumy Oblast's in Ukraine and Kursk, Tambov, Samara, Perm, Penza Oblast's, Tatarstan, Chuvashia, Udmurtia, and Bashkortostan in Russia. Northern Ukraine, Belarus', Northern and North-Eastern European Russia are inhabited by *V. b. berus*.

Habitats. *V. b. nikolskii* inhabits preglacial landscapes to south from the border of maximum glaciation in the Eastern European Plain. The distributional range embraces a woodland steppe zone, sometimes *V. b. nikolskii* penetrates to steppe zone along the river valleys (Donets River, Don River) and the hills (Donetskii Kryazh).

Distribution of *V. b. nikolskii* correlates with location of forest refugia that were existing during maximum stage of Valdai Glaciation at 18 – 20 thousand years ago in the middle part of Don and Volga river basins. Some of these refugia placing on hills were reservations of genetic diversity of *Vipera berus*.

Some authors (Andrén and Nilson, 1981; Madsen and Stille, 1988) proposed a hypothesis that the black morph of *V. berus* has some preferences under cold climate conditions in comparison with the normally colored one. However there are facts contradicting this hypothesis. The warmest part of *V. berus* range is inhabited only by melanistic adders. It may be supposed that the change to black coloration took place during the Ice Age as an adaptation to rough conditions in preglacial landscapes.

Dispersal routes. Apparently, the south-eastern part of East European Plain was occupied by *V. b. nikolskii* before the Dnepr Glaciation. The territory that was created from Dnepr and subsequent Valdai Glaciation was occupied by taiga forests and recent *V. b. berus* accordingly. To the west, the fauna dispersed from Eastern refugia. The relict *V. b. nikolskii* together with other species of the East-European faunistic complex were able to survive in refugia in the Middle Volga, Middle Don and Donets rivers, Lower Dnepr river, and Prut river valley.

Remarks. Two different morphotypes in *V. berus* are known. Typical adders have a splay edge of the snout, decreased number of the scales in pileus, one row of the shields around eye. The second morphotype is characterized by flat upper surface of the snout, sometimes the snout is slightly turned up; two rows around the eye and increased number of small shields on pileus (Gasc and Gourmain, 1968). Both morphotypes are presented in *V. b. nikolskii* populations. Also this polymerized *aspis*-like type of squamation in *V. b. bosniensis* is common. A

pileus pattern with numerous small shields is rarely observed in *V. b. berus* (Benson, 1999).

Probably, *V. b. nikolskii* is closely related to Balkan subspecies *V. b. bosniensis*. These subspecies exhibit a tendency to increase of number of prefrontal, loreal and postocular shields. In contrast to *V. b. berus*, both *V. b. nikolskii* and *V. b. bosniensis* have more intense and brighter body coloration. It is important to notice that these relict subspecies survived in Ice Age refugia and retained some ancestral characters. Moreover, *V. b. bosniensis* is allied to species of *V. aspis*-complex as distinct from *V. b. nikolskii*. Separately, we should mention presumptive close affinities between *V. b. nikolskii* and *V. barani*. Good morphological differences and genetic distance between these species were shown early after molecular analysis (Joger et al., 1997) and completed analysis of morphological characters (Franzen and Heckes, 2000) of both forms.

Acknowledgments. We are indebted to Natalia B. Ananjeva and Tatjana I. Kotenko for valuable discussion and editing of this manuscript, Valentina F. Orlova, Eugeny A. Dunaev, and Eugeny M. Pisanets for offering of collections materials and V. Vedmederija and A. Barabanov for comments. This work was funded by grant of Russian President of Russian Federation for supporting of the Leading Scientific Schools No. 1647.2003.

REFERENCES

- Al-Zavakhra Kh. A.** (1992), *Snakes of Tatarstan. Unpublished PD Thesis*, Kazan [In Russian]
- Ananjeva N. B., Borkin L. Y., Darevsky I. S., and Orlov N. L.** (1998), *Encyclopedia of Nature of the Russia. Amphibians and Reptiles*, ABF, Moscow [in Russian].
- Andrén C. and Görán N.** (1981), "Reproductive success and risk of predation in normal and melanistic color morphs of the adder, *Vipera berus*," *Biol. J. Linn. Soc.*, **15**, 235 – 246.
- Andrzejowski A.** (1832), "Reptilia inprimis Volhyniae, Podoliae et Gubernii Chersonensis," in: *Nouveaux Memoires de la Societe Imperiale des Naturalistes de Moscou. Tome II*, pp. 319 – 346.
- Atlas of the USSR** (1962), Moscow [in Russian].
- Atlas of the USSR** (1984), Moscow [in Russian].
- Bakiev A. G., Malenev A. L., Peskov A. N., Pavlov A. V., and Gridnev D. V.** (1999), "About species status of *Vipera nikolskii*," in: *Abstrs. of the 2nd Herpetol. Conf. of Volga Basin*, Tolyatti, pp. 4 – 5 [in Russian].
- Bakiev A. G., Malenev A. L., Peskov A. N., and Gridnev D. V.** (2000), "Morphological description of the adders in forest park zone of Samara," in: *Actual Problems of Herpetology and Toxinology. Collected Papers. No. 4*, Tolyatti, pp. 3 – 8 [in Russian].
- Bannikov A. G., Darevsky I. S., and Rustamov A. K.** (1971) *Amphibians and Reptiles of the USSR*, Mysl', Moscow [in Russian].
- Bannikov A. G., Darevsky I. S., Ishchenko V. G., Rustamov A. K., and Szczerbak N. N.** (1977), *Guide to Amphibians and Reptiles of the USSR*, Prosveshchenie, Moscow [in Russian].
- Benson P. A.** (1999), "Identifying individual adders, *Vipera berus*, within an isolated colony in east Yorkshire," *Br. Herpetol. Soc. Bull.*, **67**, 21 – 27.
- Bondartsev A. S.** (1954), *Colors Scale*, Izd. AN SSSR Moscow – Leningrad.
- Bozhansky A. T.** (2001), "Nikolsky's Viper, *Vipera nikolskii* Vedmederija, Grubant et Rudaeva, 1986," in: *Red Data Book of the Russian Federation. Animals*, Astrel', Moscow, pp. 348 – 349 [in Russian].
- Brandt J. F. and Ratzeburg J. T. C.** (1829), *Medizinische Zoology. Bd. 1*, Berlin, pp. 1 – 198.
- Brauner A.** (1904), *Tableaux pour la détermination des reptiles et batraciens de la Crimée et des steppes de la Russie Européenne. Du Bulletin du Club Alpin de Crimée*, Nos. 3 – 9 [in Russian].
- Brauner A.** (1906), Dritte vorläufige Mitteilungen über Reptilien und Amphibien der Suwalk – Minsk – Podolsk – Tschernigow – Cherson – Ekaterinoslaw – Gouvernements, Bessarabien und des Dnjepr — Kreises des Taurischen Gouvernements," in: *Bull. Soc. Nat. Noworossiysk. Vol. XXVIII*, pp. 1 – 17.
- Brodmann P.** (1987), *Die Giftschlangen Europas und die Gattung Vipera in Afrika und Asien*, Kümmerly und Frey, Bern.
- Bruno S. and Maugeri S.** (1990), *Serpenti d'Italia e d'Europa*, Milano.
- Charleman E. V.** (1917), "Note on the faune of Reptilia and Amphibia of the neighbourhood Kiev," in: *Contributions to the Knowledge the Fauna of South West Russia Published by the Kesslerian Ornithological Society of Kiev*, Kiev, pp. 1 – 17.
- Czernay** (1850), *Reise über die Fauna von Charkow*, Charkow.
- Czernay** (1851), "Beobachtungen gesammelt auf Reisen im Charkowschen und den angrenzenden Gouvernements in den Jahren 1848 und 1849," *Bull. Soc. Imp. Nat. Moscou*, **24**(1), 269 – 282.
- Davlyatov Y. D.** (1985), "Some results of study of the snakes venom variability," in: *Abstrs. of the 6th Herpetol. Conf. "The Problems of Herpetology"*, Leningrad, p. 65. [in Russian].
- Dwigubsky I.** (1832), *Natural History of Animals of Russian Empire*, Izd. Mosk. Univ., Moscow [in Russian].
- Forsman A.** (1993), "Growth rate in different color morphs of the adder, *Vipera berus*, in relation to yearly weather variation," *Oikos*, **66**, 279 – 285.
- Forsman A., Merila J., and Lindell L.** (1994), "Do scale anomalies cause differential survival in *Vipera berus*?" *J. Herpetol.*, **28**(4), 435 – 440.
- Franzen M. and Heckes U.** (2000), "*Vipera barani* Böhme & Joger, 1983 aus dem östlichen Pontus-Gebirge, Türkei: Differentialmerkmale, Verbreitung, Habitate," *Spixiana*, **23**(1), 61 – 70.
- Fuhn I. E. and Vancea S.** (1961), *Fauna Republicii Populare Romine. Reptilia. Vol. XIV. Fasc. 2*.
- Garanin V. I.** (1977), "On the ecology of adders in Tatarskaya ASSR and contiguous parts of Volga-Kama territory," in: *Okhrana Prirody i Biotseologiiya [Conservancy and Biotechnology]*, Kazan, pp. 76 – 79 [in Russian].

- Gasc J.-P. and Gourmain J.** (1968), "Les motifs dominants dans la disposition des plaques cephaliques chez *Vipera aspis*," *Bull. Mus. Natl. D'Hist. Nat. 2 Ser.*, **40**, 102 – 107.
- Golay Ph., Smith H. M., Broadley D. G., Dixon J. R., McCarthy C., Rage J.-C., Schätti B., and Toriba M.** (1993), *Endoglyphs and Other Major Venomous Snakes of the World. A Checklist*. Azemiops, Herpetological Data Center.
- Grubant V. N., Rudaeva A. V., and Vedmederja V. I.** (1973), "On the systematic position of black morph of common adder," in: *Abstrs. of the 3rd Herpetol. Conf. "The Problems of Herpetology"*, Leningrad, pp. 68 – 71 [in Russian].
- International code of Zoological nomenclature** (2000), Fourth Edition, St.-Petersburg.
- Joger U., Lenk P., Baran I., Böhme W., Ziegler T., Heidrich P., and Wink M.** (1997), "The phylogenetic position of *Vipera barani* and of *V. nikolskii* within the *Vipera berus* complex," in: *Herpetologia Bonnensis*, pp. 185 – 194.
- Kessler K. F.** (1853), "Amphibians and reptiles," in: *Estestv. Ist. Gub. Kievs. Uchebn. Okruga*. No. 5 [in Russian].
- Kheruvimov V. D., Sokolov A. S., Sokolova L. A.** (1977), "On sex and age determination in *Viperus berus* L.," *Zool. Zap.*, No. 6, pp. 39 – 44 [in Russian].
- Koldoba I. V.** (1983), *Comparative Analysis of Ecological and Morphological Skull Characters in some Vipera Species of Fauna of USSR* (*Vipera berus berus*, *Vipera kaznakovi*, *Vipera ursini renardi*). *Candidate's Thesis*, Izd. KhGU, Khar'kov [in Russian].
- Krassawzeff B. A.** (1932), Die Torfmoos-Viper, eine neue Varietät (*Vipera berus sphagnosa* var. *nova*)," *Zool. Anz.*, **101**(3/4), 80 – 81.
- Kren A. K.** (1939), "Vertebrates of 'Les-na-Vorskla' Reserve," in: *Uchenye Zap. LGU. No. 28. Ser. Biol. Nauk. Issue 7*, Leningrad, pp. 184 – 206 [in Russian].
- Krynicky J.** (1837), "Observationes quaedam de reptilibus indigenis," in: *Bull. Soc. Imp. Nat. Moscou. No. 3*, pp. 46 – 69.
- Lepechin I.** (1772), *Tagebuch der Reise durch verschiedene Provinzen des Russischen Reiches in den Jahre 1770*, St. Petersburg, [in Russian].
- Lindholm W. A.** (1902), "Beiträge zur Biologie einiger Reptilien des Europäischen Russlands," in: *Zool. Garten*, **XLIII**(1 – 2), 20 – 26, 41 – 56.
- Madsen T. and Stille B.** (1988), "The effect of size dependent mortality on color morphs in male adders, *Vipera berus*," *Oikos*, **52**, 73 – 78.
- Monney J.-C., Luiselli L., and Capula M.** (1995), "Correlates of melanism in a population of adders (*Vipera berus*) from the Swiss Alps and comparisons with other alpine populations," *Amphibia-Reptilia*, **16**(3), 323 – 330.
- Markov K. K., Lazukov G. I., and Nikolayev V. A.** (1965), *The Quaternary Period. Vol. 1. The Territory of the USSR*, Moscow [in Russian].
- Markova A. K., Simakova A. N., and Puzachenko A. U.** (2002), "Ecosystems of Eastern Europe during Late Glacial Maximum of Valdai Glaciation (24 – 28 thousand years ago) on floristic and teriological data," *Dokl. RAN*, **386**(5), 681 – 685 [in Russian].
- Mertens R. and Müller L.** (1940), "Die Amphibien und Reptilien Europas," in: *Abhandl. Senckenber. Naturf. Ab.* 451, Frankfurt am Main.
- Mertens R. and Wermuth H.** (1960), *Die Amphibien und Reptilien Europas*, Verlag Waldemar Kramer, Frankfurt am Main.
- Merrem B.** (1820), *Versuch eines System der Amphibien*, Marburg.
- Murzajeva S. V., Malenev A. L., and Bakiev A. G.** 1995. Differences in proteolytic activity of venom of the common adder from different localities," in: *Abstrs. of the First Conf. of Herpetol. of Volga Basin*, Tolyatti, pp. 37 – 38 [in Russian].
- Nikolsky A. M.** (1916), *Fauna of Russia and Adjacent Countries. Vol. 2. Reptilia. Ophidia*, Petrograd [in Russian].
- Nilson G. and André C.** (1997), "*Vipera nikolskii*," in: *Atlas of Amphibians and Reptiles in Europe*, Paris.
- Ognev S. I. and Worobiev K. A.** (1923). *The Fauna of the Terrestrial Vertebrates of the Governement of Woronesh*, Novaya Derevnja, Moscow [in Russian].
- Orlov B. N., Gelashvili D. B., and Ibragimov A. K.** (1990), *Poisonous Animals and Plants of USSR*, Vysshaya shkola, Moscow [in Russian].
- Pallas P. S.** (1771), *Reise durch verschiedene Provinzen des Russischen Reiches. Th. 1*, Kaiserlichen Akademie der Wissenschaften, St. Petersburg.
- Pallas P. S.** (1773), *Reise durch verschiedene Provinzen des Russischen Reiches. Th. 2*, Kaiserlichen Akademie der Wissenschaften, St. Petersburg.
- Pavlov A. V.** (2000), "On results of investigation of vipers in Tatarstan Republic," in: *Modern Herpetology. Vol. 1*, pp. 47 – 51 [in Russian].
- Paleogeography of Europe during the last one hundred thousand years** (Atlas-monograph) (1982), Nauka, Moscow [in Russian].
- Pashchenko U. Y.** (1955), *Guide to Amphibians and Reptiles*, Radyanska shkola, Kiev [in Ukrainian].
- Pengo K.** (1870), On generic and specific characters of the adder (*Pelias* (*Vipera* Daud.) *berus* Merrem)," in: *Travaux de la Societe des Naturalistes a l'Universite Imperiale de Kharkov. Vol. 2* [in Russian].
- Ptushenko E. S.** (1934), "Terrestrial vertebrates of the Kursk Oblast'. Amphibia and Reptilia," in: *Bull. Soc. Nat. Moscou. Sect. Biol. T. XLIII. Livr. 1*, pp. 35 – 51 [in Russian].
- Schreiber E.** (1875), *Herpetologia Europaea*, Braunschweig.
- Schreiber E.** (1912), *Herpetologia Europaea*, Jena.
- Shine R. and Madsen T.** (1994), "Sexual dichromatism in snakes of the genus *Vipera*: a review and a new evolutionary hypothesis," *J. Herpetol.*, **28**(1), 114 – 117.
- Shlyakhtin G. V., Ruzanova I. E., Ljubuschenko S. U., and Zavyalov E. V.** (2001). On specification of southern border of the Nikolsky's viper distribution (*Vipera nikolskii*) in Southwestern Russia," in: *The Problems of Herpetology. Proc. of the First Meeting of The Nikolsky Herpetol. Soc.*, Pushchino – Moscow, pp. 347 – 349 [in Russian].
- Sokolov A. S.** (1979), "On coloration of common adder in Tambov Oblast'," in: *Abstrs. of the Conf. of Zoologists from Teacher's Training Colleges. New Problems of Zoological Science and their Reflection in Higher Education. Part 2*, Stavropol', pp. 340 – 342 [in Russian].
- Sonnini C. S. and Latreille P. A.** (1802), *Histoire Naturelle des Reptiles. T. 3*, Paris.
- Sonnini C. S. and Latreille P. A.** (1830), *Histoire Naturelle des Reptiles. T. 3*, Paris.

- Starkov V. G. and Utkin Y. N.** (2001), "Comparison of venoms of the *Vipera* genus snakes on the data of cationic exchange chromatography," in: *Actual Problems of Herpetology and Toxinology*. No. 5, pp. 88 – 89 [in Russian].
- Starkov V. G. and Utkin Y. N.** (2003), "New data on systematic status of the vipers in Samara Oblast'," in: *Proc. of Third Regional Conf. of Herpetologists of Volga Basin*, Tolyatti, pp. 81 – 82 [in Russian].
- Steward J. W.** (1971), *The Snakes of Europe*, David and Charles, Newton Abbot, Devon.
- Strauch A. A.** (1868), "On venomous snakes inhabits in Russia," in: *Proc. of the First Congr. Russ. Naturalists*. Vol. 1, pp. 271 – 297 [in Russian].
- Strauch A. A.** (1873), "Die Schlangen des Russischen Reichs, in Systematischer und Zoogeographischer Beziehung," in: *Mem. Acad. Imp. Sci. St.-Petersbourg*. VII Ser. Tome XXI. No. 288.
- Street D.** (1979), *The Reptiles of Northern and Central Europe*, B. T. Batsford, London.
- Stumpel-Rienks S. E.** (1992), *Nomina Herpetofaunae Europaeae*, Aula-Verlag, Wiesbaden.
- Tabachishin V. G., Shlyakhtin G. V., Zavyalov E. V., Storozhilova D. A., and Shepelev I. A.** (1996), "Morphometric differentiation and taxonomic status of reptilian families Colubridae and Viperidae," in: *Fauna of Saratov Oblast. Coll. Papers*. Vol. 1. No. 2, pp. 39 – 70 [in Russian].
- Tarashchuk V. I.** (1950), "On the black vipers in the Kanev Biogeography Reserve," in: *Nauch. Zap. KGU*, 9(6), 164 – 165 [in Russian].
- Tarashchuk V. I.** (1959), *Fauna of Ukraine*. Vol. 7. *Amphibians and Reptiles*, Kiev [in Ukrainian].
- Terentyev P. V. and Chernov S. A.** (1949), *Guide to Reptiles and Amphibians*, Sovetskaya Nauka, Moscow [in Russian].
- Tsemsh I. O.** (1939), "On the sistematics and geographical distribution of reptiles and amphibians in Ukraine," in: *Univ. d'Etat de Kiev. Trav. Sci. Étudianis*. No. 4, pp. 103 – 117 [In Ukrainian].
- Vedmederja V. I., Grubant V. N., and Rudaeva A. B.** (1986), "On the name of the black adder in woodland steppe zone of European part of USSR," in: *Vestnik Khark. Univ.*, No. 288, Kharkov, pp. 83 – 85 [in Russian].
- Vedmederja V. I.** (1989), "Vipers of subgenus *Pelias*," in: *Instruction for Study of Amphibians and Reptiles*, Kiev, pp. 35 – 39 [in Russian].
- Völkl W. and Thiesmeier B.** (2002), *Die Kreuzotter: ein Leben in festen Bahnen?* Laurenti-Verlag, Bielefeld.
- Welch K. R. G.** (1983), *Herpetology of Europe and Southwest Asia: a Checklist and Bibliography of the Orders Amphisbaenia, Sauria, and Serpentes*, Malabar.
- Zinenko O. I.** (2003), "First generation hybrids between the Nikolsky's adder, *Vipera nikolskii*, and the common adder (Reptilia, Serpentes, Viperidae)," *Vestnik Zool. Kiev*, 37(1), 101 – 104 [in Russian].
- Zinenko O. I. and Ruzhilenko N. S.** (2003), "On the systematic position of adders inhabit the Kanev Natural Reserve territory," in: *Zapovidna Sprava v Ukraini*, Vol. 9. No. 1, pp. 51 – 55 [in Russian].

APPENDIX. The list of sampled localities

Vipera berus berus: Ukraine — Zakarpatskaya Oblast', Berehove and Vinogradiv Distr. (sample 1); Zakarpatskaya Oblast', V. Berezan Distr., Svalyava settl. and Perechin settl. (sample 2); Zakarpatskaya Oblast', Volovets and Turka Distr., Mezhhirya (sample 3); Zakarpatskaya Oblast', Rakhiv Distr. and Ivano-Frankivska, Verhovyna Distr. (sample 4); Volyn Oblast' (sample 5); Sumy Oblast', S. Buda Distr. (sample 16); Russia — Karelia, Segozero, Suojarvi Distr., Toyvola vill., Tolvojarvi vill., Sortavala, Risklan-sari is., Vygozero (sample 20); Leningradskaya Oblast', near Vyborg, near Zelenogorsk, Priozerskii Distr., near Vuoksa lake, near Petergof, Gatchina Distr., Siverskaya settl., Luga Distr., Lodeinoe Pole Distr., Gumbaritsy vill. (sample 9); Novgorod Oblast', Mosheno Distr., Kocherovo vill., Opochetskii Posad settl. (sample 14); Pskov Oblast', Porkhov Distr., Gvozdno vill., near Sebezh town, near Plusa st., Pechery Distr., near malye Kalki vill. (sample 6); Vologda Oblast', Darvinovskiy Resrve; Yaroslavl Oblast', Pereyaslavsky Distr., Pletschevo Lake, Rostov Distr.; Tver Oblast', Beloye Lake (sample 24); Moscow Oblast' (sample 33); Kostroma Oblast' (sample 37); Arkhangelsk Oblast', Onega Distr.; Komi, southern part; Tula Oblast', Venev Distr. and Zaokskii Distr. (sample 32); Ryazan Oblast'; Sverdlovskaya Oblast'; Tomsk Oblast' (sample 44); Belarus', Vitebsk Oblast' (sample 8); East Kazakhstan (sample 43); East Siberia (sample 45); Yakutia (sample 46);

Vipera berus nikolskii: Moldova, Strashensky Distr. (sample 7); Ukraine — near Kharkov, between Vasishchevo and Bezludovka (sample 25); Kharkov (sample 26); Kharkiv, northern vicinities (sample 27); Kharkov Oblast', Krasnokutsk Distr. (sample 21); Kharkov Oblast', Dergachi Distr. (sample 28); Kharkov Oblast', Chuguev Distr. and Pechenegi Distr. (sample 29); Kharkov Oblast', Zmiyev Distr. (sample 30); Kirovograd Oblast', Znamenka settl. (sample 15); Poltava Oblast' (sample 17); Sumy Oblast', Akhtyrka Distr. (sample 22); Lugansk Oblast' (sample 34); Russia — Kursk Oblast', Dmitriev Distr.; Belgorod Oblast' (sample 31); Voronezh Oblast', Khover Reseve (sample 35); Saratov Oblast', near Alekseevka, Arkadak Distr., near Semenovka vill. (sample 38); Volgograd Oblast', Don River, Log st., Mikhaylovka Distr., Medveditsa Riv.;

V. b. berus/V. b. nikolskii: Ukraine — Kiev Oblast', right bank of the Dnepr riv. (sample 10); Kiev Oblast', left bank of the Dnepr River (sample 11); Cherkassy Oblast', near Kanev, right bank of the Dnepr River (sample 12); Cherkassy Oblast', near Kanev, left bank of the Dnepr River (sample 13); Sumy Oblast', Putivl Distr., N. Sloboda settl. (sample 18); Sumy Oblast', Putivl Distr., Spadschina settl. (sample 19); Sumy Oblast', Sumy Distr. (sample 23); Russia — Tambov Oblast', near Kirsanov and Vorona River (sample 36); Chuvashia, Alatyry Distr., "Prisurskii" Reserve (sample 39); Udmurtia, Sumsi Distr., Kilmez vill., near Karakulino, Yashkur-Bodya Distr. (sample 41); Perm Oblast', Uktu st. and Kungur Distr., near Kishert settl. (sample 42); Penza Oblast', Lashma; Zemetchino Distr., Dolgovo settl.; near Samara (sample 40); Bashkortostan, Belaya River; Bashkortostan, Bashkirkii Reserve; Hybrids (sample 47).

MORPHOLOGICAL VARIATION AND SEX RATIO IN THE LEOPARD SNAKE (*Zamenis situla*) FROM SOZOPOL (BULGARIA)

J. Moravec¹ and W. Böhme²

Keywords: Serpentes, Colubridae, *Zamenis situla*, scalation, color polymorphism, sex ratio, Bulgaria.

INTRODUCTION

Zamenis situla (Linnaeus, 1758) (*sensu* Utiger et al. 2002) is a rare snake found throughout south and south-eastern Europe to Asia Minor and Crimea (Baran, 1976; Obst et al., 1993; Sofianidou, 1997). Until now, very little is known about its general morphological variation. The published data (e.g., Baran, 1976; Bruno, 1969; Obst et al., 1993; Szczerbak, 1966) are based mostly on small museum samples (sex often pooled), which do not allow a deeper comparison or generalization. Therefore, a unique old series of 85 museum specimens of *Z. situla* from eastern Bulgaria was examined with the aim to complete our knowledge on morphology and sex ratio in this species.

MATERIAL AND METHODS

The material consisted of 85 museum specimens of *Z. situla* (55 males, 30 females) kept in the herpetological collections of the National Museum Prague (NMP) and the Museum Koenig Bonn (ZFMK). All the individuals originate from the vicinity of Sozopol, eastern Bulgaria. They were apparently collected at the same locality just after hibernation in 1981 – 1983.

The examined animals were sexed according to external features and in dubious cases by dissection. Snout-vent length (SVL), caudal length (CL) and total length (TL) were taken by a plastic rule to the nearest 1 mm. Upper labials were counted for the left and right side separately.

Two general types of the color pattern were distinguished to describe the color polymorphism in the given population: (1) striped color morph (called “*situla*”) and (2) blotched morph (called “*leopardina*”). The latter was subdivided in to three other color subtypes: (i) one row of blotches, (ii) two complete rows of blotches, and (iii) two rows of blotches on at least one third of the body.

RESULTS AND DISCUSSION

Total length. The general size distribution (Fig. 1) shows two peaks (300 – 500 and 700 – 850 mm) and the two groups are regarded as subadults and adults, respectively (the limit was arbitrarily fixed at 600 mm TL). The ratio of subadults to the adults in the sample is 2.5:1. The actual total size range involves both the known minimum size of juveniles (276 mm) as well as the known maximum size of males (1046 mm).

Scalation. The Sozopol sample has the following scale counts (mean, range, *n*): dorsals 26.1, 23 – 27, 85; upper labials 7.7, 6 – 9, 85; ventrals of males 234.6, 223 – 242, 55; ventrals of females 243.0, 234 – 249, 30; subcaudals of males 87.7, 81 – 94, 54; subcaudals of females 77.8, 74 – 83, 29.

In comparison with the published data summarized by Obst et al. (1993) the Sozopol population has wider range of upper labials. Frequent occurrence of specimens having less than 8 upper labials at least on one head side (42.4%) seems to be typical feature of this local population.

In males the range of subcaudals exceeds considerably the upper limit published for the populations from Italy, Malta, former Yugoslavia, and Greece (82 – 85, sex not determined; Bruno, 1969) and lies at the upper values of the ranges given for Crimea (74 – 92, mean not available; Szczerbak, 1966) and “Turkey” (75 – 90, mean 86, the

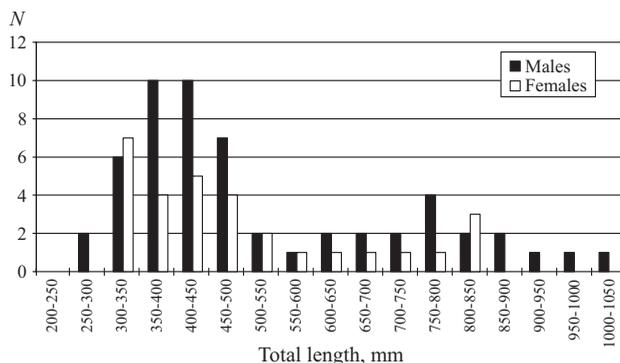


Fig. 1. Size distribution in the Sozopol population of *Z. situla*.

¹ Department of Zoology, National Museum, CZ-115 79 Praha 1, Czech Republic; E-mail: jiri.moravec@nm.cz.

² Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany; E-mail: w.boehme.zfmk@uni-bonn.de.

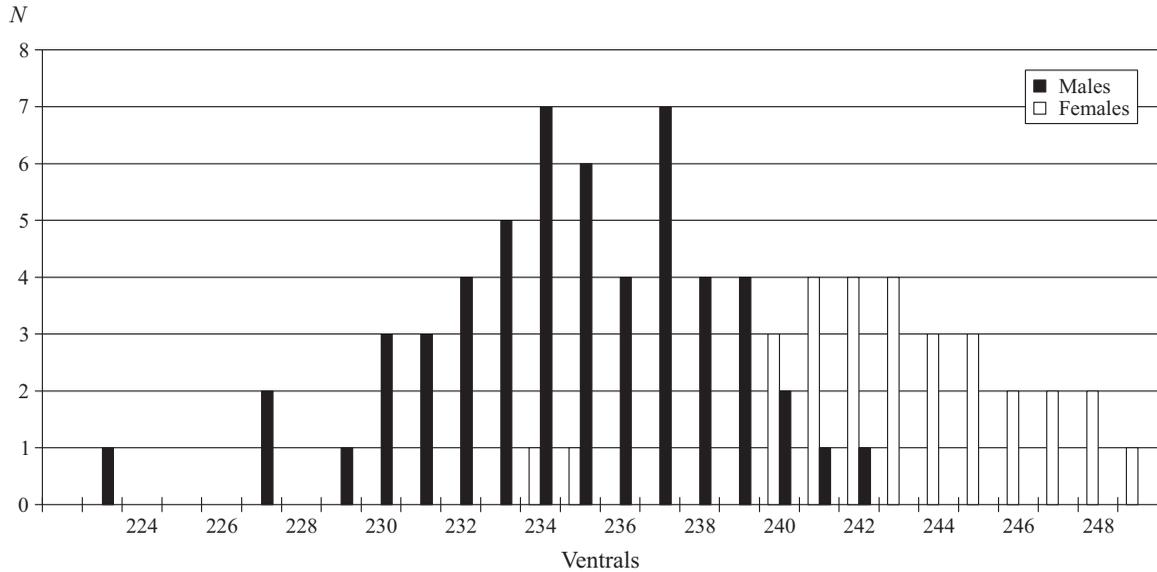


Fig. 2. Number of ventral scales in the Sozopol population of *Z. situla*.

sample does not consist completely of Turkish specimens; Baran, 1976).

Sexual dimorphism and sex ratio. Sexual differences in the number of ventral and subcaudal scales are shown in Figs. 2 and 3. The sexual dimorphism in the ratio of caudal length to the snout-vent length is obvious from Fig. 4.

Total sex ratio is 1.83:1 in favor of the males. In the subadult and adult part of the sample it is 1.65:1 and 2.43:1 respectively. Regarding the fact, that all the examined specimens were collected at the same locality just after emerging from the winter shelters, we can suppose that the obtained data may fairly well reflect the real situation in the given population. Thus, in comparison with the only published value of sex ratio in *Z. situla* (4.67:1, Crimea; Szczerbak, 1966) our data argue for a more balanced sex ratio in this species.

Color polymorphism. The striped “*situla*” color morph represents 14% of the sample, the blotched “*leopardina*” morph 86%. Specimens bearing one complete row of blotches represent 34% of the sample, the individuals with two complete rows of blotches 5% and the animals having two rows of blotches on at least one third of their body 47%. In most individuals belonging to the two last groups the blotches show a tendency to alternate to form the so-called “*hohenackeri*-pattern” (*sensu* Obst et al., 1993). Despite a slightly lesser frequency of females bearing stripes or two rows of blotches (Fig. 5), which could correspond to a cryptic function of blotched pattern (e.g., Volf et Werner 1994), no significant sexual dichro-

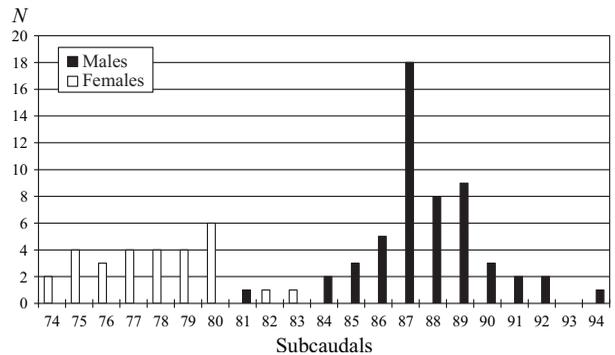


Fig. 3. Number of subcaudal scales in the Sozopol population of *Z. situla*.

matism in the dorsal pattern ($\chi^2 = 2.15$, $df = 3$, $P > 0.05$) was found.

CONCLUSION

The Sozopol population of *Z. situla* apparently represents a small marginal metapopulation existing at the north-eastern border of the species range (Obst, 1981; Sofianidou, 1997). Therefore, some of the peculiarities found in scalation and coloration (lower number of upper labials, higher number of subcaudals in males, frequent occurrence of so called “*hohenackeri*- pattern”) can reflect its probably high degree of isolation. However, only a thorough study of the morphology of *Z. situla* over its entire range will provide a picture of its general morphological variation.

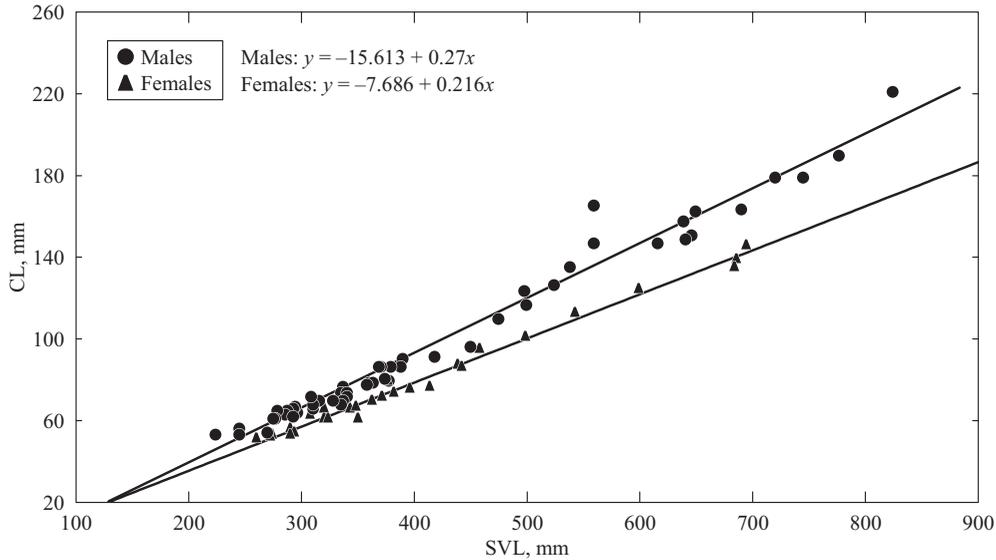


Fig. 4. Tail length as a function of snout-vent length in the Sozopol population of *Z. situla*.

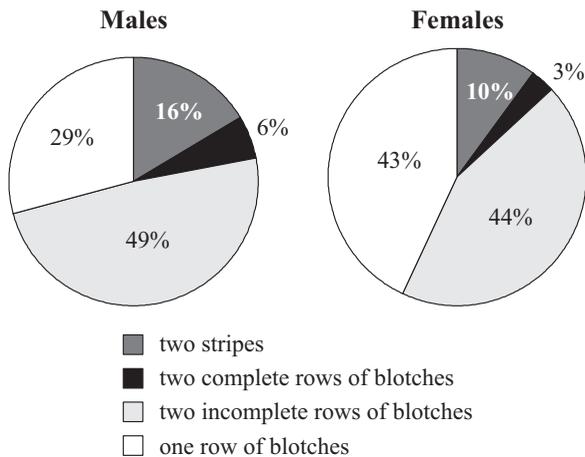


Fig. 5. Variation in dorsal pattern in the Sozopol population of *Z. situla*.

Acknowledgments. This work was supported by the Czech Ministry of Culture (projects Nos. RK01P03OMG006 and MK0CEZ99F0201).

REFERENCES

Baran I. (1976), *Türkiye yılanlarının taksonomik revizyonu ve coğrafi dağılımları* [Taxonomic Revision and Geographical

Distribution of the Turkish Snakes], TBTAK, Ankara [in Turkish].

Bruno S. (1969), "Morfologia, distribuzione e biologia di *Elaphe situla* (L.) 1758 (Reptilia, Serpentes, Colubridae)", *Atti della Accad. Scienze Natur. Catania*, **I**, 1 – 44.

Obst F. J. (1981), "Bemerkenswerte Schlangen aus Bulgarien in unserer Sammlung: *Coluber rubriceps* Venzmer und *Elaphe situla* (L.) (Reptilia, Ophidia, Colubridae)", *Faun. Abh. Staat. Mus. Tierk. Dresden*, **8**, 177 – 180.

Obst F. J., Ščerbak N. N., and Böhme W. (1993), "*Elaphe situla* (Linnaeus, 1758) — Leopardnatter," in: Böhme W. (ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/1. Schlangen (Serpentes) I*, AULA Verlag, Wiesbaden, pp. 431 – 453.

Sofianidou T. (1997), "*Elaphe situla* (Linnaeus, 1758)," in: Gasc J.-P. et al. (eds), *Atlas of Amphibians and Reptiles in Europe*, SEH, Mus. Nat. Hist. Naturelle, Paris, pp. 362 – 363.

Szczerbak N. N. (1966), *Zemnovodnye i Presmykayushchiesya Kryma* [The Amphibians and Reptiles of Crimea], Naukova dumka, Kiev [in Russian].

Utiger U., Helfenberger N., Schätti B., Schmidt C., Ruf M., and Ziswiler V. (2002), "Molecular systematics and phylogeny of Old and New world ratsnakes, *Elaphe* auct., and related genera (Reptilia, Squamata, Colubridae)", *Russ. J. Herpetol.*, **9**(2), 105 – 124.

Wolf M. and Werner Y. L. (1994), "The striped color pattern and striped/non-striped polymorphism in snakes (Reptilia: Ophidia)", *Biol. Rev.*, **69**, 599 – 610.

DIAGNOSTIC TRAITS IN THE MORPHOLOGY OF GREEN FROGS (*Rana esculenta* COMPLEX) IN THE MIDDLE DNEPR BASIN

O. Nekrasova,¹ S. Mezhzherin,¹ and S. Morozov-Leonov¹

Keywords: morphological variation, diagnostic characters, *Rana esculenta* complex.

INTRODUCTION

To solve problems of diagnostics specimens belonging to the green frogs of the *Rana esculenta* complex (*Rana ridibunda* Pall., 1771; *R. lessonae* Camerano, 1882; and their hybrid *R. esculenta* Linnaeus, 1758) on the basis of morphological traits has become possible only after successful using of the genetic markers (Mezhzherin and Morozov-Leonov, 1992). The morphological analysis of hybrid populations of green frogs was carried out, among other regions, in Poland, Hungary, France, Germany, Latvia, Russia, and Ukraine.

Morphological features are known to be subject to significant geographical variability. They depend on ploidy of hybrids and often are not fully reliable in mixed populations. Studies of diagnostic traits that allow taking into account the genetic variability and habitat choice of the pop-

ulations are of particular interest. These traits also may be the most reliable ones when distinguishing representatives of the *Rana esculenta* complex. The aim of the present study was to detect the most effective diagnostic indices using the ANOVA-MANOVA, discriminant analyses.

MATERIAL AND METHODS

Investigations were carried out in the Middle Dnepr basin (Fig. 1) from 1992 — 2002 on the basis of series of specimens that were genetically identified using electrophoresis. A total of 854 specimens of the *Rana esculenta* complex from 52 localities were analyzed. Sixteen standard morphometric indices (Terentiev, 1950; Berger, 1968, 1973; Nekrasova and Morozov-Leonov, 2001) were used (see Fig. 2): head — Lt.c./S.n., L./D.r.o., L./Sp.oc.,

¹ Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine, B. Khmel'nitskogo Vul, 15, 01601 Kiev, Ukraine; E-mail: oneks@mail.ru.

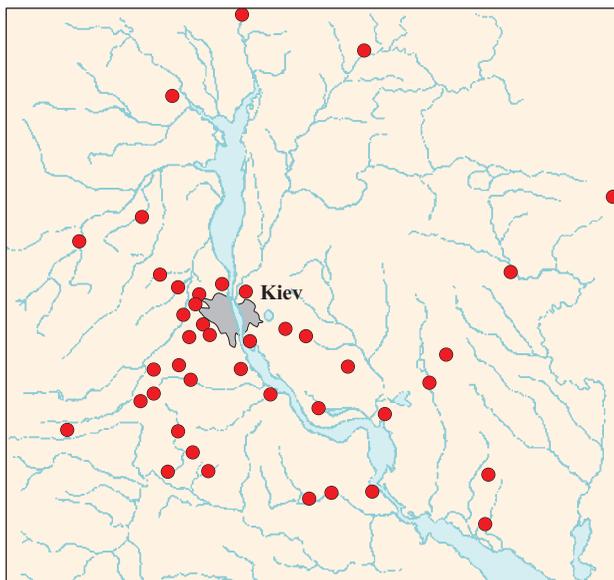


Fig. 1. Study area: the Middle Dnepr Basin.

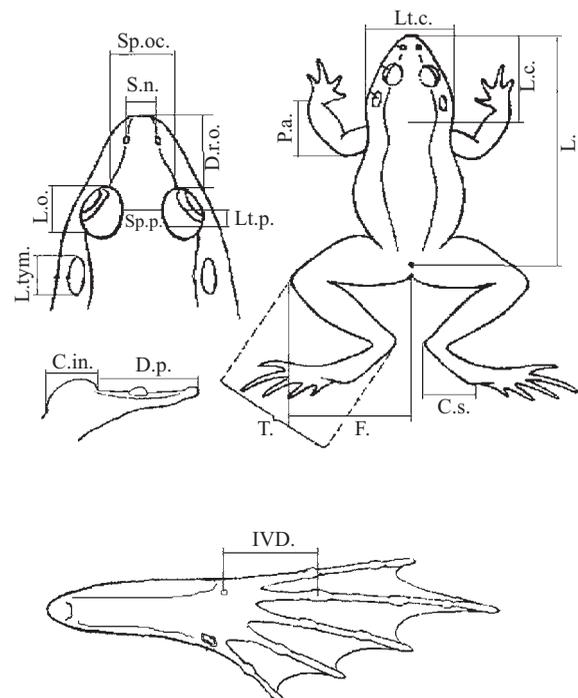


Fig. 2. Standard morphometric measurements as used in this study.

L./L.o., L./Lt.c., L./Sp.p., L./L.tym., L./S.n.; limbs — L./T., T./IVD., L./C.s., D.p./C.in., T./C.in., F./T., T./C.s., and index of Tarashchuk (1985): $I_x = T./C.in. \times D.p./C.in. \times T./C.s.$ Several coloration traits (see Nekrasova, 2002) were also recorded: (1) general body color, (2) number and size of the dorsal spots, (3) presence of dorsal-medial stripe, (4) number of stripes on the limbs (tibia, femur); (5) pigmentation of the ventral side (Figs. 2 and 3). The obtained morphometric ratios were used in standard statistical analysis using the discriminant, cluster and factor analysis modules of Statistica for Windows v. 5.0 (StatSoft, Inc., 1984 – 1995, USA).

RESULTS AND DISCUSSION

Populations of green frogs in Middle Dnepr basin are characterised by several genetic and morphological peculiarities. The most significant diagnostic indices of the

hind legs were determined green frogs genetic forms (I_x , D.p./C.in., T./C.in.). Limits of variability were specified (for most of the individuals): $I_x - R. lessonae < 21 < \text{hybrid} < 32 < R. ridibunda$; D.p./C.in. — $R. lessonae < 1.8 < \text{hybrid} < 2.3 < R. ridibunda$; T./C.in. — $R. lessonae < 6.7 < \text{hybrid} < 8.3 < R. ridibunda$.

Comparison of the results obtained using discriminant and factor analysis shows that it is possible to identify only three basic forms. The more clear results were achieved in distinguishing of parental species (Table 1): *R. ridibunda* (99%) and *R. lessonae* (97%). The hybrids were the least distinguishable (95%). The diagnostics gives the most reliable results when all the selected indices are included into the analysis. Contrary to that, the use of single traits resulted in 10 to 50% identification error. Traditionally used for diagnostics T./C.in. and D.p./C.in. provide the most accurate identification (91 and 88%, respectively).

To further improve the diagnostic resolution, the morphometric indices were compared to the body coloration pattern. The phenetic traits of *Rana esculenta* are largely intermediate between the two paternal species (Fig. 4), although some traits may be closer to one of the paternal species. This effect could be due to dominant-recessive gene interactions, which may influence the coloration pattern. It was shown that the level of the fluctuating asymmetry of dorsal spots in the hybrids exceeds one of the paternal species (Nekrasova, 2002).

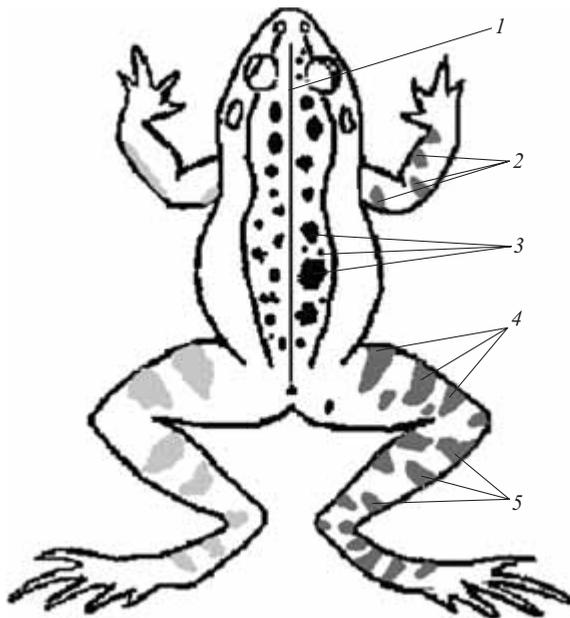


Fig. 3. Coloration traits. Right half, *Rana ridibunda*; left half, *R. lessonae*; 1, dorsal stripe; 2, basic stripes (or spots) on front limb; 3, dorsal spots; 4, main stripes (or spots) on femur; 5, main stripes (or spots) on tibia.

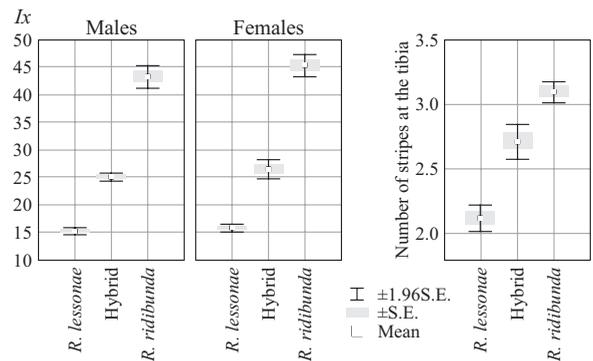


Fig. 4. Mean values (mean), standard error of mean (S.E.) and confidence interval (1.96S.E.).

TABLE 1. Ratio of Diagnosed Individuals of Paternal Species and Their Hybrids According to Different Traits (Terentiev, 1950) Combinations, Calculated by the Medium of Discriminant Analysis

Traits	3 forms, %			All, %
	<i>R. lessonae</i>	hybrid	<i>R. ridibunda</i>	
Indexes (head): L.c./Lt.c., L.c./L.o., Sp.oc./D.r.o., Lt.c./S.n., L./D.r.o., L./Lt.c., L./Sp.p., L./L.tym.	89.6	50.6	87.2	80.0
Indexes (limbs): I_x , F./T., T./IVD., 2IVD./D.p., C.s./2IVD., L./T., T./C.s., T./C.in., D.p./C.in.	96.9	95.2	99.0	98.0
Coloration (1 – 5, see <i>Material and Methods</i>)	88.6	50.0	92.9	80.2

The indexes of the head as well as the coloration traits allow for discriminating parental species at the level of 89–93%, while hybrids are only diagnosed at levels around 50%.

The following characteristics were identified to be the most significant for the diagnostics:

- Color traits and body pattern: color of vocal sacs, the location and the number of stripes on the limbs.
- Standard morphometric indexes: I_x , T./C.in., D.p./C.in.
- Other morphological characteristics: the form of internal metatarsal tubercle, position of the tight articulation (Terentiev, 1950; Berger, 1973).

REFERENCES

- Berger L.** (1966), “Biometrical studies on the population of green frogs from the environs of Poznan,” *Ann. Zool.*, **23**(11), 303–324.
- Berger L.** (1973), “Systematics and hybridization in European green frogs of *Rana esculenta* complex,” *J. Herpetol.*, **7**(1), 1–10.
- Mezhzherin S. V. and Morozov-Leonov S. Yu.** (1992), “Aber-rant phenotypes of locus Ldh-B in hybrid populations of *Rana esculenta* complex (Amphibia, Ranidae),” *Dokl. RAN*, **324**(6), 1314–1317.
- Nekrasova O. D. and Morozov-Leonov S. Yu.** (2001), “Diagnostics of green frogs complex representatives *Rana esculenta* L., 1758 (Amphibia, Ranidae) of the hybrid populations of the Dnepr basin,” *Vestn. Zool.*, **35**(5), 47–52.
- Nekrasova O. D.** (2002), “Interspecific variability and coloring polymorphism of green frogs *Rana esculenta* complex (Amphibia, Ranidae) in hybrid populations,” *Vestn. Zool.*, **36**(3), 37–44.
- Tarashchuk S. V.** (1985), “On the method of identification of the green frogs of *Rana esculenta* complex,” *Vestn. Zool.*, **19**(3), 83–85.
- Terentiev P.** (1950), *Frog*, Sovetskaya Nauka, Moscow.

THE CONTRIBUTION OF CYTOGENETICS TO THE SYSTEMATICS OF REPTILES

E. Olmo,¹ T. Capriglione,² G. Odierna,² and L. Kupriyanova³

The karyology of more than 1300 species of reptiles were studied till now, using cytological standard, banding and molecular methods. These studies gave an important contribution to the knowledge of the systematics and phylogeny of all the order of reptiles. Indeed they evidenced differences in karyotypical evolutionary rates and chromosome organisation between turtles and crocodiles on one hand and lizards and snakes on the other. Moreover, they provided information on the role of chromosome rearrangements in speciation and in other evolutionary processes. Very useful were also the results regarding the genetic mechanisms of sex determination, especially in squamates, and the identification of the steps of sex chromosomes differentiation.

Keywords: Cytogenetics, chromosome structure, chromosome evolutionary rate, speciation, sex chromosomes.

DISCUSSION

An Historical Survey

The first description of the chromosome number of a reptile dates from 1897, when Tellyesniczky counted 20 – 28 elements in *Lacerta agilis*. Subsequent pioneering work came from Loyez in 1905, and Trinci in 1908 on *Anguis*, and from Jordan in 1914 on two chelonians. In 1921 Painter, while studying the spermatogenesis of iguanids and teiids, first reported the existence of macro- and microchromosomes (Fig. 1) (see Matthey, 1949). True systematic studies started to appear in the 1930s and 1940s, after the works by the Japanese researchers Makino, Nakamura, and Oguma, and by Matthey who in 1949 published the first review of the karyology of vertebrates, “Les Chromosomes des Vertébrés,” where for the first time advanced a hypothesis on the phylogenesis of squamates based on the study of karyotypes that largely agreed with the classifications based on morphological data (see Matthey, 1949; Gorman, 1973).

Reptilian chromosome studies were boosted in the 1960s by the application to these vertebrates of the techniques developed by Ford and Hamerton, who used colchicine to arrest mitotic division and hypotonic pre-treatment to swell cells and spread the chromosomes. Reptilian cytotaxonomy thus received a marked acceleration in the 1960s and especially the 1970s (Fig. 2) (Gorman, 1973).

Among saurians, interesting investigations were conducted by Gorman on the cytotaxonomy of iguanids; by Cole and by Lowe on inter- and intraspecific variability in *Sceloporus* and *Cnemidophorus*; by Kupriyanova and Darevsky on the karyology of lacertids; by Sokolovsky on the karyology of agamids; by Max King and by Kupriyanova on skinks and by Max King on varanids and gekkonids (see Gorman, 1973; Olmo, 1986). The most significant studies of snake karyology were published by Becak and Becak and by Singh and Ray-Chaudhuri. In 1968, Wylie and co-workers determined the karyotype of *Sphenodon punctatus*. Huang and Gans wrote several reports on amphisbaenians and Cohen and Gans determined the karyotype of all living crocodiles (see Gorman, 1973). In-depth studies of the cytotaxonomy of turtles, conducted by Killebrew and, especially, the group of John Bickham, began at the end of the 1970s (see Olmo, 1986).

Another step forward in cytogenetic studies was marked in the 1970s by the development of various chromosome banding techniques, which allowed to perform more detailed analyses of chromosome morphology and to garner information on the preferential location of specific DNA sequences along the chromosomes. These techniques began to be applied to reptiles in the mid 1970s and proved to be especially helpful in the study of the cytotaxonomy of cryptodiran turtles (see Bickham, 1983).

In the 1980s, studies of reptilian chromosomes were still fairly numerous (Fig. 2). Conventional and banding techniques were employed by Bickham and colleagues (Bickham and Carr, 1983) and Bull and collaborators (Bull and Legler, 1980) to analyze the karyology of turtles; by Max King (King 1990) and Craig Moritz (Moritz and King, 1985) to investigate parthenogenetic gekkonids; by

¹ Institute of Biology and Genetics, Polytechnical University of the Marche, via Breccie bianche, I-60131 Ancona, Italy; E-mail: e.olmo@univpm.it.

² Department of Comparative and Evolutionary Biology, Naples University “Federico II,” via Mezzocannone 8, I-80134 Naples, Italy.

³ Department of Karyotaxonomy Zoological Institute Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: Vladimir@vz8122.spb.edu.

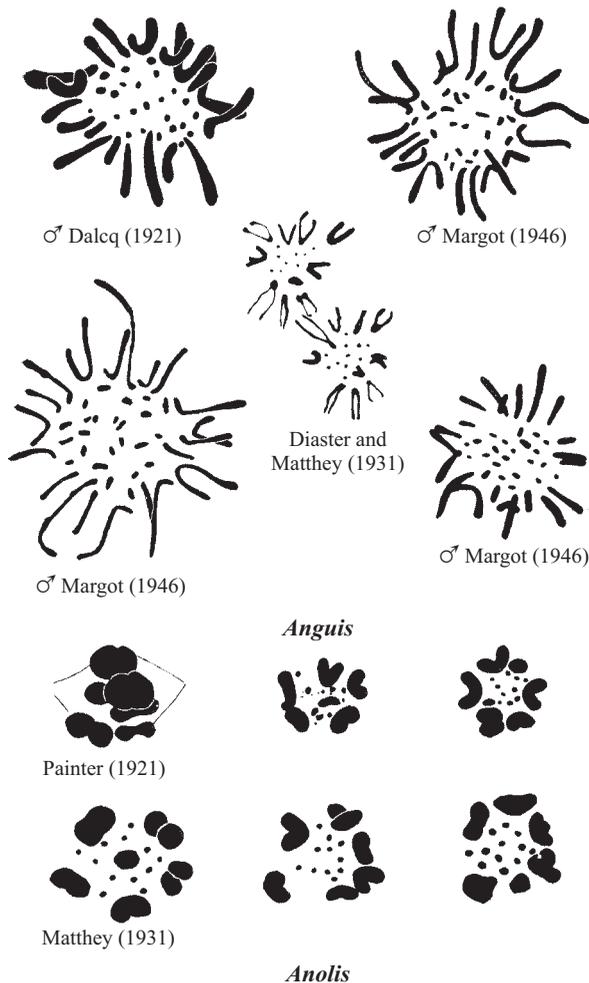


Fig. 1. Figures from some of the early studies on reptiles' karyotypes (modified from Matthey, 1949).

Kupriyanova (Kupriyanova, 1994) and by the Naples group, made up of Teresa Capriglione, Gaetano Odierna, and E. Olmo, to study the lacertids (Olmo et al., 1990) and by Donnellan (1991a, 1991b) to study the scincids. Among ophidians Gregory Mengden studied the karyology of the elapids (Mengden 1982, 2000; Olmo, 1986).

In the 1990s, research into the cytotaxonomy of reptiles declined (Fig. 2), owing especially to the development of techniques to analyze the mitochondrial genes, even though new methods of isolation and characterisation of specific DNA sequences would permit a more exhaustive study of chromosome composition, hence a more refined comparative analysis. At present, few groups are studying the karyology of reptiles with any continuity. Among these are Larissa Kupriyanova and her colleagues in Russia, Teresa Capriglione and Gaetano Odierna in It-

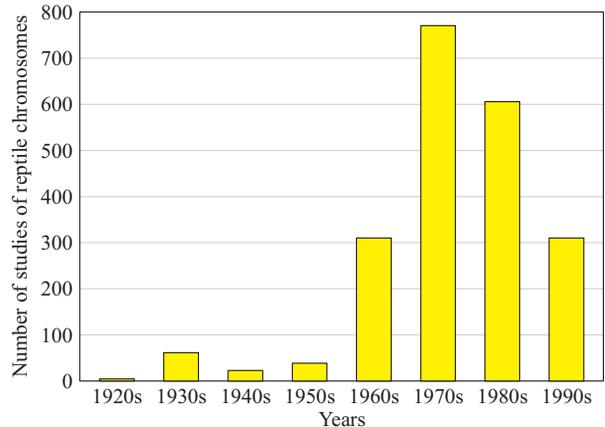


Fig. 2. Number of karyological data of reptiles (ordinate), in the various ten-year periods (abscissa).

aly, Hidetoshi Ota and his co-workers in Japan, and Yone-naga-Yassuda and his group in Brazil.

Cytogenetics, Systematic, and Phylogeny

To date, more than 500 papers have been published on reptilian karyology, and the karyotypes of over 1300 (or 18%) of the over 7900 living species have been determined (Table 1). The more comprehensive studies are those on crocodiles, of which all 23 living species have been karyotyped, and those of turtles, of which more than 50% of all species have been studied. In saurians and amphisbaenians this proportion is around 20%; and in snakes about 12%. Most of these investigations have used conventional staining methods, while banding techniques have been employed in about 23% of the species studied, especially chelonians.

A different level of karyological variability characterises turtles and crocodiles on the one hand and squamates on the other. In the first two orders, gross chromosomal morphology, and often also G-banding, vary very little at both the inter- and the intra-familial level and differences between similar species are rare (Bickham, 1984; Olmo, 1986). By contrast, in squamates differences are marked not only between lizards and snakes, but also among the various families of each suborder and among similar species of the same family, and intra-specific variability is far from being uncommon (Olmo, 1986; Olmo et al., 2002)

In the 23 species of crocodiles studied to date, only 8 different karyotypes have been identified (Cohen and Gans, 1970), all exhibiting similar G-banding patterns (King et al., 1986). The most primitive karyotype would be the one with 32 chromosomes that is found in the gavia

and in several species of *Crocodylus*, *Caiman*, and *Alligator*; the most derived would be the one with $2n = 42$, rich in uniarmed chromosomes, that is found in *Melanosuchus*, *Paleosuchus*, and *Caiman*. Karyological analysis allowed Cohen and Gans (1970) to outline a scheme of crocodile evolution that agrees quite well with the hypotheses based on morphological data (see also Bickham, 1984)

Among turtles, cryptodires are especially conservative karyologically. The study of their karyological evolution by Bickham and co-workers (Bickham and Carr, 1983) has allowed to make the most exhaustive comparative analysis at the level of suborders and to propose a karyological phyletic tree differing very little from those based on morphological data. These researchers investigated various species of all the families of the suborder, also using the banding techniques, and demonstrated that the origin of the majority of cryptodiran families has entailed few, if any, chromosome changes, and that the chromosome number 1 has probably remained unaltered for at least 100 million years (Bickham and Carr, 1983; Bickham, 1984).

In squamates, karyological studies have been more useful for the study of the taxonomic relationships between genera of the same family or species of the same genus.

Two tentative hypotheses have been advanced to account for the karyological evolution of saurians: one holds that the ancestral karyotype consisted solely of uniarmed chromosomes, the other that this karyotype was identical to the one with 12 biarmed macrochromosomes and 24 microchromosomes that is found in many living species of nearly all families. The latter hypothesis is preferred because it is the more parsimonious. Nonetheless, as G-banding studies have shown that the karyotypes of species of the same family sharing the same gross morphology exhibit several differences in G-banding (Moritz, 1986), it

is impossible to establish with any degree of certainty whether the karyological affinities of these reptiles reflect real similarities or are the result of mere evolutionary convergence. The same considerations apply to ophidians (Mengden et al., 1986).

Some of the more detailed studies on saurian karyology have been conducted on iguanids, teiids, and gekko-nids. In iguanids, the separation of the various subfamilies should have resulted, with few variations if any, from a karyotype with 12 biarmed macrochromosomes and 24 microchromosomes, which is considered basal. Within the different lineages, karyological evolution would then have followed different trends; in particular, the species-richest genera, such as *Anolis*, *Sceloporus*, and *Liolaemus*, exhibit the highest inter- and intra-specific chromosome variability noted in reptiles to date (Gorman, 1973; Bickham, 1984). These variants often characterise different populations or species and seem to be related to colonising radiations and to periods of intense speciation (King, 1993). In teiids, cytotoxic studies demonstrated earlier than other methods the wide divergence between macroteiids and microteiids, which were subsequently elevated to family rank. Karyology also evidenced two distinct groups in macroteiids: the *Dracaena* group, characterised by a typical 12 + 24 karyotype, and the *Ameiva* group with all uniarmed chromosomes. Also in this family, the genus *Cnemidophorus*, which is the most widespread and species-rich, exhibits the widest chromosome variability and frequent intra-specific differences (Gorman, 1973). Cytotoxic analysis has provided satisfactory results also in families, like lacertids, which are apparently karyologically uniform. Basing on the number and position of the nucleolar organiser, on sex chromosome morphology and on the evolution of some repetitive DNAs, the Naples group has outlined a taxonomic scheme of this family that

TABLE 1. Data on Karyology of Reptiles

	L.sp.	K. species		Banding		AgNOR		sex	xy	zw
	N	N	%	N	%	N	%	N	N	N
Chelonia	294	155	53	57	37	23	15	7	4	3
Crocodylia	23	23	100	4	18	3	14			
Rhynchoceph.	2	2	100							
Sauria	4555	867	19	189	22	165	19	185	106	79
Ophidia	2953	355	12	74	21	93	26	248		248
Amphisbaenia	138	31	22.5					1		1
Reptilia	7965	1333	18	324	23	284	20	441	110	331

L.sp., living species (from Dowling and Duellman, 1978); K. species, number (N) and percentage (%) of karyotyped species; Banding, number and percentage of species studied with banding methods; AgNOR, number and percentage of species studied with the AgNOR method for the nucleolar organiser; sex, number of species with sex chromosomes: xy male and zw female heterogamety.

Data from a database available at the web site: www.scienze.univpm.it/professori/chromorep.pdf

agrees with the morphology-based one proposed by Arnold (Olmo et al., 1991).

Among snakes, the PhD thesis of Gregory Mengden (1982) on elapids' karyology, unfortunately largely unpublished, deserves special mention. In this study Mengden evidenced in various cases a good correspondence between karyotype evolution and phylogenetic classification based on other methods, such as the enzyme electrophoresis, and demonstrated that the Australian, Asian and African elapids and American coral-snakes, though showing intra group homogeneity in standard karyology and G-banding patterns as well as in the localisation of the NOR, are however markedly different from one another (Mengden, 1982, 2000).

Chromosomes and Speciation

As mentioned above, there are several cases in which inter- and intra-specific variability has been related to speciation; the role of chromosomes in this process is among the most debated topics in karyology (King, 1993). Reptiles, mainly some groups of squamates, have proved to be a suitable model to study this issue. Especially significant in this regard are Max King's investigations on gekkonids (King, 1993). In this family, many Australian species are characterised by frequent intra-specific chromosome variability. These chromosome races often exhibit a definite trend of geographical distribution that has suggested that chromosome variations may have accompanied colonisation and adaptive radiation events. A similar intraspecific variability and trend have been recently observed also in some lacertids, like *Zootoca vivipara* (Kupriyanova and Boehme, 1997; Odierna et al., 1998)

Based on his investigations, King (1993) has proposed two models of chromosome speciation:

1. Chromosomal allopatric speciation, which would have characterised various species of *Gehyra* and *Phyllodactylus*. In this model, multiple or sequential chromosome mutations arise in peripheral, isolated populations of species and become fixed in allopatry. The population thus arisen spreads, and, in the case of secondary contact with

the parental species and formation of a hybrid zone, genetic introgression is prevented by the adverse effect of chromosome differences on the hybrid's fertility.

2. Chromosomal sympatric speciation, which in reptiles has only been described in *D. vittatus*. In this case, following the establishment within a population of one or more chromosome variants favorable in conditions of homozygosity, a new species, chromosomally differentiated from the parental species, would arise even in the absence of geographical separation. The daughter species would extend its range, displacing the parental species, because of the greater fitness of the new homozygote. The derived taxa would thus come to occupy central areas and the ancestral taxa peripheral or external areas.

Some lines of evidence that the diversification of populations or species may have been accompanied by karyotype variations preceding genetic diversification were recently found in two studies on lacertid lizards. Indeed in three Pyrenean species of *Archaeolacerta* (Odierna et al., 1996) and in several populations of the *Lacerta kulzeri* complex (in den Bosch et al., 2003) were observed marked differences in chromosomes number and/or morphology accompanied by very short genetic distances.

Chromosome variations may have also accompanied all the phases of the evolution of the class. Besides their chromosomal variability, crocodiles, turtles, snakes and lizards also differ in the chromosome changing rate, i.e., the number of hypothesised chromosome mutations per million years, which rises progressively from crocodiles to turtles, snakes, and lizards (Table 2). This parameter shows a significant, direct, logarithmic relationship with the number of living species (Fig. 3) and an equally significant, inverse correlation with the extinction rate of the various orders and suborders (Fig. 4) (Olmo et al., 2002). The two parameters are considered satisfactory measures of the evolutionary success of a given group; in reptiles a greater chromosome variability would thus have characterised the evolutionarily more successful taxa (Olmo et al., 2002).

TABLE 2. Relationship Between Chromosome Change Rate, Number of Living Species, and Extinction Rate in Reptiles*

	Time from origin, million years	The number of studied species	Karyotypes number	Chromosome change rate per million years	The number of living species	Extinction rate, %
Crocodiles	144	23	8	0.056	23	36
Turtles	208	155	36	0.173	294	27
Snakes	144	355	70	0.486	2953	6**
Lizards	144	867	214	1.486	4555	

* Modified from Olmo et al. (2002).

** This value refers to all the squamates.

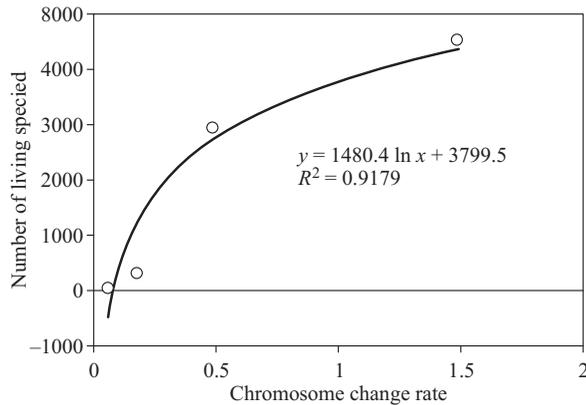


Fig. 3. A direct significant logarithmic relationship between chromosome change rate (abscissa), and number of living species (ordinate), in four taxa of reptiles.

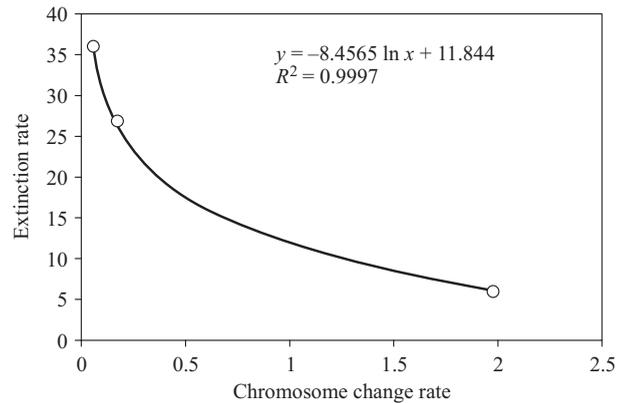


Fig. 4. An inverse significant logarithmic relationship between chromosome change rate (abscissa), and extinction rate in three reptilian orders.

Cytogenetics, Parthenogenesis, and Sex Chromosomes

Cytogenetic studies have provided important contributions on two further issues: the origin and evolution of parthenogenesis and the evolution of the sex chromosomes.

The best known karyological studies of parthenogenetic species are those by Darevsky and Kupriyanova on Caucasian lacertids, those by Cole, Cuellar, Lowe, and Peccinini-Seale on *Cnemidophorus*, and those by Craig Moritz and by Max King on the Australian parthenogenetic gekkonids (see Peccinini-Seale, 1981; Darevsky et al., 1985; Moritz and King, 1985). These studies have demonstrated that the origin of the majority of parthenogenetic saurians lies in hybridisation events between two or more bisexual species (Peccinini-Seale, 1981; Bickham, 1983; Darevsky et al., 1985; Moritz and King, 1985).

Two systems of sex determination coexist in reptiles: temperature-dependent determination, which is consistently observed in crocodiles, is prevalent in turtles, and has been demonstrated in some agamids and chamaeleonids; and a purely genotypic mode of determination, whose occurrence varies in different groups (Table 1) (Bull, 1980). Crocodiles have no sex chromosomes, in turtles they are very rare, while in saurians they have been described in 185 species, or 21% of those studied. They are most frequent in iguanids and pygopodids, which exhibit male heterogamety; in lacertids and varanids, which are characterised by female heterogamety; and in gekkonids, where male and female heterogamety seem to coexist. The sex chromosomes are especially common in snakes, which are characterised by consistent female heterogamety. Here they are more frequent in the more advanced families,

whereas in the more primitive ones they have not been observed at all, or little differentiated forms have been reported where the two homologues are homomorphic, at least in gross morphology (Bull, 1980; Bickham, 1984, Olmo, 1986). Reptiles are a useful model to study sex chromosome differentiation because, unlike those of birds and mammals, they exhibit diverse levels of differentiation.

The studies conducted so far seem to indicate that in reptiles the most common model of sex chromosome evolution entails the following steps:

- Accumulation on one of the two homologues of one or more specific sequences of highly repetitive DNA accompanied by extensive heterochromatinisation.
- Consequent dramatic reduction or elimination of recombination between the two sex chromosomes.
- Morphological modification of the heterochromatic homologue most frequently consisting of a progressive deletion that turns it into a microchromosome, but which in other cases involves a centric or tandem fusion between this homologue and an autosome or a pericentric inversion (see Olmo, 1986).

All these mechanisms have been observed in various populations of *Zootoca vivipara* (Kupriyanova and Boehme, 1997; Odierna et al., 1998)

Some Final Comments

Karyological investigations have contributed crucially to the study of reptilian taxonomy and phylogenesis, and although herpetologists at present pay few attention to these type of studies, they could still give new and important contributions.

Over the last few years, molecular biology has definitely become incorporated into cytogenetics; this has allowed more detailed analyses of the molecular organisation and evolution of the chromosomes and, in some cases, precise *in situ* localisation of specific genes. The studies of reptiles employing these new techniques are still very few, although the results that they have yielded seem to be extremely encouraging. Among these, can be reminded the Teresa Capriglione's research into highly repetitive DNA in lacertids that has provided data both on the taxonomic relationships among the various species of the family and on their possible function (Capriglione, 2000) and the effective method of comparative DNA analysis developed by Dr. Grechko and her co-workers (Ryabinin et al., 1996).

In conclusion allow us to express the hope that these new opportunities will lead to a resumption of the study of the karyology of reptiles.

Acknowledgments. Funds were obtained from the Russian Foundation for Basic Science (grant No. 02-04-48611); from Presidium St. Petersburg's Scientific Center of Russian Academy of Sciences; Min. Nauka Scien. School 1647.2003.4; from the program of Presidium of Russian Academy of Sciences "Dynamics of Genopool."

REFERENCES

- Bickham J. W.** (1984), "Patterns and modes of chromosomal evolution in reptiles," in: Sharma A. K. and Sharma K. (eds.), *Chromosomes in Evolution and Eukaryotic Groups. Vol. 2*, CRC Press, Boca Raton, pp. 13 – 40.
- Bickham J. W. and Carr J. L.** (1983), "Taxonomy and phylogeny of the higher categories of cryptodiran turtles based on a cladistic analysis of chromosomal data," *Copeia*, **1980**, 530 – 543.
- Bosch H. A. J. in den, Odierna G., Aprea G., Barucca M., Canapa A., Capriglione T., and Olmo E.** (2003), "Karyological and genetic variation in the Middle Eastern lacertid lizards, *Lacerta laevis* and *Lacerta kulzeri* complex: a case of chromosomal allopatric speciation," *Chromosome Res.*, **11**, 165 – 178.
- Bull J. J.** (1980), "Sex determination in reptiles," *Quart. Rev. Biol.*, **55**, 3 – 21.
- Bull J. J. and Legler** (1980), "Karyotypes of side-necked turtles (Testudines: Pleurodira)," *Can. J. Zool.*, **58**, 828 – 841.
- Capriglione T.** (2000), "Repetitive DNA as a tool to study the phylogeny of cold-blooded vertebrates," in: Olmo E. and Redi C. A. (eds.), *Chromosomes Today. Vol. 13*, Birkhauser Verlag, Basel – Boston – Berlin, pp. 183 – 194.
- Cohen M. M. and Gans C.** (1970), "The chromosomes of the order Crocodylia," *Cytogenetics*, **9**, 81 – 105.
- Darevsky I. S., Kupriyanova L. A., and Uzzell T.** (1985), "Parthenogenesis in Reptiles," in: Gans C. and Billett F. (eds.), *Biology of the Reptilia. Vol. 16B*, Wiley, New York, pp. 411 – 526.
- Dowling H. G. and Duellman W. E.** (1978), *Systematic Herpetology: a Synopsis of Families and Higher Categories*, HISS Publications, New York.
- Donnellan S.** (1991a), "Chromosomes of Australian lygosomine skinks (Lacertilia: Scincidae). I. The *Egernia* group: C-banding, silver staining, Hoechst 33258 condensation analysis," *Genetica*, **83**, 207 – 222.
- Donnellan S.** (1991b), "Chromosomes of Australian lygosomine skinks (Lacertilia: Scincidae). II. The genus *Lampropholis*," *Genetica*, **83**, 223 – 234.
- Gorman G.** (1973), "The chromosomes of the Reptilia, a cytotoxic interpretation," in: Chiarelli A. B. and Capanna E. (eds.), *Cytotaxonomy and Vertebrate Evolution*, Acad. Press, London – New York.
- King M.** (1990), "Chromosomal and immunogenetic data: a new perspective on the origin of Australia's reptiles," in: Olmo E. (ed.), *Cytogenetics of Amphibians and Reptiles. Advances in Life Sciences*, Birkhauser Verlag, Basel – Boston – Berlin, pp. 153 – 180.
- King M.** (1993), *Species Evolution. The Role of Chromosome Change*, Cambridge Univ. Press, Cambridge.
- King M., Honeycutt R., and Contreras N.** (1986), "Chromosomal repatterning in crocodiles: C, G and N-banding and the *in situ* hybridisation of 18S and 26S rRNA cistrons," *Genetica*, **70**, 191 – 201.
- Kupriyanova L. A.** (1994), "Structure, localization, and stability of chromosomes in karyotype evolution in lizards of the Lacertidae family," *Russ. J. Herpetol.*, **1**(2), 161 – 168.
- Kupriyanova L. A. and Boehme W.** (1997), "New data on the distribution of different forms of *Zootoca vivipara* in eastern and western Europe: chromosomal analysis," in: Boehme W., Bischoff W., and Ziegler T. (eds.), *Herpetologia Bonnensis*, SEH, Bonn, pp. 199 – 206.
- Matthey R.** (1949), *Les Chromosomes des Vertébrés*, F. Rouge, Lausanne.
- Mengden G. A.** (1982), *Chromosomal Evolution and the Phylogeny of Elapid Snakes. Ph.D. Thesis*, Australian Natl. Univ., Canberra.
- Mengden G. A.** (2000), "Australian elapid phylogeny: a summary of the chromosomal and electrophoretic data," in: Grigg G., Shine R., and Ehmann H. (eds.), *Biology of Australasian Frogs and Reptiles*, Roy. Zool. Soc. of New South Wales, pp. 185 – 192.
- Mengden G. A., Shine R., and Moritz C.** (1986), "Phylogenetic relationship within the Australasian snakes of the genus *Pseudechis*," *Herpetologica*, **42**, 215 – 229.
- Moritz C.** (1986), "The population biology of *Gehyra* (Gekkonidae): Chromosome change and speciation," *Syst. Zool.*, **35**, 46 – 67.
- Moritz C. and King D.** (1985), "Cytogenetic perspectives on parthenogenesis in the Gekkonidae," in: Grigg G., Shine R., and Ehmann H. (eds.), *Biology of Australasian Frogs and Reptiles*, Roy. Zool. Soc. of New South Wales, pp. 327 – 337.
- Odierna G., Aprea G., Arribas O. J., Capriglione T., Caputo V., and Olmo E.** (1996), "The karyology of the Iberian rock lizards," *Herpetologica*, **52**, 542 – 550.

- Odierna G., Aprea G., Capriglione T., Arribas O. J., Kupriyanova L. A., and Olmo E.** (1998), "Progressive differentiation of the W sex-chromosome between oviparous and viviparous populations of *Zootoca vivipara* (Reptilia, Lacertidae)," *Ital. J. Zool.*, **65**, 295 – 302.
- Olmo E.** (1986), "Reptilia," in: John (ed.), *Animal Cytogenetics. 4. Chordata. 3*, Gebrueder Borntraeger, Berlin – Stuttgart.
- Olmo E., Odierna G., Capriglione T., and Cardone A.** (1990), "DNA and chromosome evolution in lacertid lizards," in: Olmo E. (ed.), *Cytogenetics of Amphibians and Reptiles. Advances in Life Sciences*, Birkhauser Verlag, Basel – Boston – Berlin, pp. 181 – 204.
- Olmo E., Odierna G., Capriglione T., and Caputo V.** (1991), "A karyological approach to the systematics of Lacertidae (Reptilia, Sauria)," *Rev Esp. Herpetol.*, **6**, 81 – 90.
- Olmo E., Capriglione T. and Odierna G.** (2002), "Different genomic evolutionary rates in the various reptile lineages," *Gene*, **295**, 317 – 321.
- Peccinini-Seale D.** (1981), "New development in vertebrate cytotaxonomy. IV. Cytogenetics studies in reptiles," *Genetica*, **56**, 123 – 148
- Ryabinin D. M., Grechko V. V., Darevsky I. S., Ryskov A. P., and Semenova S. K.** (1996), "Comparative study of DNA repetitive sequences by means of restriction endonucleases among populations and subspecies of some lacertid lizard species," *Russ. J. Herpetol.*, **3**(2), 178 – 185.

STEPPE VIPER (*Vipera renardi*) IN THE NORTHERN POINT OF ITS AREA

A. Pavlov¹

Keywords: Volga-Kama Region, north of area, subspecies, *Vipera renardi bashkirovi*.

INTRODUCTION

Some morphological and ecological data on *V. renardi bashkirovi* (the extreme north population of *Vipera renardi*) are presented.

MATERIAL AND METHODS

Investigations were conducted on an archipelago of Kuibyshev reservoir from 2000 to 2002. The studied area belongs to the forest-steppe Volga Region (Zapadnoe Zakam'ye, Tatarstan, Russia) and situated at 54°55' – 55°5' N and 49°5' – 49°20' E. The island system (Spassk Archipelago) includes about 60 islands and belongs administratively to Spassk District of Tatarstan (Russia). Island plant formations (habitats of steppe viper) are described; ecology and morphology of the steppe viper population were studied.

The following morphological characters were used: L. (length of head-body), L.cd. (length of tail), Ventr. (number of ventrals), S.cd. (number of subcaudals), Sq. (number of midbody dorsal scale rows), S.orb. [number of scales in circumocular ring (right + left)], Lab. [number of supralabials (right + left)], Sublab. [number of sublabials (right + left)].

RESULTS AND DISCUSSION

The archipelago includes forest (the whole territory or 80% of it is covered by forest and bushes) and forest-meadow (most part of which are meadows) islands. The forest islands are well-preserved fragments of the former brand-leaved forests of oak, linden, maple forests, and elms. At present a half of the islands is covered mainly by meadow vegetation, which is used for haymaking and pasture. Phytocenoses of islands are presented by 32 plant formations, including xerophilous, mesophilous and hydrophilous ecological complexes.

Habitats. The central part of the archipelago is occupied by the largest two islands. The first one is about 15 km². There are remains and ruins of buildings of “old town” Spassk covered with vegetation. Natural tree vegetation is absent on the island. The vegetation includes small sites of pine, birch and balsamic poplar; on the former “old town” territory there maple and solitary apple trees occur. The main area of the island is occupied by anthropogenic-caused “bush-steppe” broom being the basic species; grass vegetation consists of forest-steppe and weed forms: *Myosotis popovii*, *Poa angustifolia*, *Astragalus cicer*, *A. danicus*, etc. The role of *Festuca valesiaca* increases with pasture intensity increase and with high load on grass vegetation soil denudation is marked. On the elevations along the border of the “old town” the *Festuca-Artemisia* cenoses are developed (Pavlov and Bakin, 2001).

Steppe viper are practically absent in the places of regular pasture. They prefer “mosaic coenoses,” open places with prevalence of *Festuca* and *Artemisia*, alternating with numerous ruins and pits covered by ruderal vegetation (*Leonurus quinquelobatus*, *Urtica dioica*, *Artemisia vulgaris*, *Conium maculatum*, etc.). For the first time steppe viper was found in the central part of the island in 2000 and that was due to the pasture reduction and recovery succession development. Broom growth serves as a shelter from high day temperatures and constant winds. Orthoptera are abundant (30 – 50 ex./m²) comprising a significant part (more than 30%) in adult snake feeding on the overgrown areas as well as on the territory of the “old town.” Orthopteran larvae are the main food of vipers up to three years. Murid rodents numerous on all islands prevail in the nutrition of the adult snakes. Population of the steppe viper on the Spassk Island is estimated as approximately 700 – 800 individuals. The second unknown early habitat of vipers was found on the island, which is about 8 km² and situated west of the Spassk Island. It is a constant habitat of another group of 500 – 900 snakes. In the southern and eastern parts the island is covered for 2/3 by forest (oak, linden etc.) alternating with plots of wet meadows. The steppe viper is spread all over its territory including forest biome where *Vipera berus* was proposed to be met. The snakes were met here in wet and swamp habitats though it is not characteristic of the species. Moreover, the

¹ Department of Basic Ecology, Ecological Faculty, Kazan State University, Kremlevskaya ul., 18, 420008 Kazan, Russia;
E-mail: zilant@ksu.ru, zilant@mi.ru

species was found on two islets being the enclaves of the Spassk Island. Their areas comprise 20 and 2.8 ha. Here the snakes keep to the wet meadows and swamp plots (*Carex acuta*, *C. juncella*, *Rumex thyrsoiflorus*, *Taraxacum* sp., etc.) from spring to autumn returning to Spassk Island for hibernation.

Morphological characters. The snakes of this population have got a number of ecological and morphological characters making this population a unique one. The discoverer of the population considered it to be a Tertiary relic (Bashkirov, 1935). On the basis of a number of morphological differences, the steppe viper of Spassk Archipelago was described as distinct subspecies *Vipera renardi bashkirovi* Garanin, Pavlov et Bakiev 2004.

Within the whole population of ursinii-complex the Spassk population vipers have the largest **total length** (up to 710 mm). This index can be compared only with the Orlov Island samples in the Black Sea (Kotenko, 1981). **Ventr.:** mean values of this indication of both sexes of the Spassk population are close to those of *V. r. renardi* taxon

(Altai) and *V. r. renardi* taxon (east) and the limit of their variation is the widest among snakes of ursinii-complex.

S.orb. (right + left): as well as number of ventrals by mean values this indication is close to those in *V. r. renardi* taxon (Altai) and *V. r. renardi* taxon (east). **Lab.:** indication meaning's variation of the males in the northern population is wider when compared with the entire *renardi* taxa (Nilson and Andrén, 2001), and mean indication values closer to east form of *V. r. renardi*. Mean values of female's sublabials is lower than that in *renardi* taxa on the whole. The number of Sublab. for both sexes is lower when with *renardi* taxa.

Color patterns. Vipers of Spassk Archipelago population have two color forms: cryptic color and melanists. The first color form is represented by several transitional variations, typical for the species. The part of melanists is prevailing.

In the Spassk population we distinguish the following basic forms of melanistic pattern of coloration: **1) perfect melanists** — fully black without any other color elements; some individuals have yellowish tail end from the bottom side; **2)** the second form of melanism includes snakes with matt (dull) **black ground and dorsal band of anthracite-black color;** **3) deep brown** vipers have a black-brown basic color of the body varying in tints. Some snakes of the group have got rare small elements varying in color scattered over the body, e.g., gray and deep (dark)-cream specks and spots. In some cases color elements of the kind shade dull dorsal band; **4)** vipers with **deep gray** form of melanism have gray-black ground and dark dorsal band.

Some individuals with white and dim-white elements on supralabial and sublabial scales are met among vipers with melanistic form of coloration. The part of melanists in the investigation area reaches 66%. It should be marked that the same correlation ($\geq 50\%$) between white and dark snakes is kept here from the first quarter of the 20th century. Bashkirov (1929) writes about Spassk snakes: "...what concerns steppe vipers... a half of them are gray, others are black or entirely black." The case when the balance between color groups in population has not been changing during 100 years is expanded by the Hardy – Weinberg Equilibrium. A conclusion of the absence of vipers' adaptive advantages with any of two color forms

TABLE 1. Some Pholidosis Characteristics of the Spassk Population of Steppe Viper

Characters	Sex	N	Range	Mean ± S.E.
L.	♀♀	73	320 – 665	533.36 ± 7.41
	♂♂	43	339 – 615	478.35 ± 9.79
L.cd.	♀♀	72	30 – 80	52.85 ± 1.15
	♂♂	42	43 – 95	65.17 ± 1.71
L. + L.cd.	♀♀	72	351 – 710	586.78 ± 8.19
	♂♂	42	388 – 670	543.00 ± 10.92
Ventr.	♀♀	45	140 – 152	147.91 ± 0.38
	♂♂	25	120 – 154	144.44 ± 1.41
S.cd.	♀♀	44	22 – 35	25.84 ± 0.44
	♂♂	24	24 – 35	32.33 ± 0.47
Sq.	♀♀	44	19 – 21	20.82 ± 0.07
	♂♂	23	20 – 21	20.96 ± 0.04
S.orb. (right + left)	♀♀	46	16 – 22	19.06 ± 0.19
	♂♂	25	17 – 22	19.64 ± 0.23
Lab. (right + left)	♀♀	47	14 – 18	17.47 ± 0.14
	♂♂	25	16 – 19	17.76 ± 0.16
Sublab. (right + left)	♀♀	45	15 – 21	18.09 ± 0.18
	♂♂	25	16 – 21	18.28 ± 0.23

TABLE 2. Basic Forms of Coloring of Spassk Population of Steppe Viper, %

Color forms	Perfect melanists	Black ground with dorsal band of anthracite-black color	Deep brown	Deep gray	Gray with dorsal band of dark color	Light ground with dorsal band of dark color	Olive ground with dorsal band of dark color
♀♀ (n = 73)	27.5	13.8	6.9	5.6	19.2	17.8	8.2
♂♂ (n = 44)	40.9	18.1	2.3	9.1	13.6	11.4	4.5

suggests itself as a consequence of the Hardy – Weinberg rule. And the nature of coloring is caused, to the great extent, by genetic mechanism. Fact of black color uniqueness for entire *ursinii*-complex is an indirect approval of it. There is only one report of *V. renardi* melanism: populations with a significant part of black individuals are known in the Krasnodar Krai of Russia (Ostrovskikh, 1997).

Meanwhile, the black color on the northern limit of spreading could help vipers to be more active in comparison with cryptic colored individuals and have great reproductive success. Such a type of life strategy is known for *V. berus* (Andrén and Nilson, 1981).

Acknowledgments. The study was supported by the Program on Global Security and Sustainability Research and Writing Initiative of the John D. and Catherine T. MacArthur Foundation.

REFERENCES

- Andrén C. and Nilson G. (1981), “Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*,” *Biol. J. Linn. Soc.*, **15**, 235 – 246.
- Bashkirov I. S. (1929), “On the steppe viper (*Coluber renardi* Christoph) in Tatarstan Republic,” in: *Coll. Papers of Student's Study Group. Vol. 3*, Kazan, pp. 143 – 144 [in Russian].
- Bashkirov I. S. (1935), “Relic elements in the fauna of Zgyguli,” *Byull. Mosk. Obshch. Isp. Prirody. Otd. Biol.*, **44**(5), 240 – 245 [in Russian].
- Garanin V. I., Pavlov A. V., and Bakiev A. G. (2004) “*Vipera renardi* (Christoph, 1861),” in: *Snakes of Volga-Kama Region*, Samara Sci. Center, Samara, pp. 61 – 91 [in Russian].
- Kotenko T. I. (1981), “On *Vipera ursinii renardi* in the south of Ukraine,” in: Darevsky I. S., Ananjeva N. B., Barkagan Z. S., Borkin L. Ya., Sokolova T. M., and Shcherbak N. N. (eds.), *The Problems of Herpetology. Abstrs. of 5th Herpetol. Meeting*, Nauka, Leningrad, p. 73 [in Russian].
- Nilson G. and Andrén C. (2001), “The meadow and stepper vipers of Europe and Asia — the *Vipera (Acridophaga) ursinii* complex,” *Acta Zool. Academ. Hung.*, **7**(2 – 3), 87 – 267.
- Ostrovskikh S. V. (1997), “Different form of melanism and its development with age in the populations of stepper viper *Vipera renardi* (Christoph, 1861),” *Russ. J. Herpetol.*, **4**(2), 186 – 191.
- Pavlov A. V. and Bakin O. V. (2001), “Records of *Vipera ursinii* on islands of Kuibyshev reservoir,” *The Problems of Herpetology. Proc. of the 1th meet. of the Nikolsky Herpetol. Soc.*, Pushchino – Moscow, pp. 220 – 222 [in Russian].

ARE *Leptodactylus didymus* AND *L. mystaceus* PHYLOGENETICALLY SIBLING SPECIES (AMPHIBIA, ANURA, LEPTODACTYLIDAE)?

R. O. de Sá,¹ W. R. Heyer,² and A. Camargo¹

Keywords: *Leptodactylus fuscus*, sibling species, molecular analyses, sequence data, 12S rDNA, 16S rDNA, ND1, phylogenetic analyses.

INTRODUCTION

The *Leptodactylus fuscus* species group consists of 25 currently recognized species; within this species group and distributed throughout the Amazon Basin, Atlantic Forests, Gran Chaco, and cerrados is the *L. mystaceus* species complex. This species complex consists of *L. didymus*, *L. elenae*, *L. mystaceus*, *L. notoaktites*, and *L. spixi*. Adult morphologies have been used to distinguish these species from each other except for *L. didymus* and *L. mystaceus* (Heyer, 1978; Heyer et al., 1996). *Leptodactylus didymus* and *L. mystaceus* are morphologically indistinguishable; the species are recognizable only by the characteristics of their advertisement calls: non-pulsed in *L. didymus* and pulsed in *L. mystaceus* (Heyer et al., 1996).

Traditionally, *L. mystaceus* and *L. didymus* have been considered “sibling species.” The concept of “sibling species” was originally introduced by Mayr (1942: 151) to describe pairs or groups of morphologically identical or nearly identical species; however, in subsequent work Mayr (1976) interchangeably used the terms “sibling and cryptic species” to describe morphologically similar species. Mayr (1942: 151) considered sibling species to be important in understanding the full complexity of animal speciation. In order to differentiate these two terms, herein we take a narrow cladistic methodological approach (i.e., dichotomous speciation) by which we restrict the term “sibling” species to two taxa that share a most recent common ancestor; whereas, the term cryptic (derived from the Greek *Kruptos*, meaning ‘hidden’; Allaby, 1991) species refers to “hidden” diversity and does not necessarily imply close phylogenetic relationship. Thus, the sibling species pair of *L. didymus* and *L. mystaceus* assumes two postulates: (1) the taxa shared a most recent common ancestor not shared with other species in the *L. mystaceus* species complex and (2) the two taxa could represent a recent speciation event (i.e., not enough time has passed to reach

morphological differentiation, although this is not a requisite).

Herein, we analyze the genetic diversity among taxa in this species complex to determine if the sibling species *L. didymus* and *L. mystaceus* are sister taxa. If the assumptions about sibling species are correct, then we would expect that the two taxa involved would be genetically closer between themselves than with any other closely related species.

MATERIAL AND METHODS

Molecular sequence data were obtained for *L. didymus*, *L. elenae*, *L. mystaceus*, *L. notoaktites*, and *L. spixi*; in addition, data were collected for *L. fuscus* and *L. mystacinus* (other *fuscus* species group members) to use as outgroups. We obtained a total of 2553 base pairs (bp) for each taxon, 786 bp corresponding to the 12S rDNA gene, 814 bp to the 16S rDNA gene, and 953 bp to the ND1 gene. The sequence data have GenBank accession numbers are AY948952 – 948959, AY905695, AY905716-17, AY911264, and AY911285-911286. Voucher specimens are presented in the *Appendix*. Sequences were aligned using Clustal X (Thompson et al., 1997). Alignment of ND1 coding sequences included the known complete ND1 coding sequence for *Rana catesbeiana* (Nagae, 1988). Maximum Parsimony (MP) and Maximum Likelihood (ML) exhaustive search analyses were performed with PAUP* (Swofford, 1998). ML analyses used the GTR+G model recommended by Modeltest 3.04 (Posada and Crandall, 1998), with empirical base frequencies. Analyses were performed using the two *Leptodactylus* taxa as outgroups and also tested the effect of alternatively using a single outgroup taxon at the time on the recovered trees. ND1 sequences also included *Rana* as an outgroup and for MP analyses, the third position was down-weighted relatively to first and second positions and gaps positions were alternatively treated as missing data and as a fifth character; transition substitutions were down-weighted relative to transversion substitutions.

¹ Department of Biology, University of Richmond, Richmond, VA 23173, USA; E-mail: rdesa@richmond.edu.

² Amphibians & Reptiles, MRC 162, PO Box 37012, Smithsonian Institution, Washington, DC 20013-7012, USA; E-mail: heyerr@si.edu.

RESULTS

The results of the different phylogenetic analyses are best illustrated by the trees in Fig. 1. Neither in the MP (with gaps as a fifth character, Fig. 1A) or the ML (Fig. 1B) analyses do *L. didymus* and *L. mystaceus* exhibit sister species relationships within the *mystaceus* species complex. Support for clades was assessed using bootstrap (1000, pseudoreplicates; Felsenstein, 1985), decay indices enforcing topological constraints (Bremer, 1988), and Bayesian posterior probabilities (Bayes et al., 2001) (Fig. 1).

Analyses of data partitions separately (i.e., 12S, 16S, and ND1 data matrices) and alternatively using the two *Leptodactylus* outgroups or using only one at the time (either *L. fuscus* or *L. mystacinus*) also resulted in tree topologies where *L. didymus* and *L. mystaceus* do not exhibit sister taxa relationships. MP weighted analyses as well as alternative treatment of gaps as missing data in combined and separate analyses also retrieved similar trees in which *L. didymus* and *L. mystaceus* do not exhibit sister taxa relationships.

DISCUSSION

The present molecular analyses of the *L. mystaceus* complex shows that *L. didymus* and *L. mystaceus* are not sibling species as defined in this paper (i.e., a sister species relationship was not recovered in any of the analyses), despite their being morphologically indistinguishable. The topology recovered enforcing a sister taxa relationship between *Leptodactylus didymus* and *L. mystaceus* is 5 steps longer than the most parsimonious tree; however a Kishino – Hasewaga test comparing the two likelihood topologies was not statistically significant. These two taxa are treated as distinct species based on their call differences (Heyer et al., 1996), a common isolating mechanism occurring in anurans. The genetic differentiation between these two species is comparable (about 10%) to that between each of them with other species in the complex that

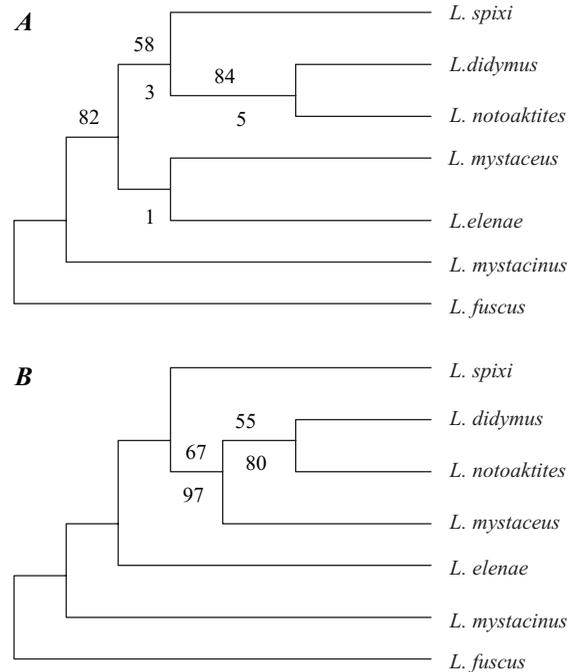


Fig. 1. A. MP most parsimonious tree recovered (length = 1000, C.I. = 0.75, gaps as fifth character. Bootstrap support above 50% is indicated above branches, number below branches corresponds to clade decay indexes. **B.** ML tree, bootstrap support above 50% is indicated above branches, number below branches corresponds to Bayesian posterior probabilities.

have differentiated morphologically (9 – 12% between *L. mystaceus* and other species, 8 – 12% between *L. didymus* and other species, see Table 1).

These results are interesting because they highlight an unusual case among vertebrates in which the species involved show behavioral (e.g., call) and genetic (Table 1) differentiation, but do not differ morphologically.

Two alternative hypotheses need to be considered to explain this case.

1. Morphological convergence. Either *Leptodactylus didymus* and *L. mystaceus* are morphologically converging on each other or they both may be converging onto

TABLE 1. Genetic distances among taxa analyzed in this study.

	<i>spixi</i>	<i>didymus</i>	<i>fuscus</i>	<i>mystaceus</i>	<i>notoaktites</i>	<i>elenae</i>	<i>mystacinus</i>
<i>spixi</i>	0.00						
<i>didymus</i>	0.10	0.00					
<i>fuscus</i>	0.11	0.13	0.00				
<i>mystaceus</i>	0.10	0.12	0.14	0.00			
<i>notoaktites</i>	0.07	0.08	0.12	0.09	0.00		
<i>elenae</i>	0.09	0.11	0.12	0.10	0.08	0.00	
<i>mystacinus</i>	0.11	0.13	0.13	0.12	0.11	0.10	0.00

the morphology of at least a third species occurring in the Amazon basin. This morphological convergence could be justified if either one of these two species, or a third unidentified taxon at this point, are proven to produce skin toxins that would make them, if not toxic, at least strongly distasteful, giving them a selective advantage by avoiding predation. Alternatively, their morphological characteristics may be providing unique camouflage advantages in the habitat they occupy. Extensive field-work would be needed to test either of these alternatives.

2. Retention of ancestral morphological patterns. The two taxa involved are exhibiting morphological adult patterns inherited from a) a most recent common ancestor to both of them or b) to an ancestor to the *L. mystaceus* species complex, or a subclade of it. We have no evidence in support of the first alternative. Our data show that the two taxa involved are not sibling species; that is they do not share a most recent common ancestor. There is also no evidence in support of the second scenario, particularly considering that all other taxa in the *L. mystaceus* species complex can be differentiated morphologically among themselves and from the *L. didymus* – *L. mystaceus* pair.

Acknowledgments. We thank the various colleagues in South American and North American Universities and museums who have provided us with *Leptodactylus* samples. Simon Loader provided helpful suggestions on this manuscript. Financial support was provided by the National Science Foundation (award #9815787 and #0342918, subsequent REU amendments) to RdS and WRH and by the Neotropical Lowlands Research Program, Smithsonian Institution (R. Vari, P. I.)

APPENDIX. Voucher specimen data used in molecular analyses.

Leptodactylus didymus. USNM 268970, Peru, Madre de Dios, Tambopata Reserve.

Leptodactylus elenae. USNM 319643, Argentina, Salta, Embarcacion, 4.0 km NE of junction with road into, on National Route 34.

Leptodactylus fuscus. MZUSP 67073, Brazil; Roraima; Caracaranã, near Normandia.

REFERENCES

- Allaby M. (1991), *The Concise Oxford Dictionary of Zoology*, Oxford Univ. Press, New York, pp. 1 – 508.
- Bremer K. (1988), “The limits of amino acid sequence data in Angiosperm phylogenetic reconstruction,” *Evolution*, **42**, 795 – 803.
- Felsenstein J. (1985), “Confidence limits on phylogenies: an approach using the bootstrap,” *Evolution*, **39**, 783 – 791.
- Heyer W. R. (1978), “Systematics of the *fuscus* group of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae),” *Nat. Hist. Mus. Los Angeles County Sci. Bull.*, **29**, 1 – 85.
- Heyer W. R., García-Lopez J. M., and Cardoso A. (1996), “Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species,” *Amphibia-Reptilia*, **17**, 7 – 31.
- Huelsenbeck J. P. and Ronquist F. (2001), “Mr. Bayes: Bayesian inference of phylogenetic trees,” *Bioinformatics*, **17**, 754 – 755.
- Mayr E. (1942), *Systematics and the Origin of Species from the Viewpoint of a Zoologist*, Dover Publication, Inc., reprint of Columbia Univ. Press. Cambridge (MA), pp. 1 – 334.
- Mayr E. (1976), “Sibling or cryptic species among animals,” in: *Evolution and the Diversity of Life*, Belknap Press, Cambridge, pp. 509 – 514.
- Nagae Y. (1988), “The primary and secondary structures of the ribosomal RNAs of *Rana catesbeiana* mitochondrion,” *J. Nippon Med. Sch.*, **55**, 417 – 430.
- Posada J. C. and Crandall K. (1998), “Modeltest: testing the model of DNA substitution,” *Bioinformatics*, **14**, 817 – 818.
- Swofford D. L. (1998), *PAUP: Phylogenetic Analysis Using Parsimony (* and other methods)*. Version 4, Sinauer Associates Inc., Sunderland (MA).
- Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., and Higgins D. G. (1997), “The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools,” *Nucleic Acids Res.*, **24**, 4876 – 4882.

Leptodactylus mystaceus. MZUSP 70371, Brazil, Pará, Serra de Kukoinhokren.

Leptodactylus mystacinus. RdS 789, Uruguay, Departamento de San Jose, Sierra de Mahoma.

Leptodactylus notoaktites. USNM 303191, Brazil, São Paulo, ca. 5 km S of Luiz Antonio, Fazenda Jatai.

Leptodactylus spixi. USNM 534008, Brazil, Sergipe, Crasto.

PATERNAL INHERITANCE OF MOLECULAR MARKERS IN RAT SNAKES INTERSPECIES BREEDING

O. Sideleva,¹ S. Ryabov,² N. Ananjeva,¹ and N. Orlov¹

Keywords: hybrids, rat snake, Colubridae, RAPD, interspecific breeding.

INTRODUCTION

One of the important contributions of evolutionary biology is investigation of the factors of speciation in natural populations. One of such factors is hybridogenesis between already existing species. However, accurately estimating of interspecific hybrids in nature is frequently difficult. Incomplete knowledge of pedigrees in natural populations limits the investigation of hybrids. Snakes breeding in captivity provide a model system for dissolving such evolutionary problem. For understanding of heredity principles and hybridization mechanisms, experiments with rat snakes from the captivity were carried out.

Rat snakes (*Elaphe* genus, Colubridae family) are presented by more than 40 species distributed in the North and Central America, in the mainland and island Asia, in the Philippines, in Southern and Central Europe (Schultz, 1996).

MATERIAL AND METHODS

Parental forms of rat snakes in the present study comprised of eight different species: *Elaphe guttata*, *E. obsoleta*, *E. schrenckii*, *E. climacophora*, *E. situla*, *E. persica*, *E. dione*, *E. bimaculata*, originated from wild populations from different localities around the world. Genealogy of each individual and each family group were exactly and detailed documented. Each specimen was described in details (color and pattern, pholidosis characters) and photographed by digital camera. It allows creating computer database where all the parameters and images (color pictures demonstrating color and pattern) were included. The blood was collected from the caudal vein and preserved in 96% ethanol, or dried on filter paper. Genomic DNA was extracted from the blood using a standard proteinase K phenol-chloroform procedure. PCR with RAPD primers was carried out as described in our previous papers

(Sideleva et al., 2003). Amplification of the RAPD bands was carried out with primers AA2M2 (5'-GAG CGA CCC AGA GCG G-3') and L-45 (5'-GTA AAA CGA CGG CCA GT-3'), developed by S. Bulat with co-authors (1996). Molecular study was conducted in "Taxon"-group of Zoological Institute, Russian Academy of Sciences.

The band sharing statistic was used to calculate the proportion of shared bands between two individuals:

$$SI = 2N_{AB}/(N_A + N_B),$$

where SI is Similarity Index, N_A and N_B are the numbers of bands present in individuals A and B respectively, and N_{AB} is number of shared bands (Hoggren, 1995).

RESULTS AND DISCUSSION

RAPD pattern in all studied rat snakes has considerable differences in non-related family groups. Summarized number of DNA fractions of different length composed 13 – 15 but there was only one common RAPD band for all studied individuals. In the average the identity of RAPD genotype of all individuals composed about 7%. Our data show that molecular RAPD-DNA markers mostly have paternal inheritance in hybrids from the interspecific breeding of rat snakes of *Elaphe* genus.

Hybrids of first and second generations of interspecific breeding *E. guttata* × *E. obsoleta* inherit RAPD pattern of farther *E. obsoleta*. Sometimes individual DNA fractions appear in offspring but are absent in both of parents. Similarity Index for parental individuals belonging to different species was 0.46; for brother and sister of *E. obsoleta* — 0.88, for F2 brother and sister — 0.89. Close meaning of this index (SI = 0.89) was noted for the paired comparison grandfather – grandson and grandfather – granddaughter.

RAPD pattern of offspring of parents: female of *E. schrenckii* and hybrid male (*E. climacophora* × *E. schrenckii*) carries paternal DNA bands distribution, and in some cases they lacked one father DNA fraction.

¹ Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: osideleva@mail.ru.

² Tula Exotarium, Oktyabrskaya ul., 26, 300002 Tula, Russia; E-mail: rept@tula.net.

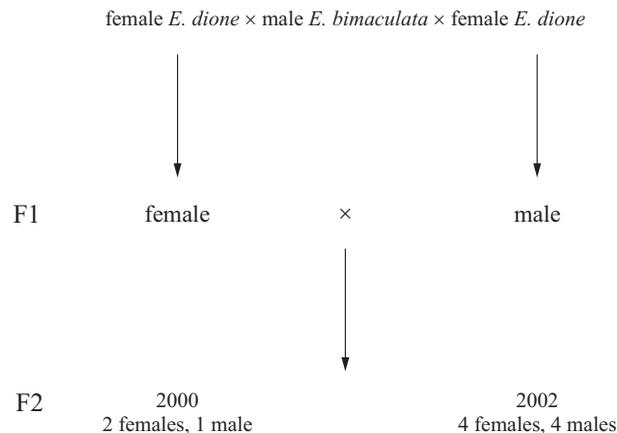


Fig. 1. Genealogy of the family group 4 (*Elaphe dione* × *E. bimaculata*).

However, DNA markers typical for maternal individuals never appeared. SI for parental individuals participating in crossbreeding is 0.33. Maximal value of SI was recorded for two sister individuals, having 75% of *E. schrenckii* genotype and 25% of *E. climacophora* genotype (SI = 0.97). SI values between each of these sisters and their farther were slightly lower (0.90 and 0.88).

One more family group of rat snakes was presented by parents belonging to different species: female of *E. situla* was breed with male of *E. persica*, resulted by hybrids of the first generation. All cross-breedings of F1 hybrids resulted of the lack of offspring. Hybrids of the first generation inherit RAPD pattern of farther's individual *E. persica* after the amplification with L-45 primer. PCR with second primer show that both offspring RAPD bands of their parents and only individual bands were presented. SI, calculated for father and his two offspring, comprised 1.0 for L-45 primer and 0.18, 0.20 for AA2M2 primer. The average value of SI for comparisons father – son and father – daughter were 0.61 and 0.64, respectively. Siblings have the maximal SI values (0.93 and 0.80), like in all studied family groups of rat snakes.

The data about family groups *Elaphe guttata* × *E. obsoleta*, *E. climacophora* × *E. schrenckii*, and *E. situla* × *E. persica* are described more detailed in our previous paper (Sideleva et al., 2003).

More complicated situation was presented in three generations of hybrids between taxonomically related species *Elaphe dione* and *E. bimaculata*.

Family group *E. dione* × *E. bimaculata* included parents belonging to different species of the rat snakes, hybrids of the first generation after the breeding of father (*E. bimaculata*) with different females of *E. dione*, as well

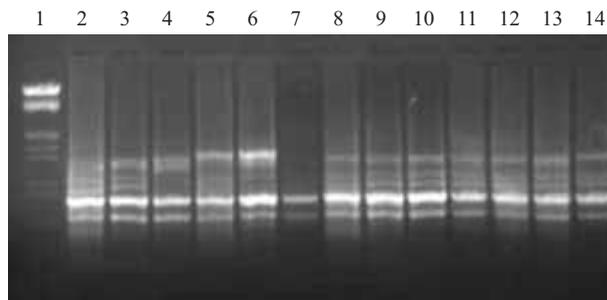


Fig. 2. Results of PCR with universal primer L-45 in family group *Elaphe dione* × *E. bimaculata*. 1, DNA marker λ /Pst; 2, male *E. bimaculata* (grandfather); 3, F1 hybrid male (father); 4, F1 hybrid female (mother); 5, female *E. dione* (grandmother); 6, 7, F2 hybrid females, born in 2000; 8 – 14, F2 hybrids, born in 2002.

as hybrids of the second generation born in 2000 and 2002 (Fig. 1). In total 15 individuals were analyzed.

SI for the parents belonging to different species was 0.60. Hybrids of the first generation like in all families described above inherited RAPD pattern of *E. bimaculata*, except one band absent in both offspring. SI for a pair of F1 siblings composes 1.00, despite of the origin from the different mothers-females of *E. dione*. Relationships between father and children was 0.99 whereas for children and mother SI = 0.44.

Analysis of RAPD-genotype in hybrid individuals of the second generation revealed different pattern according to different primers used for PCR. Thus, for primer L-45 identical pattern of DNA-bands comprised for all offspring born in 2000 (SI = 1.00 for all siblings). At the same time SI for parents and children was 0.67, and for the pairs: grandchildren – grandfather was 0.60, grandchildren – grandmother was 0.50. All grandchildren of second hatch (2002) had absolutely identical RAPD-genotype, the same with their parents (SI = 1.00). SI for pair comparison grandchildren – grandfather *E. bimaculata* was 0.99, and for grandchildren – grandmother *E. dione* was 0.67 (Fig. 2). Comparison of the second generation hybrids were born in 2000 and 2002, show the index of their similarity as 0.67.

Pattern of DNA fractions after amplification with universal primer AA2M2 in the second generation of hybrids were differentiated into two genotypes which were recorded in offspring in equal ratio (SI = 0.50). SI for the pairs RAPD genotype No. 1/parents composed 0.36, RAPD genotype No. 2/parents was 0.31. Comparison of individual with RAPD genotype No. 1 with male *E. bimaculata* (grandfather) show SI = 0.63, and for offsprings with genotype No. 2 was 0.88. Common DNA fractions for hybrid individuals of the second generation and female of *E. dione* were not found (Fig. 3).

Thus, the general tendency of paternal RAPD pattern inheritance was permanent, despite of some differences in studied family groups. Distinctive feature in family group *E. dione* × *E. bimaculata* is the revealing of two equal RAPD genotypes among the offspring of the second generation.

Inheritance of the morphological characters in this family group also had some specific features. The hybrids of the first generation were colored like father species (*E. bimaculata*), and had a pattern intermediate between parental individuals. Most of hybrids of the second generation (8 among 11, or 72.7%), had color and pattern similar with their parents (F1 hybrids). At the same time, one male of F2 phenotypically corresponded to *E. dione*, and two hybrid males (18.2%) differed by a new appearing bright coloration and pattern which were not similar with both parental species.

There were no differences in subcaudals between parents and offspring. At the same time we found the differences in the important identification character — coloration of supralabial shields. In *E. dione* supralabials are white whereas in *E. bimaculata* these shields are brightly-yellow. In all children of the first generation supralabials are white. We revealed the following distribution of this character among the hybrids of the second generation: 5 white: 4 light-yellow (intermediate coloration): 2 brightly-yellow. This proportion allows as suggesting that at least 3 genes are involved in determinations of this character.

In our study was shown that morphological character (the number of subcaudal shields) is inherited by interspecies hybrids from parental individual with greater number of shields irrespective of crossing direction. In further breeding of hybrids with forms carrying lesser number of scales, the hybrids of the next generation demonstrate consecutive reduction of the scale number.

Another studied morphological characters, coloration and pattern, have complicated polygenic inheritance. Shape and color of pattern are inherited irrespective from the color of background. There are at least four independent characters: coloration itself (background), type of pattern, frequency of elements in color pattern, color of bands or spots.

The data show that molecular RAPD-DNA markers have paternal inheritance in hybrids from the interspecific breeding. Especially interesting is inheritance of the complex of paternal RAPD fragments that could show the possibility of linkage between these specific markers.

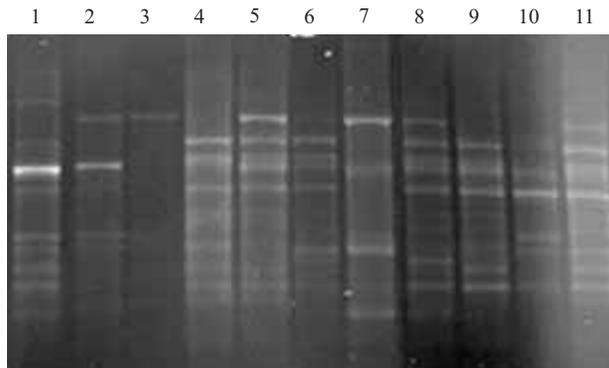


Fig. 3. Results of PCR with AA2M2 universal primer in family group *Elaphe dione* × *E. bimaculata*. 1, male *E. bimaculata* (grandfather); 2, F1 hybrid female (mother); 3, female *E. dione* (grandmother); 4, 5, F2 hybrid females, born in 2000; 6 – 11, F2 hybrids, born in 2002.

The distinguishing of hybrid rat snakes in natural populations is possible with application of the complex analysis of morphological and molecular data. This study found the opportunity to determine the hybrid origin of individual by combination of morphological characters which are similar with maternal species or intermediate and molecular characters which comes from the paternal species.

Acknowledgments. This project is supported by Grant of President of Russian Federation for leading scientific schools NS 1647.2003.4 and by the Russian Fund for Fundamental Research (grant No. 05-04-48147).

REFERENCES

- Bulat S. A. and Mironenko N. V.** (1996), "Identification of fungi and analysis of their genetic diversity by polymerase chain reaction (PCR) with genespecific and nonspecific primers," *Russ. J. Genet.*, **32**, 143 – 159.
- Hoggren M.** (1995), *Mating Strategies and Sperm Competition in the Adder (Vipera berus)*. Ph.D. Thesis, Uppsala University, Uppsala, Sweden.
- Schultz K.-D.** (1996), *A Monograph of the Colubrid Snakes of the Genus Elaphe*, Fitzinger, Koelz Sci. Books, Havlickov Brod, Czech Republic.
- Sideleva O. G., Ananjeva N. B., Ryabov S. A., and Orlov N. L.** (2003), "The comparison of morphological and molecular characters inheritance in family groups of rat snakes of genus *Elaphe* (Serpentes: Colubridae)," *Russ. J. Herpetol.*, **10**(2), 149 – 156.

ON THE TAXONOMIC STATUS OF THE COMMON ADDER OF THE PARTIALLY WOODED STEPPE OF THE OKA – DON PLAIN

A. S. Sokolov¹

Morphological characters of the typical form of the common adder, *Vipera berus*, and its black form, so-called “*Vipera nikolskii*,” distributed in the partially wooded steppe of the Oka – Don plain, were investigated. Taxonomic status of black form is discussed.

Keywords: Serpentes, Viperidae, *Vipera berus*, morphological characters, taxonomy.

INTRODUCTION

Until quite recently it was believed that the wooded and partially wooded zones within the limits of the former USSR are inhabited with only one species of vipers — common adder *Vipera berus* (Linnaeus, 1758) (Terentjev and Chernov, 1949; Bannikov et al., 1971, 1977; Shcherbak and Shcherban, 1980). The publication of Grubant et al. (1973) had broken out the stability of this idea and gave “reasons to put into discussion to resurrect for the black viper its specific name *Vipera prester* (L.).” Investigating the morphological characters of the black type of vipers from the Kharkov Oblast’ and the common adder from the north of the Sumy Oblast’, the investigators “revealed the significant differences” between these types of the vipers. The authors were guided by the following distinctive characters: the body length of the new-born females and males; the maximum body length of the mature females and males; the number of ventralia for males and females separately; the number of dorsale rows around the middle of the body; the number of supralabialia. Grubant et al. (1973) believed that “the additional criteria which makes the determination more simple” is the height of the second and the third supralabialia. According to their data, the second supralabialia of the common adder is usually higher than the third, and as for the black type, it’s vice versa — the second supralabialia is lower than the third. In another paper (Vedmederja et al., 1986) the same authors suggested another variant of the specific name for the viper from the partially wooded steppe of the European part of the USSR — *Vipera nikolskii*. They motivated their suggestion by the fact that the *Vipera nikolskii* “is distributed to the south from the line: Kanev – Kursk – Tambov – Buzuluk (this type is not found to the west and to the east of this line),” and “the name *Coluber prester* Linne, 1761 was given for the black specimens from the Sweden and is not suitable for the viper from the partially wooded

steppe.” In this article the number of differential characters had grown up to seven, and, besides the already mentioned, another three are described: the number of scales around the eyes; the ratio of height to the width of praenasa; the ratio of length to the width of frontale. The investigation of the two “species” of vipers [*Vipera berus* (L.) and *Vipera nikolskii* sp.n.] revealed their significance differences on these characters (the Student’s criteria varies from 5.70 up to 23.62). Unfortunately, the localities of the analysed samples are not mentioned in the paper, and the summarizing of data for males and females for all criteria, besides the number of ventralia, deprived the possibility to compare it with our data.

The papers of Ukrainian zoologists were really revived the interest to the problem among the number of herpetologists. But the opinions about “finding out” the new species had differed, as it often occurs in such cases. Some of the scientists (Ananjeva et al., 1998; Orlova and Semenov, 1999; Bozhanskiy, 2000) included *Vipera nikolskii* Vedmederja, Grubant et Rudaeva, 1986 into the list of species of herpetofauna of the former USSR. Others (Bakiev et al., 2000; Sokolov and Lada, 2000) had doubts in the reasons for separating the viper from partially wooded steppe into the separate species.

MATERIAL AND METHODS

For study of intraspecific variability of the common adder we investigated the peculiarities of the external morphology of the subspecies *V. berus berus* — the “typical” form from the Ryazan Oblast’, from the northwest of Russia and from Bashkortostan and additionally of black viper of partially wooded steppe from Tambov and Voronezh Oblast’s (Oka – Don plain). Fourteen characters recommended for investigation of the common adder of the subgenus *Pelias* (Vedmederja, 1989) were analyzed: the number of ventralia (1), subcaudalia (2), rows of dorsales around the middle of the body (3), supralabialia on the left (4), and on the right (5), sublabialia on the left (6), and on

¹ Tambov State University, Department of Zoology, 392622 Tambov, Russia; E-mail: vipera5@rambler.ru.

TABLE 1. Comparison of Common Adder from Different Parts of Specific Range on Some Morphological Characters

Characters	Tambov Oblast' – – Voronezh Oblast'		Tambov Oblast' – – Ryazan Oblast'		Tambov Oblast' – – Northwest of Russia		Tambov Oblast' – – Bashkortostan		Northwest of Russia – – Ryazan Oblast'		
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	
Males											
1	1.61	>0.05	7.76	<0.001	7.35	<0.001	5.04	<0.001	1.09	>0.05	
2	0.86	>0.05	3.31	<0.01	2.53	<0.05	3.05	<0.01	1.31	>0.05	
3	2.11	<0.05	2.93	<0.01	3.80	<0.001	1.40	>0.05	0.18	>0.05	
4	0.21	>0.05	4.00	<0.001	1.69	>0.05	1.56	>0.05	2.15	<0.05	
5	0.38	>0.05	3.48	<0.001	1.67	>0.05	1.31	>0.05	2.27	<0.05	
6	1.43	>0.05	0.33	>0.05	1.81	>0.05	1.29	>0.05	1.30	>0.05	
7	0.95	>0.05	0.94	>0.05	3.53	<0.001	1.20	>0.05	2.12	<0.05	
8	2.24	<0.05	0.59	>0.05	1.10	>0.05	2.30	<0.05	0.28	>0.05	
9	1.18	>0.05	0.99	>0.05	3.63	<0.001	3.56	<0.001	4.68	<0.001	
10	1.30	>0.05	7.69	<0.001	0.89	>0.05	1.95	>0.05	7.02	<0.001	
11	0.03	>0.05	3.60	<0.001	5.69	<0.001	4.59	<0.001	0.97	>0.05	
12	0.27	>0.05	0.98	>0.05	2.36	<0.05	1.69	>0.05	0.89	>0.05	
13	0.95	>0.05	2.53	<0.05	2.64	<0.05	3.55	<0.001	0.27	>0.05	
14	2.47	<0.05	3.42	<0.01	0.11	>0.05	2.88	<0.01	3.25	<0.01	
Females											
1	1.44	>0.05	3.63	<0.001	3.94	<0.001	4.60	<0.001	0.93	>0.05	
2	0.86	>0.05	3.29	<0.01	5.34	<0.001	4.92	<0.001	0.69	>0.05	
3	0.21	>0.05	3.79	<0.001	4.81	<0.001	2.43	<0.05	0.09	>0.05	
4	1.53	>0.05	1.27	>0.05	0.84	>0.05	1.43	>0.05	1.51	>0.05	
5	2.31	<0.05	0.27	>0.05	0.60	>0.05	1.78	>0.05	0.15	>0.05	
6	2.05	<0.05	1.88	>0.05	2.52	<0.05	1.71	>0.05	0.24	>0.05	
7	1.64	>0.05	1.48	>0.05	2.61	<0.05	2.41	<0.05	0.42	>0.05	
8	0.92	>0.05	0.60	>0.05	2.98	<0.01	2.71	<0.01	3.61	<0.001	
9	1.68	>0.05	0.04	>0.05	3.29	<0.01	3.54	<0.001	2.62	<0.01	
10	2.73	<0.01	6.45	<0.001	0.48	>0.05	1.05	>0.05	6.00	<0.001	
11	3.11	<0.01	2.14	<0.05	5.04	<0.001	4.86	<0.001	1.86	>0.05	
12	2.24	<0.05	0.30	>0.05	3.24	<0.01	2.86	<0.01	3.37	<0.001	
13	0.29	>0.05	0.50	>0.05	0.73	>0.05	1.97	>0.05	0.03	>0.05	
14	1.84	>0.05	1.21	>0.05	1.65	>0.05	2.21	<0.05	2.37	<0.05	

the right (7), the ratios of the body's length to the tail's length (8), the head's width to its length (9), the head's length to its maximum width (10), the length of frontale to its width (11), the length of frontale to the distance between front edge of frontale and upper edge of rostrale (12), the height of second supralabialia to the height of third one (13), the height of praenasale to its width (14).

Totally 260 specimens were investigated. 106 specimens (46 females and 60 males) from the Tambov Oblast' and 18 ones (10 females and 8 males) from the Voronezh Oblast' were belonging to the black type of the common adder (so-called "*Vipera nikolskii*"). 30 vipers (11 females and 19 males) from the Ryazan Oblast', 58 ones (27 females and 31 males) from the northwest of Russia and 48 ones (22 females and 26 males) from the Bashkortostan were belonging to the typical form (*Vipera berus*). All the characters were studied separately for males and females. The collections are stored in the Zoological museum of

Tambov State University (samples from Tambov, Voronezh, and Ryazan Oblast's), in the Zoological Institute, Russian Academy of Sciences (samples from the northwest of Russia — Leningradskaya and Pskov Oblast's and Karelia) and in the Zoological Museum of Moscow State University (samples from Bashkortostan). Standard statistical parameters (min – max are limits, *M* is mean value, S.E. is standard error, S.D. is standard deviation, *t* is the Student's criteria) were used (Lakin, 1968).

RESULTS AND DISCUSSION

The results of comparison of the mentioned features in the samples from different parts of the viper's specific range are shown in Table 1. The first impression from the achieved results seems to leave no doubts in the rightness of herpetologists from Kharkov. The males from Tambov

and Ryazan Oblast's are distinguished in nine characters, and differences on five of them have the maximum level of significance ($P < 0.001$). The females from these regions also have morphological differences, though more modest. The comparison of black type of the common adder with the "typical" form inhabiting the northwest of Russia and Bashkortostan gives just the same results. Well, does *Vipera nikolskii* really exist? The analysis of the degree of stability of the characters differentiating the samples under comparison makes us refrain from a positive answer. The stable differences between the "black" and "typical" males in all the three compared groups is noticeable in characters 1, 2, 11, and 13. As for the females from these groups, the differences here are noticeable in characters 1, 2, 3, and 11. So, both sexes of the two types of the common adder in all compared groups are distinguished for certain only in 1, 2, and 11 characters. As for the rest characters (4, 5, 6, 7, 8, 9, 10, 12, and 14), they can not be called "working" because there is no stability in their manifestation. The analysis of the column of the Table 1 showing the results of the comparison of the "typical" form of the common adder from Ryazan Oblast' and from the northwest of Russia testifies that the males are distinguished in six, and females — in five characters. It must be noted that the females of "black" form from Tambov Oblast' are distinguished from the "typical" females from Ryazan Oblast' also in five characters. The comparison of samples of "black" type (from Tambov and Voronezh Oblast's) has also revealed the significant differences in some characters, including the 11 (only the females are distinguished in this character). Moreover, we compared the black vipers from Tambov and Kharkov Oblast's in the character 1 (the number of ventralia). Comparing our data (number of ventralia in males $M \pm m = 149.02 \pm 0.33$, in females 152.76 ± 0.40) with the materials given by Grubant et al. (1973) we identified that the degree of difference between the males isn't significant ($t = 1.68$), but as for the females, it exceeds the third level ($t = 5.07$; $P < 0.001$). Vedmederja et al. (1986) gave a bit different information about the number of ventralia in *Vipera nikolskii* sp.n., and it naturally had resulted in the change of the before established degree of difference of the character in the samples of vipers under comparison. In this case the difference had become significant for males ($t = 2.33$; $P < 0.05$) as well as for females ($t = 2.49$; $P < 0.05$).

The question arises: which of the analyzed characters must be included in the identification of viper's forms? The number of ventralia? The number of subcaudalia? The form of frontale? In spite of the high level of significance of differences, these characters are highly variable, and this fact practically deprives them the possibility to be

used while identifying the analyzed types of the common adder. Besides, the available materials show that many of the analyzed characters have more or less stable connection with the latitude of the region. We compared the six samples of *V. berus* from the different parts of the specific range, and the comparison had revealed the high variability between populations. If the investigations will be continued, I believe the results will be almost the same.

To summarize, the common adder in their great specific range, naturally formed a number of types adequate to the conditions of the environment of the certain region. The level of genotypic and phenotypic differences between such forms is not the same. The problem of separating of new "races," "morphs," "subspecies," "species" is not new and will probably be open for discussion for a long period of time (for certain reasons). According the recommendations for the description of new taxons (Shcherbak, 1989), the number and degree of significance of differences of the mentioned above characters are certainly not enough for attaching the status of the species to the black form of *Vipera berus*. In my opinion, the total combination of these characters allows to separate the black form of the common adder as a subspecies. It will be natural to remain the specific name suggested by Vedmederja et al. (1986) as a subspecies name — *Vipera berus nikolskii*.

REFERENCES

- Ananjeva N. B., Borkin L. J., Darevsky I. S., and Orlov N. L. (1998), *Amphibians and Reptiles*, ABF, Moscow [in Russian].
- Bakiev A. G., Malenev A. L., Peskov A. N., and Gridnev D. V. (2000), "Morphological characteristics of vipers from the forest-park zone of Samara," in: *The Actual Problems of Herpetology and Toxinology. Vol. 4*, Tolyatti, pp. 3 – 8 [in Russian].
- Bannikov A. G., Darevsky I. S., and Rustamov A. K. (1971), *Amphibians and Reptiles of the USSR*, Mysl', Moscow [in Russian].
- Bannikov A. G., Darevsky I. S., Rustamov A. K., Ishchenko V. G., and Shcherbak N. N. (1977), *Key to Amphibians and Reptiles of the USSR Fauna*, Prosveshchenie, Moscow [in Russian].
- Bozhanskiy A. T. (2001), "*Vipera nikolskii*," in: *The Red Data Book of the Russian Federation. Animals*, AST – Astrel', Tver', pp. 348 – 349 [in Russian].
- Grubant V. N., Rudaeva A. V., and Vedmederja V. I. (1973), "On the taxonomic status of the black form of the common

- adder,” in: *Problems of Herpetology*, Nauka, Leningrad, pp. 68 – 71 [in Russian].
- Lakin G. F.** (1968), *Biometry*, Vysshaya Shkola, Moscow [in Russian].
- Orlova V. F. and Semenov D. V.** (1999), *Amphibians and Reptiles*, AST, Moscow [in Russian].
- Shcherbak N. N.** (1989), “The procedures of description of new taxons,” in: *The Guide for Investigation of Amphibians and Reptiles*, Kiev, pp. 89 – 92 [in Russian].
- Shcherbak N. N. and Shcherban M. I.** (1980), *Amphibians and Reptiles of the Ukrainian Carpathians*, Naukova Dumka, Kiev [in Russian].
- Sokolov A. S. and Lada G. A.** (2000), “Reptiles,” in: *Red Data Book of the Tambov Oblast. Animals*, Proletarskii Svetoch, Tambov, pp. 231 – 237 [in Russian].
- Terentjev P. V. and Chernov S. A.** (1949), *Key to Reptiles and Amphibians*, Sovetskaya Nauka, Moscow [in Russian].
- Vedmederja V. I.** (1989), “Vipers of the subgenus *Pelias*,” in: *The Guide for Investigation of Amphibians and Reptiles*, Kiev, pp. 35 – 39 [in Russian].
- Vedmedreja V. I., Grubant V. N., and Rydaeva A. V.** (1986), “On the problem of specific name for the black adder of the partially wooded steppe of the European part of the USSR,” *Trudy Khark. Univ.*, **288**, 83 – 85 [in Russian].

TAXONOMICAL ANALYSIS OF MORPHOLOGICAL VARIETY OF THE SAND LIZARD (*Lacerta agilis*) IN UKRAINE

O. A. Tytov,¹ V. N. Peskov,^{1,2} and A. U. Brovko¹

Keywords: pholidosis, morphology, variety, *Lacerta agilis*, Ukraine.

INTRODUCTION

The four subspecies *L. agilis agilis*, *L. a. chersonensis*, *L. a. euxinica*, and *L. a. exigua* of the sand lizard occur in the Ukraine (Darevsky et al., 1976; Scherbak and Scherban, 1980; Kotenko and Taraschuk, 1982, Kalyabina-Hauf and Ananjeva, 2004). The age and sex composition of populations and questions in connection with taxonomic varieties of the sand lizard in Ukraine are still not clearly understood.

MATERIAL AND METHODS

232 lizards of pure subspecies populations were analyzed to resolve these questions. We have studied 57 lizards of *L. a. agilis* from Zakarpatskaya Oblast', 49 lizards of *L. a. chersonensis* from Zhytomir Oblast', 58 lizards of *L. a. euxinica* from Odessa Oblast', 68 lizards of *L. a. exigua* from Donetsk Oblast'. From each lizard 17 pholidoses, 3 pattern and 18 morphometry features were recorded. We used one- and multimeasured methods for statistical analysis of these data.

The differences were analyzed as integral indices by the Zarapkin Distances and Squared Mahalanobis Distances (The Mahalanobis distance is similar to the standard Euclidean distance measure, except that it takes into account the correlation between variables). The distance matrixes were analyzed by klaster, factor and discriminant analyses.

RESULTS AND DISCUSSION

Young males and females of the sand lizard clearly differ from matures by body proportions. Nevertheless, with age body proportions of lizards from subadultus to senex change gradually. This property is more typical for

L. a. chersonensis and *euxinica* subspecies in comparison with *L. a. agilis* and *L. a. exigua*. Among these age groups there are few lizards with intermediate properties of the same linear dimensions and body proportions. Young lizards have relatively bigger head and eyes and more longer and wider muzzle in comparison with matures. In body proportions they almost have no reliable differences. The reliability to distinguish age groups could be confirmed by the discriminant analysis. It is known that the sex differences increase with the age. For example the Squared Mahalanobis Distances of *L. a. agilis* young males and females is 28.05, while the old males and females of this subspecies have much more Squared Mahalanobis Distances — 40.14. Taxonomical differences were registered in character and degree of sex dimorphism by all features and by some of them as well. For example the differences between the old males and females of *L. a. exigua* are two times as little as the same differences of *L. a. agilis* lizards.

According to the results of discriminant analysis of *L. agilis* males and females by pholidoses and pattern features the maximal differences were registered between *L. a. agilis* and *L. a. exigua* subspecies, and minimal between *L. a. chersonensis* and *L. a. euxinica* subspecies (Table 1).

The most similar by phenotype *L. a. chersonensis* and *L. a. euxinica* subspecies have reliable difference in ratio combinations of postnasalia and frenale scutums ($P < 0.001$) and bilateral combinations of postnasalia ($P < 0.001$) and frenale ($P < 0.01$) scutums. With reliable level 95% ($P < 0.05$) these subspecies have reliable difference by number of subciliaria scutums and scutums which adjoin the 4th submaxilaria scutum, by ratio of central white strip and lateral lines morphs. *L. a. agilis* and *L. a. exigua* have reliable difference by 16 (it is 80%) pholidoses features. For other comparison pairs 13 – 16 features registered the reliable difference.

The morphometry features have had the same result for males and females of all four subspecies. The maximal differences in body proportions were registered between *L. a. agilis* and *L. a. chersonensis*, *L. a. agilis* and *L. a. exigua* (Table 2).

¹ Biological Department, International Solomon University, Sholudenko str. 1-b, 01135 Kiev, Ukraine;

E-mail: oleks@walla.co.il, vladimir.peskov@ukr.net, brovko@i.com.ua

² Zoological Institute, NASU, B. Khmelnytsky str. 15, 01601 Kiev, Ukraine.

TABLE 1. Squared Mahalanobis Distances in Compare of Four Sand Lizard Subspecies by the 17 Pholidoses and 3 Pattern Features

Females	Males			
	<i>agilis</i>	<i>chersonensis</i>	<i>euxinica</i>	<i>exigua</i>
<i>agilis</i>	—	19.03	24.07	31.15
<i>chersonensis</i>	23.04	—	8.94	22.45
<i>euxinica</i>	19.63	10.77	—	16.96
<i>exigua</i>	31.81	16.53	16.25	—

The minimal differences were registered for *L. a. chersonensis* and *L. a. euxinica*. *L. a. chersonensis* occupies a middle part of all subspecies distribution and by body proportions this subspecies is intermediate form among other three subspecies. By the all of body proportions *L. a. euxinica* (which is synonymized with *L. a. chersonensis* in 1984 (Bischoff, 1984)), is a reduced copy of *L. a. chersonensis*, that can be explained by means of ecological peculiarity (living on the sand) of subspecies (Kotenko and Taraschuk, 1982). Although *L. a. euxinica* has shorter, lower and narrower head, it almost hasn't differences from *L. a. chersonensis* by such head features as width of brain part, eye and ear-drum diameter, that has shown on some of mutual pattern adaptations to arid conditions.

Each subspecies can be characterized by the following features: *L. a. euxinica* has the most narrow and low head, short extremities and narrow anal scutum. Females have a big ear opening. *L. a. exigua* has the longest extremities, short frontal and anal scutums, little eye and ear opening. Females have a wide anal scutum. *L. a. agilis* has the longest neck, narrow pileus, and wide distance between nostrils and the biggest height of head. Males have long and wide frontal and anal scutums. Females of *L. a. chersonensis* have the longest head and the biggest one in comparison with the other subspecies female length of frontal, anal and width of frontal scutums.

The discriminant analysis of young and mature *L. a. agilis* and *L. a. exigua* subspecies lizards has shown, that Squared Mahalanobis Distances value used to be determined by taxonomical lizard's attribute, then by sex of animals and their age. By the results of our work the following conclusions could be made.

1. The age differences of the sand lizard in body proportions can be divided into the two age groups: young (subadultus and adultus 1) and mature (adultus 2 and senex).

2. *L. a. euxinica* in comparison with *L. a. chersonensis* has the most variable age groups by morphology. The increasing of these subspecies differentiation level with the age has been registered.

3. The sex differences become evident both for the body proportions and individual morphometry features. The level of sex differences increases with the age.

TABLE 2. Squared Mahalanobis Distances in Compare of Four Sand Lizard Subspecies by the 18 Morphometry Features

Females	Males			
	<i>agilis</i>	<i>chersonensis</i>	<i>euxinica</i>	<i>exigua</i>
<i>agilis</i>	—	33.00	15.41	31.75
<i>chersonensis</i>	29.79	—	12.75	28.49
<i>euxinica</i>	23.36	8.22	—	17.89
<i>exigua</i>	24.19	29.73	26.93	—

4. In pholidoses and body proportions features *L. a. agilis* and *L. a. exigua* are the most variable subspecies and the least variable are *L. a. chersonensis* and *L. a. euxinica*.

5. We think that level and degree of *L. a. agilis*, *L. a. exigua*, and *L. a. chersonensis* divergence confirm their subspecies status by the all folidoses features and body proportions. *L. a. chersonensis* and *L. a. euxinica* have a less degree of difference, so we think that they should be considered as the biotopical forms, which are at the initial forming stage by morphology divergence level. The *L. a. chersonensis* subspecies by both the body proportions and pholidoses features will keep intermediate status for a long time yet.

Acknowledgments. Authors would like to thank the Director of Zoological museum of the National Museum of Natural History Prof. Eugen Pisanets and senior reserch scientist of the Institute of Zoology of National Academy of Science of Ukraine Dr. Tatjana Kotenko for offered an opportunity to work with scientific collections. Special sincere gratitude to International Solomon University rector, Professor Alexandr Rozenfeld for the sponsor assistance.

REFERENCES

- Bischoff W.** (1984), “*Lacerta agilis* Linnaeus 1758 — Zauneidechse,” in: Böhme W. (ed.), *Handbuch der Amphibien und Reptilien Europas*, AULA Verlag, Wiesbaden, pp. 23 – 68.
- Darevsky I. S. et al.** (1976), “Systematic and intraspecific structure,” in: A. V. Yablokov (ed.), *Sand Lizard*, Nauka, Moscow, pp. 53 – 96 [in Russian].
- Kalyabina-Hauf S. A. and Ananjeva N. B.** (2004), *Phylogeography and Intraspecific Structure of Wide Distributed Sand Lizard, Lacerta agilis L., 1758 (Lacertidae, Sauria, Reptilia). Case Study of Mitochondrial Cytochrom b Gene*, St. Petersburg [in Russian].
- Kotenko T. I. and Taraschuk C. V.** (1982), “New subspecies of the sand lizard in USSR fauna *Lacerta agilis euxinica* Fuhn et Vancea, 1964 (Reptilia, Lacertidae),” *Vestnik Zool.*, 6, 33 – 37 [in Russian].
- Scherbak N. N. and Scherban M. I.** (1980), *Amphibians and Reptiles of Ukrainian Carpathians*, Naukova Dumka, Kiev [in Russian].

ARE THERE MORPHOLOGICAL DIFFERENCES BETWEEN TWO GENETICALLY DIFFERENTIATED CLADES IN THE ADDER *Vipera berus berus*?

S. Ursenbacher,¹ I. Sasu,¹ M. Rossi,¹ and J.-C. Monney²

The morphologies of 164 adders (*Vipera berus berus*) were analyzed in order to assess if morphological differences occur between the Northern and the Italian clades. Pholidosis and some corporal proportions were measured, corresponding to 17 parameters. Several parameters show significant differences between clades (e.g., subcaudals, labials, sublabials, parietal scales). Moreover, discriminant analyses separate both clades with high degrees of accuracy of a correct classifying.

Keywords: *Vipera berus*, morphology, discriminant analysis.

INTRODUCTION

The adder, *Vipera berus* (Linnaeus, 1758), has the most widespread terrestrial snake in the world (Saint Girons, 1978). Despite this large distribution area, only three sub-species are recognised: *V. b. berus*, *V. b. bosniensis* (Boettger, 1880), *V. b. sachalinensis* (Zarevsky, 1917). Moreover, the nominal subspecies is present from France to middle Russia, with only a low level of morphological variation over approximately 10,000 km (Saint Girons, 1978). Homogeneity within this subspecies was also confirmed by 2 different studies based on genetic markers (Joger et al., 2003; Ursenbacher et al., submitted), except adders in Italy, Northern Slovenia, southern Austria and extreme southeastern Switzerland (the Italian clade). Given the high degree of genetic differentiation (the split between the two clades occurred more than 1 Million years ago), the aim of this short note is to compare the morphology of these two clades.

MATERIAL AND METHODS

Measurements. We measured 164 *Vipera berus berus* deposited in the Natural History Museum of Geneva, Switzerland (MHNG): 101 (54 females and 47 males) from the Northern clade and 63 (35 females and 28 males) from the Italian clade described in Ursenbacher et al. (submitted) and Joger et al. (2003). Clade was assigned according to the location of the collected animal. The number of ventrals (V), subcaudals (C), apicals (A), parietals (P) intercanthals scales (I), as well as, on both sides, the

total number of loreals (L), canthals (CA), peri-oculars (PO), supralabials (SUP), sublabials (SUB) and parafrontals scales (PA) were counted according to the method used by Saint Girons (1978).

The number of rows between the peri-oculars and the supralabials was also counted on both sides (RO). Altogether, 12 parameters describing head scale patterns were measured. Moreover, the total length (LOT.C), the length of the tail (LO.Q), the snout-vent length (SVL), and head length (LO.T) and width (LR.T) were measured; from these, the proportion of length composed of the tail (%TAIL = LO.Q/LOT.C) and the head (%HEAD = LO.T/LOT.C) and the relative width of the head (%W.T = LR.T/LO.T) were calculated. Only sub-adults and adults, defined by a total length >300 mm were included in analyses.

Statistical analyses. All statistical analyses were done using SPSS 11.0.1 for MacOSX (SPSS Inc.). Analyses were conducted with data for males and females together, as well as for each sex separately. First the SVL were compared between clades. Subsequently, comparisons between clades for all parameters were done using Student's

TABLE 1. Probabilities of *t*-Tests or the Wilcoxon Tests for Parameters with at Least One Significant Value

	Female and male probability		Female probability		Male probability	
V	0.020	*	0.062	NS	0.064	NS
C	0.000	***	0	***	0	***
L	0.001	***	0.029	*	0.009	**
SUP	0.012	*	0.122	NS	0.003	***
SUB	0.002	***	0.017	*	0.012	*
I	0.001	***	0.005	***	0.089	NS
P	0	***	0.002	***	0.013	*
%TAIL	0.119	NS	0.036	*	0.358	NS

NS, $P > 0.05$; * $p = 0.05$; ** $p = 0.01$; *** $p = 0.005$.

¹ Laboratoire de Biologie de la Conservation, Institut d'Ecologie, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland; E-mail: sylvain.ursenbacher@unil.ch.

² Centre de Coordination pour la Protection des Amphibiens et des Reptiles de Suisse (KARCH), CH-3005 Bern, Switzerland.

t-test, Welch's *t*-test and Wilcoxon's test. Scale pattern measurements and ratios (%TAIL, %HEAD, %W.T) were analyzed with discriminant.

RESULTS

Comparison between clades. SVL did not differ between clades either when males and females were analyzed together ($Z = -0.507, p = 0.612$) or when considered separately ($Z = -0.269, p = 0.788$ for females, $Z = -0.991, p = 0.321$ for males). All significant differences between the two clades are shown in Table 1. Significant differences for both sexes both combined and split were observed for the number of subcaudals (C), loreals (L), sublabials (SUB) and parietals (P). Only males showed a difference in supralabials (SUP), while only females differed in intercanthals scales (I) and the proportion of the tail (%TAIL). When males and females were combined, the number of ventral scales (V) differed significantly between clades although neither males nor females alone showed a significant difference.

Discriminant analyses. The result of the discriminant analysis grouping males and females is shown in Fig. 1. Group membership was predicted correctly in 68.5 to 85.7% of cases. When the same analyses were done on females and males separately, the predicting accuracy was 83.3 – 88.6% for females and 91.5 – 92.9% for males.

DISCUSSION

The Northern and Italian clades seem to have several morphological differences as shown above, some of which

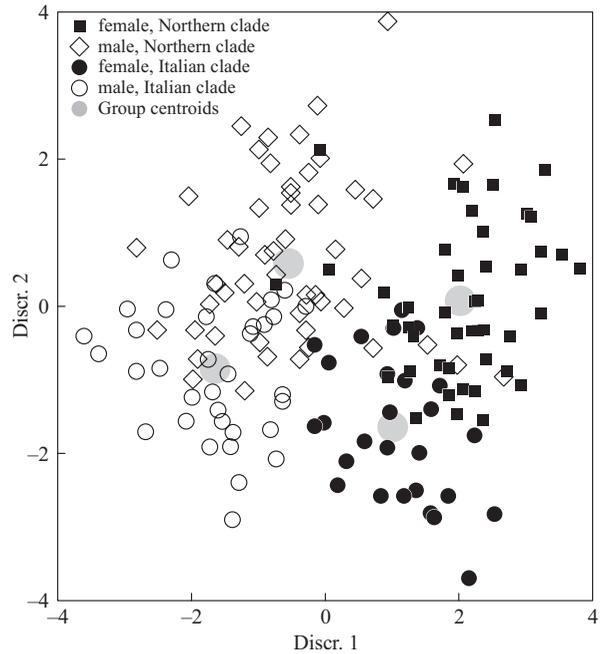


Fig. 1. Discriminant analysis with all animals (Discr. 1: 77.8% of the variance; Discr. 2: 20.8% of the variance; Wilks' $\lambda = 0.1836, p < 0.0005$).

might be useful to morphologically determine the clade of an animal. Adders from the Italian clade have a lower number of subcaudals (about 3 subcaudals less than the Northern clade), higher number of loreals and sublabials, a lower number of intercanthals, and the parietals are less split (Table 2). The number of ventral scales also seems to be lower in the Italian clade, but this difference is not significant when the analysis is conducted on males and females separately. Saint Girons (1978) has already ob-

TABLE 2. Measurements on Females and Males of Each Clade, with the Mean, Standard Deviation, Minimum and Maximum Observed

Group		V	C	L	SUP	SUB	I	P
Females								
Italian clade <i>n</i> = 35	Mean	145.77 ± 3.23	27.63 ± 2.13	6.71 ± 2.47	17.63 ± 0.84	20.89 ± 1.37	6.91 ± 2.62	1.97 ± 0.38
	Minimum	137	23	2	16	19	2	0
	Maximum	153	34	11	19	24	14	3
Northern clade <i>n</i> = 54	Mean	147.15 ± 3.55	30.50 ± 3.59	5.54 ± 2.40	17.17 ± 1.61	20.00 ± 2.06	8.70 ± 3.26	2.46 ± 0.86
	Minimum	139	21	1	12	15	4	2
	Maximum	155	39	11	20	25	18	7
Males								
Italian clade <i>n</i> = 28	Mean	142.43 ± 3.73	34.36 ± 2.39	5.89 ± 2.60	17.93 ± 1.15	21.21 ± 2.17	6.82 ± 2.63	2.00 ± 0.27
	Minimum	134	29	2	16	15	3	1
	Maximum	149	39	12	22	26	12	3
Northern clade <i>n</i> = 47	Mean	144.11 ± 3.54	36.81 ± 2.71	4.47 ± 1.99	17.11 ± 1.03	19.96 ± 1.77	7.91 ± 2.69	2.47 ± 0.95
	Minimum	135	30	0	15	16	2	2
	Maximum	152	42	9	19	23	16	6

served a lower number of ventrals in adders belonging to the Italian clade. Scali and Gentilli (1999) also showed a lower number of ventrals in specimens from the Po plane (northern Italy).

However, the number of analyzed animals is not very high and the majority of adders of the Italian clade come from the Graubunden region (extreme southeastern Switzerland), and might be different from adders from the rest of the distribution of the Italian clade. Due to a low number of animals per location, it is not possible to analyze the present dataset in detail. The number of samples of each clade, especially animals from Russia, Scandinavia, Italy, Slovenia and Austria should also be increased for better resolution of the local morphological differences. Finally, adding more morphological characters such as dorsal pattern (see Nilson and Andrén, 2001, for *V. ursinii* group) might also reveal more differences within *V. b. berus*. Nevertheless, these preliminary analyses suggest morphological differentiation in *V. b. berus* following genetic splitting.

Acknowledgments. This work was funded by grants from the Swiss National Foundation (grant No. 3100-059132.99/1). We acknowledge the Muséum d'Histoire Naturelle of Geneva (Switzerland) and its curator, Mrs. Fisch-Muller, for the loan of its collection, as well as Philippe Golay and two anonymous referees for their comments.

REFERENCES

- Boettger O.** (1880), "Die Reptilien und Amphibien von Syrien, Palästina und Cypern," *Ber. senckenb. naturf. Ges Frankfurt*, **1879/1880**, 132 – 219.
- Joger U., Kalyabina S., Schweiger S., Mayer W., Orlov N. L., and Wink M.** (2003), "Phylogeny of the Eurasian *Vipera* (Subgenus *Pelias*)," in: *Abstrs. of the 12th Ordinary General Meeting, August 12 – 16, 2003*, Soc. Eur. Herpetol., St. Petersburg.
- Linnaeus** (1758), *Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*, Vol. 1, 10th Edition.
- Nilson G. and Andrén C.** (2001), "The meadow and steppe vipers of Europe and Asia — the *Vipera* (*Acridophaga*) *ursinii* complex," *Acta Zool. Hung.*, **47**, 87 – 267.
- Saint Girons H.** (1978), "Morphologie externe comparée et systématique des Vipères d'Europe (Reptilia, Viperidae)," *Rev. Suisse Zool.*, **85**, 565 – 595.
- Scali S. and Gentilli A.** (1999), "Morphometric analysis and sexual dimorphism of extinct adders (*Vipera berus*) of the Po plane (Northern Italy)," in: Miaud C. and Guyétant R. (eds.), *Current Studies in Herpetology. Proc. of the 9th Ordinary General Meeting of the Soc. Eur. Herpetol.*, Soc. Eur. Herpetol., Le Bourget du Lac, pp. 391 – 396.
- Ursenbacher S., Carlsson M., Helfer V., Tegelstrom H., and Fumagalli L.** (submitted), "Phylogeography and Pleistocene refugia of the Adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data," *Mol. Ecol.*
- Zarevsky S. F.** (1917), "Formes nouvelles du genre *Vipera*, trouvées dans l'empire russe: *Vipera tigrina* n.sp. and *Vipera berus* var. *sachalinensis* var nova?" *Ann. Mus. Zool. Acad. Petrograd*, **21**.

MOLECULAR-GENETIC CHARACTERISTICS OF LIZARD RIBOSOMAL DNA

A. S. Voronov,¹ G. A. Voronova,¹
N. S. Kupriyanova,¹ and A. P. Ryskov¹

Keywords: lizards, ribosomal DNA, internal transcribed spacers.

INTRODUCTION

Remarkable changes in sequence composition of different genome components took place in the process of transition between cold- and warm-blooded vertebrates. The possible way to study this transition on the molecular level today is to compare mammalian or avian DNA sequences with homologous sequences in *Xenopus*, the only cold-blooded vertebrate having enough sequences in data banks. Ribosomal DNAs (rDNAs), and particularly internal transcribing rDNA spacers (ITS-1 and ITS-2), are commonly used in comparative genomic studies of different taxa providing valuable data for phylogenetic and systematic constructions. Unfortunately, information on sequence organization of rDNA in reptiles is also very limited. Here, we present first data on sequence organization of rDNA monomers and its ITS-1 and ITS-2 regions for several lizard species.

MATERIAL AND METHODS

Darevski nairensis, *Darevski valentini*, *Lacerta strigata*, and *Laudakia caucasia* total genomic DNAs were isolated from the lizard blood samples, according to Mathew (1984). Two oligonucleotide primers 5'-AGTCCCTGCCCTTTGTACACA-3' and 5'-GCCGCGTCTGATCTGAGGTC-3' were designed and used for PCR amplification of the rDNA segment, containing ITS-1 and ITS-2. The oligonucleotide probes RI, RII, RIII, and RIV used in blot-hybridization experiments were the same as described earlier (Kupriyanova et al, 1996). PCR amplification and blot hybridization were carried out as previously described (Kupriyanova et al., 1999). Comparison between 3'18S-ITS-1-5.8S-ITS-2 regions of the *L. strigata*, *Xenopus laevis*, and *Rattus norvegicus* was performed after their alignment with the use of "DNA Star" program (Clustal).

RESULTS AND DISCUSSION

Figures 1 and 2 show restriction maps and blot-hybridization patterns obtained for *D. nairensis* and *Laudakia caucasia* lizard rDNAs. Hybridizations were carried out with the use of oligonucleotide probes complementary to the most conservative transcribed regions of rDNA and different restriction enzymes. The conservative EcoR I sites characteristic of other vertebrate 18S and 28S rDNAs are found in all lizards studied. Positions of some other restriction sites are similar for *D. nairensis* and *D. valentini* rDNA, but differ for rDNA of *Laudakia caucasia*. The blot-hybridization data obtained allow to conclude that rDNA monomers in *D. nairensis* and *D. valentini* are about 15 – 16 kb and rDNA monomers in *Laudakia caucasia* are about 10 kb in length.

To study rDNA regions containing 3' end of 18S rDNA, ITS-1, 5.8S rDNA, and ITS-2 in different lizard species, PCR amplification with primers to conservative 18S and 28S rDNA sequences was used. Here, only completed data for *L. strigata* are presented. The amplified 2 kb DNA fragment of *D. strigata* was cloned into T-Easy vector (Fermentas), sequenced and compared with homologous segments of *Xenopus laevis* and *Rattus norvegicus* rDNAs (Fig. 3). Our calculations showed that total percentage of similarity of 3'-18S, 5.8 rDNA, and ITS-1 + ITS-2 regions between these organisms is about 89, 94, and 16.5%, respectively. It is interesting that the great bulk of the coincident nucleotides in ITS-1 + ITS-2 is "G" and "C" whereas only 6 coincident "A" and 4 coincident "T" are detected among 302 identical positions in ITS regions. Surprisingly, but similarity between ITS regions of *L. strigata* and *Rattus norvegicus* comprises 45% and this is much higher than pairwise similarity between ITS regions of *L. strigata*/*Xenopus laevis* and *Xenopus laevis*/*Rattus norvegicus* that comprise 21 and 24% respectively. Of course, rDNA sequence information for other lizard species is necessary to make evolutionary comparisons, and this work is now in progress.

¹ Institute of Gene Biology, Russian Academy of Sciences, Vavilov str. 34/5, 119334 Moscow, Russia; E-mail: ryskov@mail.ru.

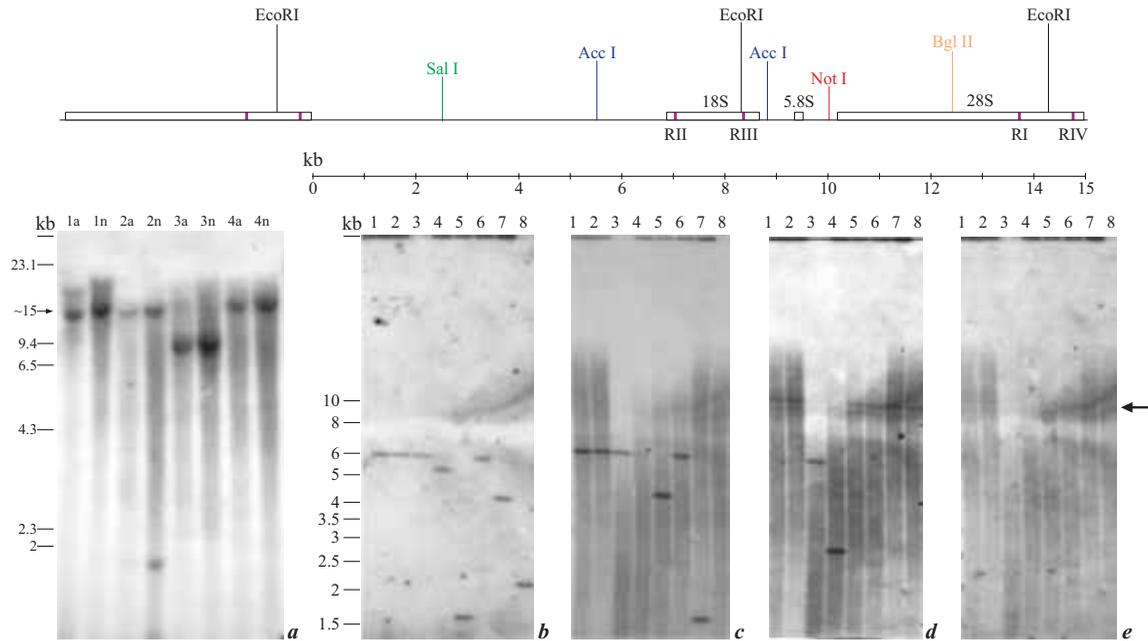


Fig. 1. Organization of a 15-kb repeating ribosomal DNA unit of rock lizards. Identical results were obtained for *D. narensis* and *D. valentini* lizards. *a*: *D. narensis* lizard DNA after digestion with HindIII (1); BamHI (2); EcoRI (3); KpnI (4) was blot-hybridized with labelled oligonucleotide RII. Identical sizes of the hybridizing fragments on lanes 1a – 1n, 2a – 2n, and 4a – 4n let to conclude that putative length of repeating rDNA unit in rock lizards approximates to 15 kb (shown by the arrow). *b – e*: Digestion of *D. narensis* genomic DNA with EcoRI (1); EcoRI + Pst I (2); EcoRI + SalGI (3); EcoRI + AccI (4); EcoRI + BglII (5); EcoRI + EcoRV (6); EcoRI + Not I (7); EcoRI + Xho I (8) and successive blot-hybridization with RI (*b*), RIII (*c*), RII (*d*), or RIV (*e*). Positions of the probes used for hybridization are shown on the rDNA restriction map above. The right arrow in (*e*) shows location of the 10 kb hybridizing fragments.

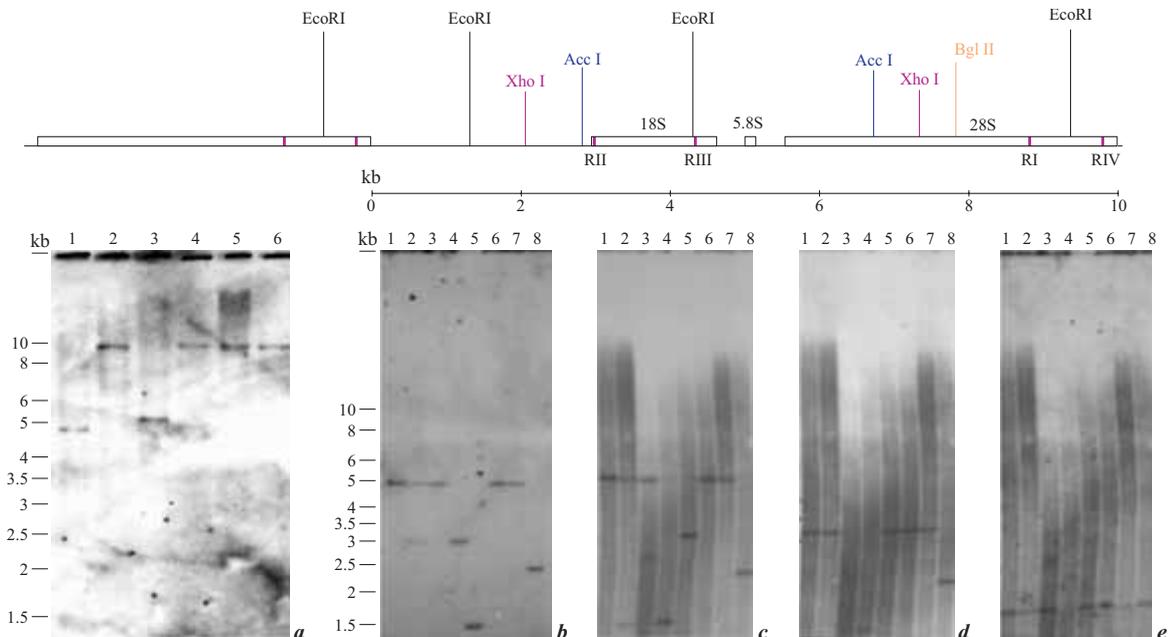


Fig. 2. Organization of a 10 kb repeating ribosomal DNA unit of *Laudakia caucasica*. *a*: *Laudakia caucasica* DNA after digestion with EcoRI (1); Bgl II (2); BamHI (3); Pvu II (4); KpnI (5); HindIII (6) was blot-hybridized with labelled oligonucleotide RII. Identical sizes of the hybridizing fragments on lanes 2 and 4 – 6 let to conclude that putative length of repeating rDNA unit in *Laudakia caucasica* lizards approximates to 10 kb. *b – e*: Digestion of lizard genomic DNA with EcoRI (1); EcoRI + SalGI (2); EcoRI + Pst I (3); EcoRI + AccI (4); EcoRI + Bgl II (5); EcoRI + EcoRV (6); EcoRI + NotI (7); EcoRI + Xho I (8) and successive blot-hybridization with RI (*b*), RIII (*c*), RII (*d*), or RIV (*e*). Positions of all these specific oligonucleotide probes are shown on the rDNA restriction map.



Fig. 3. Alignment of the rDNA regions containing 3' end of 18S rDNA, ITS-1, 5.8S rDNA, and ITS-2 from *Rattus norvegicus* (Rat), *Lacerta strigata* (Str), and *Xenopus laevis* (X.L.) using “DNA Star” program (Clustal). The identical nucleotide positions are shown in dark gray, pairwise homology in light gray, and absence of homology in black.

Acknowledgments. This research was partly supported by grants from Russian Foundation for Basic Research (grant No. 02-04-48516); Ministry of Industry, Science and Technology (1995.2003.4 and 43.073.1.1.2501); Academy Programm on Physical and Chemical Biology (10002-251/II-10/143-142/010403-046); State Programms on Integration (CH0064/885 and YA0033/1990). Authors are thankful to Dr. I. A. Martirosyan for a kind gift of DNA samples of some lizards.

REFERENCES

- Arribas O. J. (1999), "Phylogeny and relationship of the mountain lizards of Europe and the Near East and their relationships among the Eurasian Lacertid lizards," *Russ. J. Herpetol.*, **6**(1), 1 – 22.
- Darevsky I. S. (1993), "Evolution and ecology of parthenogenesis in reptiles," in: Adler K. (ed.), *Current Research in Biology of Amphibians and Reptiles*, Oxford, pp. 209 – 257.
- Kupriyanova N. S., Netchvolodov K. K., Kirilenko P. M., Kapanadze B. I., Yankovsky N. K., and Ryskov A. P. (1996), "Intragenomic polymorphism of ribosomal RNA genes for human chromosome 13," *Mol. Biol.*, **30**, 51 – 60 [in Russian].
- Kupriyanova N. S., Netchvolodov K. K., Kirilenko P. M., and Ryskov A. P. (1999), "Microsatellite (TTGC)₄, specific for the intergenic spacer of human and chimpanzee rDNA," *Mol. Biol.*, **33**, 314 – 318 [in Russian].
- Mathew C. G. P. (1984), in: Walker J. M. (ed.), *Methods in Molecular Biology*, L. Humana Press, New York, pp. 31 – 34.

CONTACT ZONE BETWEEN TWO SUBSPECIES OF THE SAND LIZARD: *Lacerta agilis exigua* EICHW., 1831 AND *Lacerta agilis chersonensis* ANDR., 1832 IN THREE REGIONS OF THE LEFT-BANK UKRAINE

O. I. Zinenko,¹ P. L. Drabkin,² O. M. Rudyk³

Keywords: *Lacerta agilis*, subspecies, zone of intergradation, Ukraine.

INTRODUCTION

The Left-bank Ukraine is known as a territory, where contact zone of two subspecies of *L. agilis* exists (Sukhov, 1928; Sukhov, 1948; Tarashchuk, 1959; Szczerbak, 1966; Darevsky et al., 1976). But in present time only general or occasional observations were conducted. General direction of contact zone spread was suggested as “from Kursk between Kharkov and Poltava to Dnepropetrovsk” (Sukhov, 1948), “to the west of the line passing through Vorozhba station (Sumy Oblast’) and Dnepropetrovsk” (Tarashchuk, 1959), “in Sumy and Kursk Oblast’s and in western parts of Kharkov and Poltava Oblast’s...” (Szczerbak, 1966); “Kursk – Dnepropetrovsk – Crimean peninsula” (Darevsky et al., 1976). Views of different authors on its width of transition are different and range from completely negating its existence (Sukhov, 1948), considering it comparatively narrow (Bischoff, 1988), to believing it to be very extended (Darevsky et al., 1976).

Populations of both subspecies with mixed characteristics exist very close to each other in several locations in Russia and Ukraine (Sukhov, 1928; Pereleshin, 1928; Szczerbak, 1966; Darevsky et al., 1976; Gavrilenko, 1970). A hybrid specimen with morphology of *L. a. chersonensis* but sequence of mitochondrial gene cytochrom b of *L. a. exigua* was found to the northeast of Tula (Kalyabina-Hauf and Ananjeva, 2004).

Drabkin and Bobylev (1989) investigated about 20 populations in the 200 km section of contact zone in Central Ukraine and estimated the width of transition as about 40 – 50 km.

MATERIAL AND METHODS

More than 1000 specimens collected from 100 sites in a section of the contact zone of two subspecies of the sand

lizard — *L. a. exigua* and *L. a. chersonensis* with general extent approximately 300 km in Kharkov, Poltava, and Dnepropetrovsk Oblast’s were analyzed by external morphology features (Fig. 2).

In addition, collections of Museum of Nature at Kharkov University were analyzed. Control samples of *L. a. chersonensis* from sites in the vicinities of Lebedin, Sumy Oblast’ and in the vicinities Nemishaevo, Kiev Oblast’ (70 and 300 km away from the contact zone respectively); *L. a. exigua* from sites in the vicinities of Slatino, Kharkov, and Balakleya, Kharkov Oblast’ (20, 40, and 120 km from contact zone, respectively), and also from a site in Rostov Oblast’, Russia (300 km from the contact zone) were used.

Subspecies in contact zone are clearly distinguished by the type of dorsal pattern. *L. a. exigua* has a pronounced unbroken occipital line and between 14 and 17 scales (average 15 – 16) between parietal lines in the middle of the body (a point determined by dividing the total number of ventral scales rows by 2). Occipital line in *L. a. chersonensis* is absent or broken; there are between 8 and 12 scales (average 10 – 11) between parietal lines in the middle of the body. According to these two signs hybrid indexes (HI) for each specimen were calculated.

$$HI_1 = (L.o._n - L.o._{ch}) / (L.o._{ex} - L.o._{ch}) \times 100\%,$$

$$HI_2 = (S.l.p._n - S.l.p._{ch}) / (S.l.p._{ex} - S.l.p._{ch}) \times 100\%,$$

where $L.o._n$ is presence of occipital line in investigated specimen (0, absent; 1, broken; 2, unbroken and well pronounced); $L.o._{ch} = 0.23529$ is presence of occipital line in *L. a. chersonensis* (the number determined as the mean for the population from Nemishaevo, Kiev Oblast’, approximately 300 km from contact zone); $L.o._e = 2$ is presence of occipital line in *L. a. exigua*; $S.l.p._n$ is number of scales between parietal lines in the middle of the body in investigated specimen; $S.l.p._{ch} = 10.231$ is number of scales between parietal lines in the middle of the body in *L. a. chersonensis* (the number determined as the mean for the population from vicinities of Kolontayev village, left bank of Merla river, Krasnokutsk dis-

¹ Museum of Nature at the Karazin Kharkiv National University, Trinkler st., 8, 61022 Kharkiv, Ukraine;

E-mail: zinenkoa@yahoo.com, zinenko@au.ru.

² Sevastopolskaja str., 30, appt. 35, Dnipropetrovsk, Ukraine.

³ University of California at San Francisco, 2340 Sutter Str., Box 0875, San Francisco, CA 94115, USA;

E-mail: olmrudyk@yahoo.com, amrudik@cc.ucsf.edu.

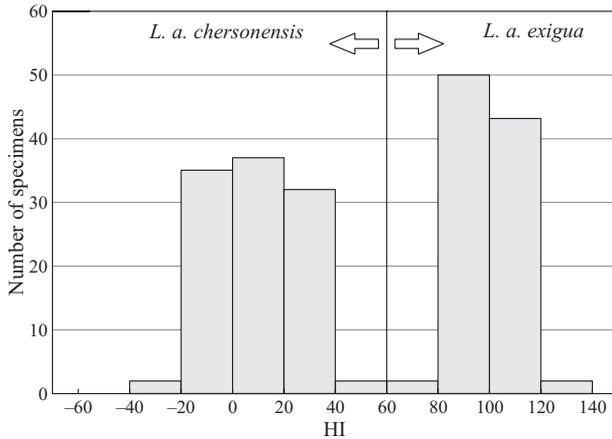


Fig. 1. Distribution of HI in control extracts of *L. a. chersonensis* and *L. a. exigua*.

trict, Kharkov Oblast', site No. 59); and $S.l.p._{ex} = 15.727$ is number of scales between parietal lines in the middle of the body in *L. a. exigua* (the number determined as the mean for the population from vicinities of Chernoglazovka village, Zolokhov district Kharkov Oblast', site No. 96).

Population's hybrid indexes were calculated as the mean of individual HI of specimens. Correlation between population hybrid indexes HI_1 and HI_2 calculated on different signs is high (0.92), so we used the mean. Distribution of HI in control samples *L. a. chersonensis* and *L. a. exigua* is shown in Fig. 1. As we have taken the extreme manifestations of signs as typical for subspecies in order to include slightly deviated populations in the range, value HI of "pure" populations of *L. a. chersonensis* appears slightly bigger than 0% (most frequently 4.5 – 13%), and in *L. a. exigua* slightly smaller than 100% (92 – 97%).

We used 10 – 15 specimens from each site. After investigation the lizards were released in the place of capture. In several cases, when the capture success was low or when we used museum collections, the number of investigated specimens per site was somewhat lower.

RESULTS AND DISCUSSION

On the investigated territory the contact zone is situated on the Dnepr Lowland, Poltava Plane, and southwest edge of Middle Russian Height and spreads from southwest to northeast. *L. a. chersonensis* is distributed to the west and northwest from the contact zone, *L. a. exigua* inhabits the territory to the east and southeast of the contact zone.

The contact zone begins from the left bank of the Dnepr River in the Dnepropetrovsk vicinities (to the south

of Dnepropetrovsk the natural border between subspecies is the Dnepr) and passes to the north, northeast and east along the Oril', the Vorskla and the Kolomak rivers. In the vicinities of Pokrovka village (Kolomak district, Kharkov Oblast') and Alekseevka village (Krasnokutsk district, Kharkov Oblast') the contact zone turns to the north, crosses the watershed of the Kolomak and the Merla rivers and switches from the left to the right side of the Merla valley in the area of confluence with the Sukhoi Merchik river. Then, it goes along the Merla – Vorskla watershed and further in Russia passes through Borisovka (territory "Wood on the Vorskla" of natural reserve "Belogor'e," the Vorskla river valley) (Fig. 2).

In spite of a complete set of specimens with intermediate characteristics in the contact zone and absence of evident barriers between subspecies, the zone is very small and is not more than 20 – 30 km wide. Abrupt replace of one subspecies by another takes place within the zone 10 to 15 km wide, in some cases even narrower (sites Nos. 22 – 25, 33 – 36, 47 – 50, 79 – 80) (Fig. 2).

Variability of external morphology patterns seems to depend on the level of isolation and migration of specimens from neighboring populations. Distribution of hybrid index in populations in contact zone is unimodal, but its range depends on structure of the site, too. Distribution of HI on site No. 44, the territory connected with neighboring lizard populations, and on site No. 61, the territory isolated from other populations for quite a long period (at least 25 years) by landscapes that make an insurmountable barrier for lizards is displayed on Fig. 3.

It is generally believed that disjunctions of ranges and subspecies' differentiation in Europe have taken place under the influence of great climate changes in Pleistocene. The total range of species have been divided and isolated in refugia, where populations diverged and gave birth to new taxa (Hewitt, 1999). According to Darevsky et al. (1976) ancestors of *L. a. chersonensis* survived cold periods in the Balkan refugium, and *L. a. exigua* stepped back to Caucasus. Then, after the warming of climate, lizards dispersed from these two refugia, met and have created a wide intergradation zone. But the intergradation zone was found to be not as wide as it was expected. Small width of intergradation zone may indicate that it is relatively young or that there is certain isolation between these subspecies.

An extremely interesting fact is the coincidence of the contact zone with the orographic (relief) borders. The contact zone in the northern part of Kharkov Oblast' parallels the southwestern edge of Middle Russian Height. The contact zone in Poltava and Dnepropetrovsk Oblast's is associated with relief, too. Here the transition from *L. a. exigua* to *L. a. chersonensis* often occurs when absolute altitude

decreases from 100 – 130 m in water shed to 70 – 100 m in the Dnepr, the Oril', and the Vorskla valleys. This dependence could be the result of ecological differences between subspecies.

The borders of phenetically similar lizard population groups coincide with elevations — Middle Russian, Donetskii Kryazh, etc. (Baranov, 1982); our data corroborates the same tendency in the subspecies case. Present reconstructions of ecosystems that existed during the last glacial maximum (24 – 12 thousand years ago) show that forest refugia existed on the East European plane and were associated with the same elevations (Markova et al., 2002). Fossil remnants of *Lacerta agilis* prove that lizards did not disappear completely in Eastern Europe during the whole Pleistocene (Ratnikov, 2002).

In accordance with our hypothesis the presence of the occipital line in the Eurasian green lizards (*Lacerta sensu stricto*) is one of the characteristic features of continental taxa and its absence is common to the members of *Lacerta sensu stricto* comparatively more adapted to humid and warm climate (Rudyk, in preparation). If their ranges overlap, taxa with pronounced occipital line can occupy more dry areas and terrains. It follows from the data published for Romania (Fuhn and Mertens, 1959), Greece (Strijbosch, 2001), Turkey (Schmidtler, 2001), and from our observations in Ukraine.

Thus, during glacial periods *L. a. exigua* probably could survive in small refugia in eminent territories, such as Central Russian Uplands, and after the postglacial softening of climate recolonize those territories faster than *L. a. chersonensis*. A "softer" *L. a. chersonensis* was stepping back to the territories with a milder climate in the South and in the West. Spreading of *L. a. chersonensis* after warming took place mainly along the valleys of big rivers and flatlands.

A remarkable corroboration of such hypothesis is the similar distribution in investigated region several taxa of amphibians and reptiles, which could be split into two groups:

1) new colonists from the west — *Vipera berus* Linnaeus, 1758, *L. a. chersonensis*, *Lacerta viridis* Laurenti, 1768, western form of *Pelobates fuscus* Laurenti, 1768 (Borkin et al., 2003);

2) taxa that probably survived in refugia in Eastern Europe — *Vipera nikolskii* Vedmederya, Grubant et Rudaveva, 1986, *L. a. exigua*, eastern form of *P. fuscus* (Borkin et al., 2003).

The distribution of species of the first group is connected with young and homogenous postglacial landscapes, while the second one is connected with older landscapes of former refugia.

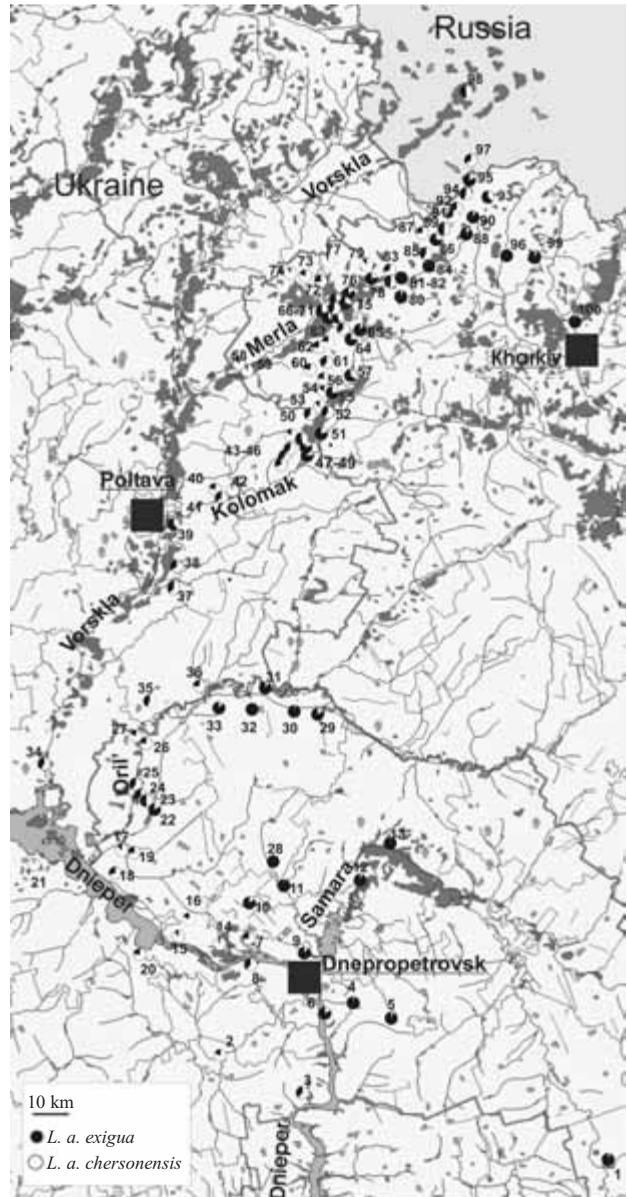


Fig. 2. The contact zone of *L. a. chersonensis* and *L. a. exigua* with collecting sites.

An interesting fact that supports the hypothesis of similar colonization history of *V. nikolskii* and *L. a. exigua* in Left-Bank Ukraine is the discovery of *L. a. chersonensis* – *L. a. exigua* hybrid population (population HI = 15.2%, several specimens are well-pronounced hybrids with HI up to 37.8%) together with *V. nikolskii* in the vicinities of Lubny, Poltava Oblast', 122 km to the west of contact zone. Also in the vicinities of Lubny specimens with the sign peculiar to *L. a. exigua*, namely females

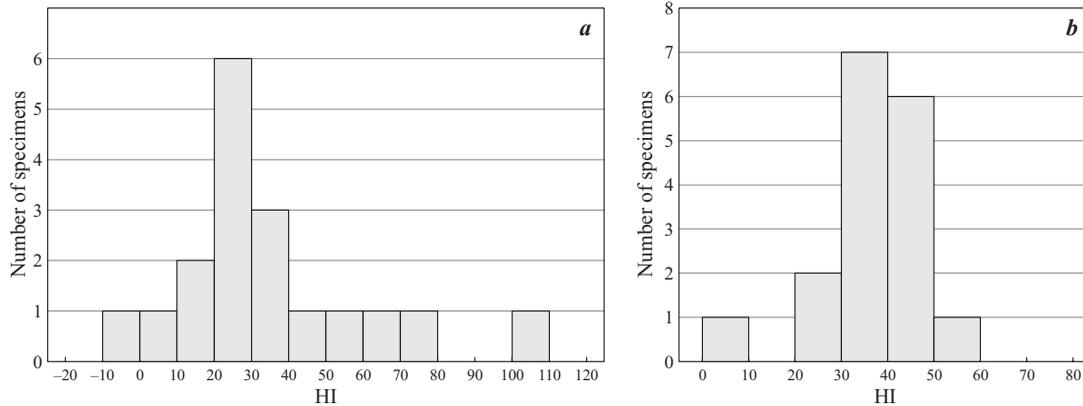


Fig. 3. Distribution of hybrid index (HI) in population from site No. 44, mean HI for population 36.3% (a), and site No. 61, mean HI for population 38.8% (b).

with green back (abber. *viridicapitilis*, Sukhov, 1928) are common.

REFERENCES

- Baranov A. S.** (1982), "Phenogeography and reconstruction of species history," in: *Fenetika Populatsii [Fenetics of Populations]*, Nauka, Moscow, pp. 201 – 214 [in Russian].
- Bischoff W.** (1988), "Zur Verbreitung und Systematik der Zauneidechse, *Lacerta agilis* Linnaeus, 1758," *Mertensiella*, **1**, 13.
- Borkin L. J., Litvinchuk S. N., Rosanov J. M., Khalturin M. D., Lada G. A., Borissovsky A. G., Faizulin A. I., Kotserzhinskaya I. M., Novitsky R. V., and Ruchin A. B.** (2003), "New data on the distribution of two cryptic forms of the common spadefoot toad (*Pelobates fuscus*) in Eastern Europe," *Russ. J. Herpetol.*, **10**(2), 115 – 122.
- Darevsky I. S. et al.** (1976), "Systematic and intraspecies structure," in: Jablovok A. V. (ed.), *The Sand lizard. Monographic Description of Species*, Nauka, Moscow, pp. 53 – 95 [in Russian].
- Drabkin P. L. and Bobylev U. P.** (1989), "Phenogeographical investigation of *Lacerta agilis* in subspecies' *L. a. exigua* and *L. a. chersonensis* contact zone (left-bank Ukraine)," *Vopr. Gerpetol.*, **7**, 81 – 82 [in Russian].
- Fuhn J. E. and Mertens R.** (1959), "Studien an *Lacerta trilineata* aus Rumanien mit Beschreibung einer neuen Unterart," *Senck. Biol.*, **40**, 25 – 42.
- Gavrilenko N. I.** (1970), *Vertebrates of Poltava Town and Their Urbanization*, Izd. KhGU, Kharkov [in Russian].
- Hewitt G. M.** (1999), "Post-glacial re-colonization of European biota," *Biol. J. Linn. Soc.*, **68**, 87 – 112.
- Kalyabina-Hauf S. A. and Ananjeva N. B.** (2004), *Phylogeography and Intraspecies Structure of Wide Distributed Sand Lizard, Lacerta agilis L., 1758 (Lacertidae, Sauria, Reptilia). Case Study of Mitochondrial Cytochrom b Gene*, St. Petersburg [in Russian].
- Markova A. K., Simakova A. N., and Puzachenko A. U.** (2002), "Ecosystems of Eastern Europe during Late Glacial Maximum of Valdai glaciation (24 – 28 thousands years ago) on floristic and teriological data," *Dokl. RAN*, **386**(5), 681 – 685 [in Russian].
- Pereleshin S.** (1928), "Essay on a biometrical analysis of the term "underspecies" (subspecies). Referring to the relationship between *Lacerta agilis agilis* Wolf and *L. a. exigua* Eichw.," *Rev. Zool. Russe*, **8**(1), 39 – 71 [in Russian].
- Ratnikov V. Yu.** (2002), "Late cenozoic amphibians and reptiles of the East-European Plain," *Trudy NII Geologii VGU (Voro-nezh)*, **10**, 138 [in Russian].
- Szczerbak N. N.** (1966), *Amphibians and Reptiles of Crimean Peninsula*, Naukova dumka, Kiev [in Russian].
- Schmidtler J. F.** (2001) "Zur Verbreitung, Habitatwahl und Chorologie der türkischen Smaragdeidechsen (*Lacerta sensu stricto*)," *Mertensiella*, **13**, 165 – 187.
- Strijbosch H.** (2001). "Zur unterschiedlichen Habitatwahl von *Lacerta trilineata* und *Lacerta viridis* in Ostgriecheland," *Mertensiella*, **13**, 159 – 164.
- Sukhov G. F.** (1928), "Materials to investigation of herpetofauna of Poltava Oblast," *Zb. Poltav. Muz.*, **1**, 251 – 256 [in Ukrainian].
- Sukhov G. F.** (1948), "Review of lizards *Lacerta* (Sauria) subgenus in the USSR," *Trudy Zool. Inst. AN SSSR*, **7**, 101 – 117 [in Russian].
- Tarashchuk V. I.** (1959), *Fauna of Ukraine. Vol. 7. Amphibians and Reptiles of Ukraine*, Izd. AN UkrRSR, Kyiv [in Ukrainian].

GEOGRAPHICAL COMPARISON AND BODY SIZE DIFFERENTIATION IN THE EUROPEAN WHIP SNAKE, *Hierophis viridiflavus*, FROM CENTRAL AND SOUTHERN ITALY.

M. A. L. Zuffi,¹ S. Fornasiero,¹ P. Carlino,¹ A. Bonacci,²
C. Corti,³ E. Sperone,² and S. Tripepi²

Significant geographic differences have been found, using standard head and body parameters, between separated populations of the European whip snakes from central, continental southern Italy and Sicily. Sexual Size Dimorphism is very marked. Whip snakes of southern continental Italy and Sicily are shorter than those from central Italy, while head shape did not differ significantly within the studied populations. All the measured southern snakes were melanistic. Data set is congruent with the hypothesis of a subspecific differentiation of the European whip snake southern populations.

Keywords: Geographic variation, Italy, *Hierophis viridiflavus*.

INTRODUCTION

The European Whip snake is the most widespread Italian colubrid species distributed throughout the country. This snake is also present in northern Spain, central and southern France, southern Switzerland, and to the northern Adriatic coast of the former Yugoslavia (Heimes, 1993). The different Italian habitats, that range from the alpine, through the continental and to insular ones, along a thousand kilometer transect, may have led to the evolution of different body shapes patterns (Nagy et al., 2002; Scali et al., 2002). We have preliminarily studied the overall external morphology of the whip snakes from one of the most important Mediterranean “bottle neck” areas, southern Italy and Sicily. The latter have been compared with the snakes of a population of Tuscany (Central Italy). The aim of our study was to test if body shapes differences are present within the Italian peninsular populations (King, 1989; Scali et al., 2002; Boback, 2003), due to the particularly long isolation in the different habitats.

MATERIAL AND METHODS

We studied the overall external morphology (snout to vent length, SVL, total length, ventral and subcaudal scales, head shape, dorsal and ventral color) of about 200

adult snakes, longer than 500 mm SVL, from southern peninsular Italy and Sicily. The latter have been compared with a population of central Italy (Tuscany) (for details, see Scali et al., 2002). The examined specimens come from field studies and herpetological collections. Each variable has been natural log transformed before analysis, tested for normality and processed using SPSS version 8.0. Two samples *t*-test for unpaired samples has been performed to verify the degree of sexual dimorphism on the separate variables. Analysis of Covariance has been then performed to assess the influence of different factors (sex, locality, area) and the covariation of independent variables on selected dependent variable. Post Hoc tests on Ancova results have been then performed to determine the actual contribution to differences among the selected samples.

RESULTS

A marked Sexual Size Dimorphism was evident in all the considered populations, with males larger than females (SVL_{males} = 829.7 ± 172.1, *n* = 144; SVL_{females} = 751 ± 102.6, *n* = 48; *t*-test = 3.818, *df* = 190, *P* < 0.0001) and with lower number of VS (VS_{males} = 201.45 ± 5.89, *n* = 130; VS_{females} = 214.63 ± 6.56, *n* = 46; *t*-test = -12.65, *df* = 174, *P* < 0.0001). Southern Italian males (females have been excluded due to the small sample size of some localities) were significantly smaller than those of central Italy and characterised by a significant lower number of ventral scales (FSVL = 5.568, *P* = 0.001, *df* = 3; FVS = 60.559, *P* < 0.0001, *df* = 3) (Fig. 1), they also showed a melanistic pattern at the adult stage (ANCOVA_{VS},

¹ Museum Natural History and Territory, University of Pisa, via Roma 79, I-56011 Calci (Pisa), Italy; E-mail: marcoz@museo.unipi.it.

² Department of Ecology, University of Calabria, I-87036 Arcavacata di Rende (Cosenza), Italy.

³ Department of Animal Biology and Genetics, University of Florence, Via Romana 17, I-50125 Florence, Italy.

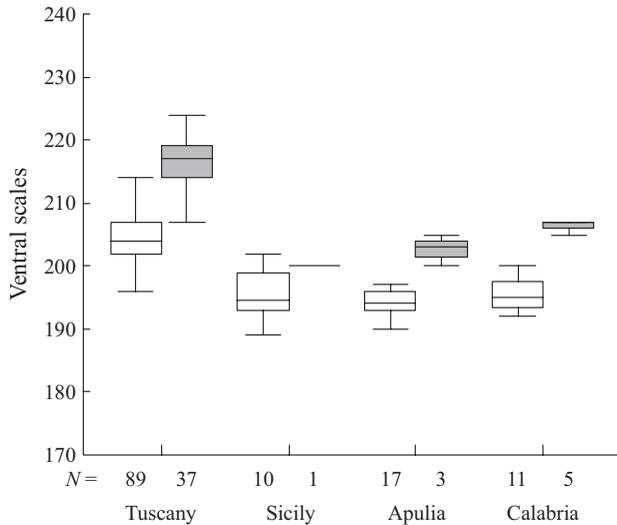


Fig. 1. Box plot of ventral scales of *Hierophis viridiflavus* males (white box) and females (gray box).

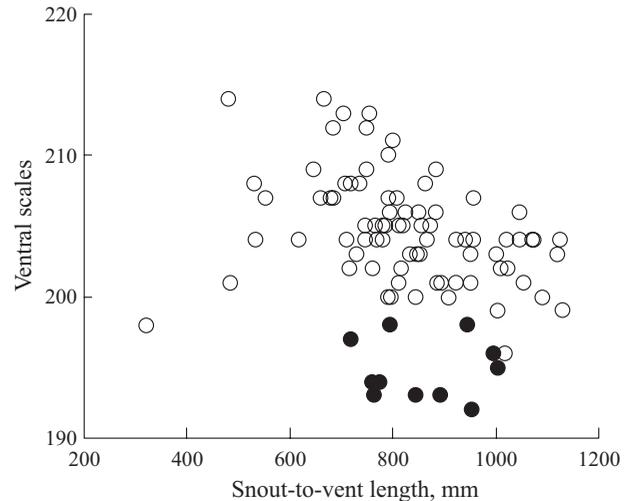


Fig. 2. Ventral scales-SVL relationship vs normal (white circles) and melanic (black circles) patterns in males *Hierophis viridiflavus*.

$P = 0.0001$; $\text{factor}_{\text{color pattern}}, P < 0.0001$; $\text{covariate}_{\text{SVL}}, P < 0.0001$; Fig. 2). Within the considered southern populations, we did not find any difference between the Apulian and the Calabrian populations for both the considered parameters (Lesser Significant Difference, LSD, post-hoc test, $P > 0.05$); while we found that the Sicilian whip snakes were shorter than those of the other southern Italian populations (LSD post-hoc tests of Sicily vs. Apulia and Calabria, $P = 0.028$ and $P = 0.003$ respectively). Head shape of whip snakes differs on the whole, but not significantly between all the considered populations (ANCOVA $_{\text{head width}} = 84.770$, $P < 0.0001$, $\text{factor}_{\text{area}} = 0.522$, $P = 0.669$; $\text{covariate}_{\text{head length}} = 322.95$, $P < 0.0001$), suggesting any geographical effect. Body structure of southern Whip snakes is then particularly differentiated with respect to the Tuscan population, with no clear evidence of a geographical cline in body shape or body size. The body shape differences found (i.e., ventral scales, melanic pattern) in the southern Italian Whip snakes is in accordance with previous studies (Scali et al., 2002), that have hypothesised that the southern Italian populations may likely belong to a different subspecies (*H. v. xanthurus*).

DISCUSSION

The homogeneity of the examined parameters within the different southern Italian populations suggests that these populations do not show any significant latitudinal or longitudinal effect (Scali et al., 2002). The morphologi-

cal differences found could likely be due to the effect of a long term isolation of these snakes in the southern Italian glacial refugia, that may likely have led to a differentiation that could support the attribute of subspecific status. Also the occurrence of the melanic pattern, shared by all the southern examined snakes, may reveal a common history of the differentiation patterns. New open questions for future researches are the proximate and ultimate causes of the evolution of the melanic pattern, the reduction of ventral scales, sexual size dimorphism, and the variability of the head shape in this species.

REFERENCES

- Boback S. M.** (2003), "Body size evolution in snakes: evidence from island populations," *Copeia*, **2003**, 81 – 94.
- Heimes G.** (1993), "*Coluber viridiflavus* Lacépède, 1789. Gelbgrüne Zornnatter," in: Böhme W. (ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/I. Schlangen (Serpentes). I*, Aula-Verlag, Wiesbaden, pp. 177 – 198.
- King R. B.** (1989), "Body size variation among island and mainland snake populations," *Herpetologica*, **45**, 84 – 88.
- Nagy Z. T., Joger U., Guicking D., and Wink M.** (2002), "Phylogeography of the European Whip Snake *Coluber (Hierophis) viridiflavus* as inferred from nucleotide sequences of the mitochondrial cytochrome b gene and ISSR genomic fingerprinting," in: *Proc. of the 11th Ord. Gen. Meeting Soc. Eur. Herpetol. (SEH)*, žalec, Slovenia, *Biota*, 3(1 – 2), 109 – 118.
- Scali S., Corti C., Gentili A., Luiselli L., Razzetti E., and Zuffi M. A. L.** (2002), "Continental versus Mediterranean European Whip snake (*Hierophis viridiflavus*): a morphometric approach," *Biota*, 3(1 – 2), 161 – 166.

**ECOLOGY,
FAUNISTIC,
CONSERVATION,
AND CAPTIVE BREEDING
OF AMPHIBIANS
AND REPTILES**

ON THE DISTRIBUTION OF *Pelodiscus sinensis* (WIEGMANN, 1834) (TESTUDINES: TRIONYCHIDAE) IN THE RUSSIAN FAR EAST

E. V. Adnagulov¹ and I. V. Maslova²

Keywords: *Pelodiscus sinensis*, Russian Far East, distribution.

INTRODUCTION

For the first time the chinese soft-shell turtle, *Pelodiscus sinensis* (Wiegmann, 1834) in the Amur River basin was noted after the results of R. Maack's expeditions (Maack, 1859, 1861). More detailed data appear after A. T. Buldovsky's (1936) and M. V. Okhotina's (1959) papers.

In 1970 – 1980s some data on the distribution of turtles in the south of the Far East (Tagirova, 1978, 1981; Khozatsky and Nesov, 1979) were published. A turtles population in the Bidzhan River (Middle Amur River area) was discovered in 1998 (Tarasov et al., 1998; Tarasov and Adnagulov, 1999). However the knowledge about this interesting and declining species is still quite poor.

MATERIAL AND METHODS

Study of soft-shell turtle in the Russian Far East was conducted in 2001 using the analysis of different publications, as well as results of the field work in Primorskii and Khabarovsk Krai's, and Jewish Autonomous Oblast' (JAO). The turtle records were documented by activity remains and clutches on beaches. Some information was obtained by questioning of local people.

At present the range *Pelodiscus sinensis* within the studied area consists in a few populations, and seems to have more disruptive pattern as compared to the data of previous publications.

RESULTS AND DISCUSSION

At least seven populations are known and named after their geographical localization (Fig. 1).

1. **Razdol'naya.** Razdol'naya River (South of Primorskii Krai). Perhaps, it is the smallest population of *P. sinensis* within Russia. Information on turtles is referred

to the vicinity of Pokrovka (about 100 km upstream of Razdol'naya River mouth). M. V. Okhotina writes "dead turtles were met on the Amurskii Bay shores after high floods in some years" (Okhotina, 1959: 142). According to some interrogated data, soft-shells are breeding in reservoirs in the Chinese part of Razdol'naya River (Chinese name is Suifung He) (V. A. Kostenko, 2001, personal communication).

2. **Khanka.** Lake Khanka and lower parts of some of its tributaries, Ilistaya, Mel'gunovka, Spasovka, Troits-

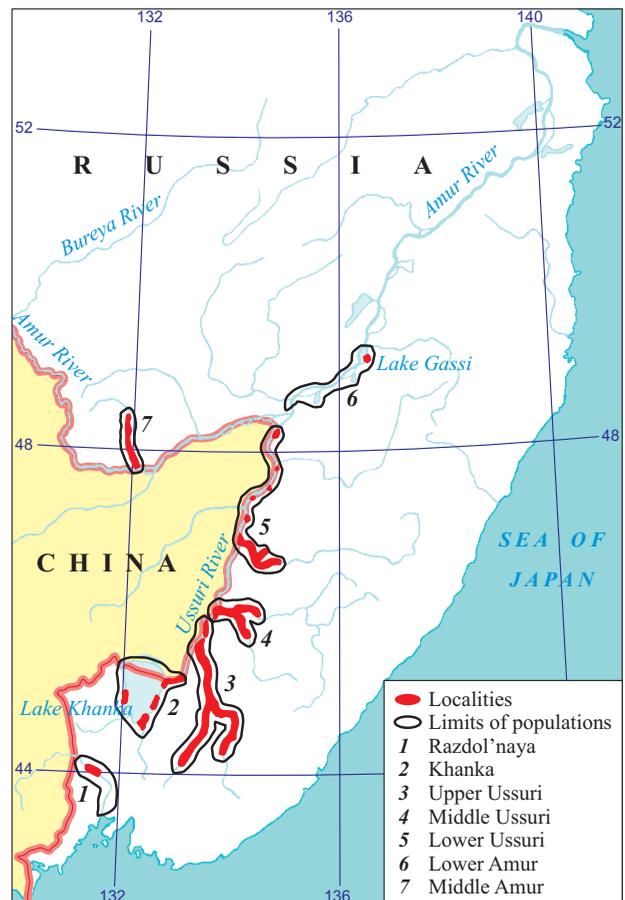


Fig. 1. Geographical localization of *Pelodiscus sinensis*.

¹ Institute of Water and Ecological Problems FEB RAS, Kim Yu Chen str., 65, 680000 Khabarovsk, Russia; E-mail: rfe_herps@mail.ru.

² Nature Reserve "Khankaiskii," Ershov str., 10, Spassk-Dal'nii, 692245 Primorskii Krai, Russia; E-mail: Irinarana@yandex.ru.

kaya, Tur, and an upper part of the Sungacha River. There are few locations in Lake Khanka mostly noted as the turtle habitats, Sosnovyi Island, Cape Przheval'sky, Luzanova Sopka Peninsula, Lakes Gniloi Ugol, and Trostnikovoe. This is a well known population described by different authors (Przevalsky, 1870; Emelianov, 1923; Buldovsky, 1936; Okhotina, 1959; Khozatsky and Nesov, 1979; Bannikov, 1984; Darevsky and Orlov, 1988; Cherepanov, 1990; Maslova, 2002; etc.).

3. **Upper Ussuri.** Upper Ussuri River and some of its tributaries (Arsen'evka and lower Sungacha River). According interrogated data turtles are met in the Arsen'evka River about 120 km upstream of its mouth. It is the one of the mostly poorly studied populations.

4. **Middle Ussuri.** Turtles inhabit Middle Ussuri River, as well as lower of its tributaries (Bol'shaya Ussurka and Malinovka Rivers). This population seems be one of the most endangered and declining due to illegal hunting by Chinese immigrants (Maslova, 2002).

5. **Lower Ussuri.** Turtles are met in lower Ussuri River up to its mouth, lower Bikin River and some small right tributaries. The first descriptions of *P. sinensis* (as *Trionyx maackii* Brandt) within Amur River basin are referred to this population (Maack, 1859). Information on the turtles in lower parts of some rivers (e.g., Khor River) remains doubtful because those rivers do not present optimum habitats (relatively fast stream, up to 1.5 – 1.8 m/sec and relatively low maximum temperature, up to +15 – 16°C).

6. **Lower Amur.** Data on this population includes records of turtles living in the Amur River basin about 400 km downstream of the Ussuri River mouth. The most known is the Lake Gassi population (Buldovsky, 1936; Tagirova, 1979, 1981; etc.). Information on the turtles' distribution up to the Amur River mouth is doubtful.

7. **Middle Amur.** Bidzhan River (a left tributary of Amur River, JAO). This population was discovered a few years ago (Tarasov and Adnagulov, 1999; Adnagulov et al., 2001), but there are references in some earlier publications (Gorobeiko, 1994). Turtles inhabit the middle and lower Bidzhan River about 160 km upstream of its mouth. Perhaps the turtles live in other adjacent rivers and in other parts of the Russian Far East (Zeya and Bureya Rivers, Amurskaya Oblast') that should be confirmed by special surveys.

Recent *P. sinensis* distribution depends on two main factors: suitable climatic conditions and alluvial accumulations. Recent turtles' populations may be divided into two groups according their hydrological and geomorphological features as well as their habitat in plain streams (Nos. 1, 2, 5, 6, and 7) or semi-mountain (Nos. 3 and 4).

In some plain biotopes (e.g., Bidzhan River) up to 7 habitats were discovered, which are of the most serious importance for *P. sinensis* in both aquatic (overwintering, feeding and migrating) and terrestrial (basking and breeding) life history (Adnagulov et al., 2001).

The number of turtles decreases in many areas due to illegal hunting of adult animals for sale and clutch destruction. In some locations the nesting sites (sand and gravel bars) are destroyed by excavation of ground for construction industry needs.

Acknowledgments. The work was supported by USAID (grant No. 118-A-00-95-00303-00) and WWF (grant No. LR 19).

REFERENCES

- Adnagulov E. V., I. G. Tarasov, and N. V. Ivanova (2001), "On the distribution and biology of *Pelodiscus sinensis* in the Middle Amurland," in: Ananjeva N. B. et al. (eds.), *The Problems of Herpetology. Proc. 1st Meet. Nikolsky Herpetol. Soc., December 4 – 7, 2000*, Izd. MGU, Pushchino – Moscow, pp. 10 – 13 [in Russian].
- Bannikov A. G. (1984), "Dal'nevostochnaya cherepakha [Chinese soft-shell turtle]," in: A. G. Borodin et al. (eds.), *The Red Data Book of USSR. Vol. 1. Animals*, Lesnaya Promyshlennost', Moscow, pp. 178 – 179 [in Russian].
- Buldovsky A. T. (1936), "The biology and economic value of the Ussuri (Amur) Turtle *Amyda maackii* (Brandt)," in: *Proc. of Far Eastern Branch of the USSR Academy of Sciences*, Izd. AN SSSR, Moscow – Leningrad. Vol. 1, pp. 62 – 102 [in Russian with English summary].
- Cherepanov G. O. (1990), "K biologii dal'nevostochnoi cherepakhi na ozere Khanka [On the biology of chinese soft-shell turtle in the lake Khanka]," *Byull. LGU. Ser. 3*, 2(10), 23 – 28 [in Russian].
- Darevsky I. S. and Orlov N. L. (1988), *Redkie i Ischezayushchie Zhivotnye: Zemnovodnye i Presmykayushchiesya [Rare and Decreasing Animals: Amphibians and Reptiles]*, Vysshaya Shkola, Moscow [in Russian].
- Emelianov A. A. (1923), "Presmykayushchiesya i zemnovodnye Primor'ya [Reptiles and amphibians of Primor'e]," in: *Primor'e: Yego Priroda i Khozyaistvo [Primor'e: Its Nature and Economics]*, Goskniga, Vladivostok, pp. 128 – 140 [in Russian].
- Gorobeiko V. V. (1994), "Amfibii i reptilii [Amphibians and reptiles]," in: *Fauna of the Jewish Autonomous Region. Part 1*, Institute of the Complex Analysis of Regional Problems, Far-East Branch, Russian Academy of Sciences, Birobidzhan [in Russian].
- Khozatsky L. I. and Nesov L. A. (1979), "Rasprostranenie dal'nevostochnoi cherepakhi v Primor'e i Priamur'e i zadachi ee okhrany [Distribution of chinese soft-shell turtle in Primor'e and Priamur'e and problems of its conservation]," in: *Herpetology*, Izd. KGU, Krasnodar, pp. 89 – 93 [in Russian].

- Maack R.** (1859), *Puteshestvie na Amur [Trip to the Amur]*, K. Wulf, St. Petersburg [in Russian].
- Maack R.** (1861), *Puteshestvie po Doline Reki Ussuri [Trip to the Valley of the Ussuri River]*, V. Bezobrazov, St. Petersburg [in Russian].
- Maslova I. V.** (2002), “The new data on distribution of *Pelodiscus sinensis* at Primorskii Krai,” in: Artamonov S. D. and Kolyada A. S. (eds.), *Animal and Plant World of the Far East. Vol. 6. Contribution to the Animal Ecology and Systematics*, Izd. UssGPI, pp. 65 – 73 [in Russian with English summary].
- Okhotina M. V.** (1959), “K utochneniyu granits arealov nekotorykh amfibii i reptilii Primor’ya [On the definition of ranges’ borders of some Amphibians and Reptiles in Primor’e],” *Soobshch. V. L. Komarov’s DVF AN SSSR*, **11**, 139 – 143 [in Russian].
- Przevalsky N. M.** (1870), *Puteshestvie v Ussuriiskom Krae 1897 – 1869 [Travels in the Ussuri Border Region in 1867 – 1869]*, St. Petersburg [in Russian].
- Tagirova V. T.** (1978), “Rasprostranenie i chislennost’ dal’nevostochnoi cherepakhi v Priamur’e [Distribution and number of chinese soft-shell turtle in Priamur’e],” in: *Herpetofauna of the Far East and Siberia, Institute of Biological and Pedology Sciences of Far Eastern Scientific Center of Acad. Sci. USSR*, Vladivostok, pp. 33 – 34 [in Russian].
- Tagirova V. T.** (1981), “Rasprostranenie i biologicheskie osobennosti dal’nevostochnoi cherepakhi” [Distribution and biological features of chinese soft-shell turtle],” in: *Animal Ecology in the South of Far East*, Izd. BGPI, Blagoveshchensk, pp. 50 – 64 [in Russian].
- Tarasov I. G., Adnagulov E. V., and Bylkov A. F.** (1998), “K faune amfibii i reptilii Evreiskoi Avnonomnoi Oblasti” [Contribution to the fauna of amphibians and reptiles in the Jewish Autonomous Region],” in: *Biological Diversity of Siberian Animals. Abstrs. of the Sci. Conf. to the 110th Anniv. Regular Zool. Investigations and Zool. Education in Siberia, October 28 – 30, 1998*, Izd. TGU, Tomsk, pp. 110 – 111 [in Russian].
- Tarasov I. G. and Adnagulov E. V.** (1999), “On the Ecology of the chinese soft-shell turtle, *Trionyx sinensis* Wiegmann, 1835 in the Jewish Autonomous Region, Russian Far East,” in: *Proc. of the 10th Ord. Meet. Soc. Eur. Herpetol., September 6 – 10, 1999*, Irakleio (Greece), pp. 153 – 154.

Triturus alpestris inexpectatus: NORMAL DEVELOPMENTAL STAGES MORPHOLOGY AND TEMPERATURE INFLUENCE

A. Bonacci,¹ M. T. Rizzuti,¹ and S. Tripepi¹

Keywords: *Triturus alpestris inexpectatus*, embryonic development, temperature influence, newt.

INTRODUCTION

Temperature is one of the most important environmental variables affecting embryonic development of aquatic species, as fish, amphibians and aquatic insects (Gillooly et al., 2002; Bermudes and Ritar, 1999). Temperature appears to be an important adaptive feature for developmental rate of most amphibians (Giacoma and Balletto, 1988). Embryonic development can be successful only within a thermal preferendum in which developmental rate increases as temperature rises (Duellman and Trueb, 1986; Brown, 1976). Bachmann's equation better describes amphibian adaptation to temperature (Bachmann, 1969).

Alpine newt (*Triturus alpestris*, Laurenti 1768) in Calabria is represented by *T. alpestris inexpectatus* (Dubois and Breuil, 1983), which is an endemic subspecies placed in only five sites on the Catena Costiera (Cosenza, Italy) to altitudes ranging from 800 to 1200 m.

The aim of this work is to describe embryogenesis and passive larval life and determine thermal preferendum of *T. alpestris inexpectatus* development (Tripepi et al., 98; Bonacci et al., 2001).

MATERIAL AND METHODS

All the adult specimens of *T. alpestris inexpectatus* come from the same place: San Benedetto Ullano (Cosenza, Italy). After spawning, eggs have been collected and placed in constant-temperature incubators. Data, drawn from observations at temperatures of 13 and 25°C, have been used to determine developmental stages. The relationship between temperature and development was obtained from experimental temperatures of 13, 20, 25, and 27.5°C. Equation is:

$$\Delta D = \Delta t(T - T_0),$$

where ΔD is a constant and represents the developmental progress between two prearranged stages: stage 1, first cleavage, and stage 20, closure of neural tube (Bachmann, 1969), °C · h; Δt is the time interval between the two stages; T is the

developmental temperature, °C; and T_0 is the temperature threshold under which the embryo does not develop.

Finally, a linear relationship between developing rate and temperature has been observed inverting the Bachmann's equation and a linear regression analysis has been used to describe this relationship.

RESULTS

Morphology. Based on external morphology criteria, 44 developmental stages have been described using comparative tables, drawn by employing bibliographic data (Gallien and Bidaud, 1959; Harrison, 1969; Hepperlein and Junginger, 1982). Development has been divided in three phases: cleavage, gastrulation and organogenesis. The first phase includes stages from 1 (uncleaved egg) to 9 (late blastula). The second phase includes stages from 10 (beginning of gastrulation) to 13 (late gastrula); the stage 12 shows the formation of yolk plug (Fig. 1A). The third phase consists of neurula, from stage 14 (early neurula) to stage 23 (completed neurula), tailbud, from 24 to 30, and organogenesis, which includes stages from 31 to 44. Figure 1B and C show the formation of neural plate (stage 15) and the appositions of the neural folds in the spinal cord region (stage 17). Figure 1D shows the complete formation of neural tube (stage 20). In Fig. 1E (stage 26) the embryo is still bent on itself and the gill buds and the optic capsules are visible. In the stage 29 (Fig. 1F) the trunk and the tailbud go on stretching while a slight pigmentation begins to appear on the embryo dorsal region. In the stage 42 (Fig. 1G) larval pigmentation is more extensive, the gills are longer and the bifurcation of fingers is evident. Figure 1H shows the stage 44, in which the fourth finger bud appears and balancers have disappeared. At this stage yolk is consumed and the larva goes from passive to active life.

Temperature influence. Through this study it has been possible to find the time interval, expressed in hours, from stage 1, uncleaved egg, to stage 44, four fingers larva, at 13 and 25°C. At 13°C, the time needed is equal to 1901 h and 46 min; at 25°C, the time of development is equal to 475 h and 45 min. As it is shown in Fig. 2A, our

¹ Dipartimento Ecologia, Università della Calabria, I-87036 Rende (Cosenza), Italy; E-mail: s.tripepi@unical.it.

results reveal that different phases have different periods, even if the general trend is the same for all. In addition, it is noted that the early two development phases are faster than the organogenesis. Introducing experimental values in Bachmann's equation (Bachmann, 1969), it can allow us to calculate the value of the specific species constant ΔD (developmental rate), which results equal to 900.85 ($^{\circ}\text{C} \cdot \text{h}$). The temperature-threshold, T_0 , is equal to 8.49 $^{\circ}\text{C}$. Being the developmental rate R the reciprocal of the time interval Δt , there is a linear relationship between developmental rate and temperature T :

$$R = \frac{1}{\Delta t} = \frac{T - T_0}{\Delta D}.$$

This relation is the equation of a straight line on the temperature-developmental rate plane (Fig. 2B). It is characterized by the temperature-threshold, T_0 , which corresponds to the intercept of the rate-temperature line with temperature axis, and by $1/\Delta D$ which represents the line angular factor. The optimum development temperature, $T_0 + 10$, is 18.49 $^{\circ}\text{C}$. The statistical reliability has been checked by a linear regression analysis (r , correlation factor = 0,99). In addition, Bachmann equation can be shown as a hyperbolic relationship between temperature and development time (Fig. 2C): the development time decreases as temperature increases.

DISCUSSION

From a morphological point of view the only difference in embryonic development of *T. alpestris inexpectatus* compared to *T. carnifex* (Bonacci et al., 2001), *T. italicus* (Tripepi et al, 1998) and *T. alpestris alpestris* (Epperlein and Junginger, 1982) is a variation in number of stages, which strongly depends on the observation criteria adopted by researcher.

A tight relation between the embryonic developmental pattern of Urodeles (and Amphibians in general) and environmental temperature has been confirmed in our studies. Temperature variations do not seem to affect the general developmental pattern and the relationships among the different stages of development, but simply reduce or accelerate developmental time.

Comparing developmental duration and related parameters (ΔD , T_0 , and $T_0 + 10$ values) of *T. alpestris inexpectatus* with those of other newts living in Calabria (*T. italicus* and *T. carnifex*) we have observed evident differences (Table 1).

The minor temperature-threshold found in *T. alpestris inexpectatus* and *T. carnifex* is determined by a spreading

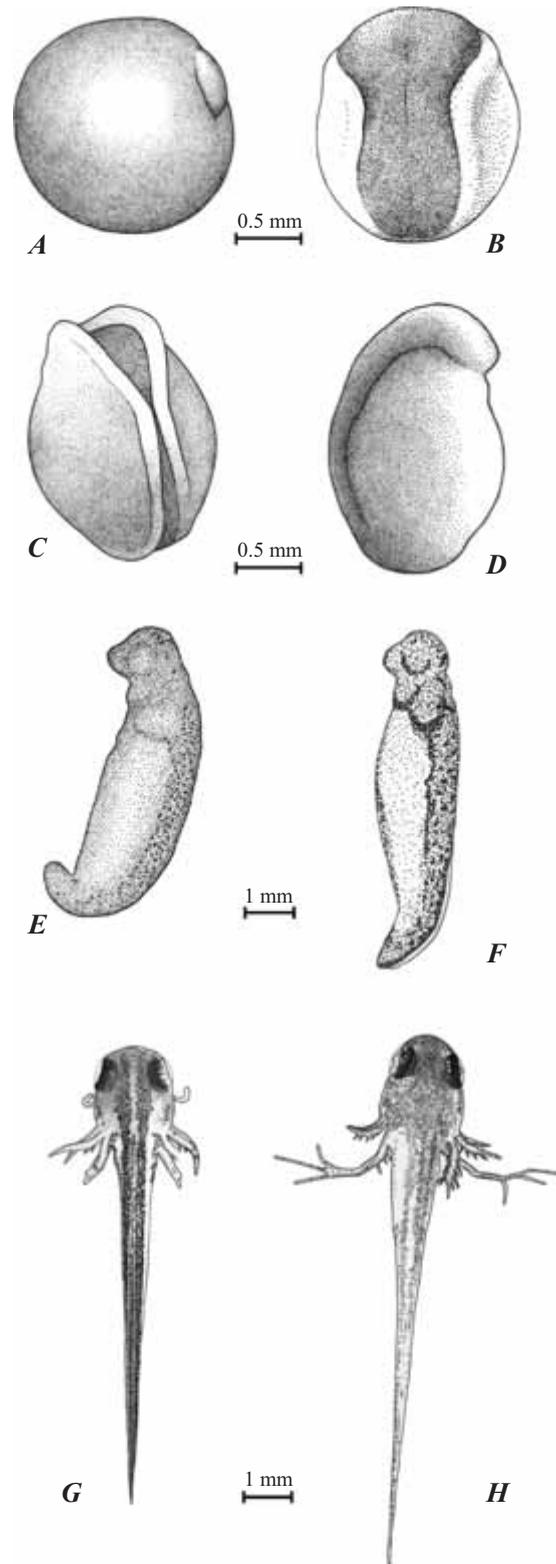


Fig. 1. Schematic drawing of developmental stages of *Triturus alpestris inexpectatus*. A, stage 12; B, stage 15; C, stage 17; D, stage 20; E, stage 26; F, stage 29; G, stage 42; H, stage 44.

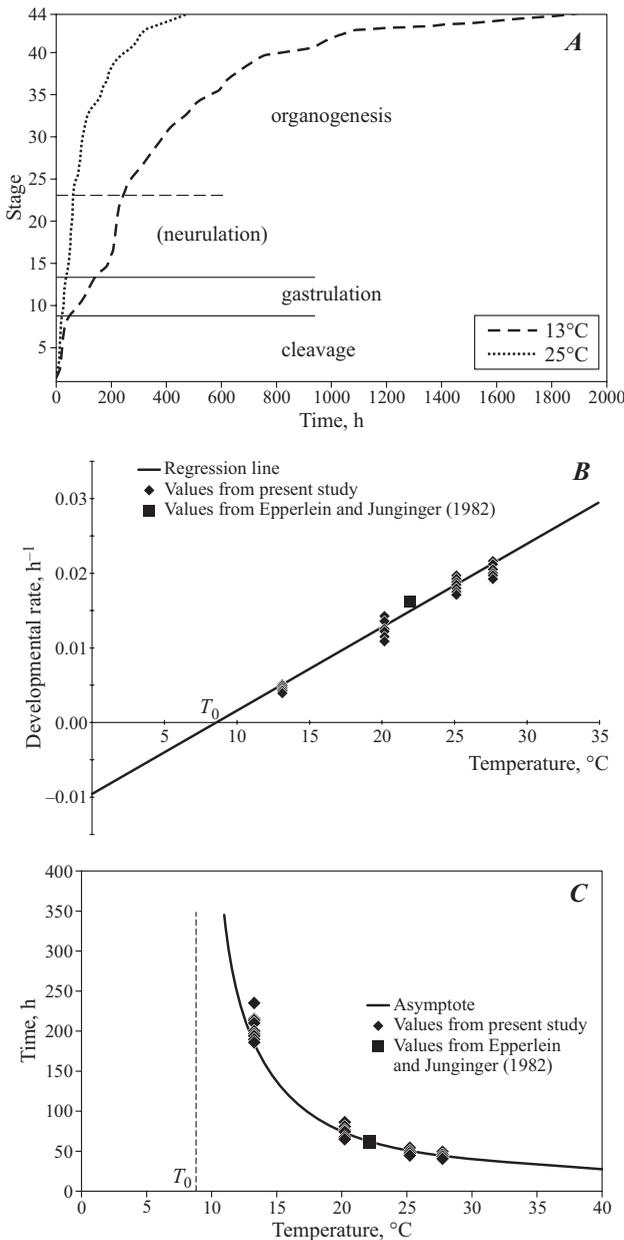


Fig. 2. *A*, developmental curves at temperatures of 13°C and 25°C; *B*, linear relationship between developmental rate and temperature; *C*, hyperbolic relationship between temperature and time of development.

of these species to higher altitudes if compared to *T. italicus*. This last species often lives in syntopy with *T. alpestris inexpectatus* but a high population density was found at a low altitude (Tripepi et al., 1998). Their differences in ΔD value are given by their different ecological features. According to Bachmann's theory, a warm-adapted species as *T. italicus* develops faster in a more variable reproduc-

TABLE 1. Comparison of the Developmental Temperature-Related Parameters in the Calabrian Newts

Species	ΔD , °C · h	T_0 , °C	$T_0 + 10$, °C	Source of data
<i>Triturus alpestris inexpectatus</i>	900.85	8.49	18.49	Present paper
<i>Triturus italicus</i>	670.06	10.4	20.4	Tripepi et al., 1998
<i>Triturus carnifex</i>	1100	8.2	18.2	Bonacci et al., 2001

tive environment, such as tanks or pools whose drainage is very fast. *T. alpestris inexpectatus* prefers a bigger and stable environment situated at higher altitudes such as mountain ponds which are unlikely to drain during summer.

Acknowledgments. We thank Dr. Maria Domenica Alfano for her help with drawing some figures.

REFERENCES

- Bachmann K. (1969), "Temperature adaptation of amphibian embryos," *Am. Nat.*, **103**, 115 – 130.
- Bermudes M. and Ritar A. J. (1999), "Effects of temperature on the embryonic development of the striped trumpeter (*Latris lineata* Bloch and Schneider, 1801)," *Aquaculture*, **17**, 245 – 255.
- Bonacci A., De Luca L., Rossi F., and Tripepi S. (2001), "Influenza della temperatura sullo sviluppo embrionale dell'urodela *Triturus carnifex*," *Proc. of III Natl. Congr. "Societas Herpetologica Italica," Pianura*, **13**, 159 – 161.
- Brown H. A. (1976), "The time-temperature relation of embryonic development in the northwestern salamander, *Ambystoma gracile*," *Can. J. Zool.*, **54**, 552 – 558.
- Dubois A. and Breuil M. (1983), "Decouverte de *Triturus alpestris* (Laurenti, 1789) en Calabre (Sud de Italie)," *Alytes*, **1**, 9 – 18.
- Duellmann W. E. and Trueb L. (1986), *Biology of Amphibians*, McGraw-Hill Book Company, New York.
- Epperlein H. H. and Junginger M. (1982), "The normal development of the newt, *Triturus alpestris* (Daudin)," *Amphibia-Reptilia*, **2**, 295 – 308.
- Gallien L. and Bidaud O. (1959), "Table chronologique du developpement chez *Triturus helveticus* Razoumowsky," *Bull. Soc. Zool. Fr.*, **84**, 22 – 32.
- Giacoma C. and Balletto E. (1988), "Phylogeny of the salamandrid genus *Triturus*," *Boll. Zool.*, **55**, 337 – 360.
- Gillooly J. F., Charnov E. L., West G. B., Savage V. M., and Brown J. H. (2002) "Effects of size and temperature on developmental time," *Nature*, **417**, 70 – 73.
- Harrison R. G. (1969), "Harrison stages and description of the normal development of the spotted salamander, *Ambystoma punctatum* (Linn.)," in: Harrison R.-G. (ed.), *Organization and Development of the Embryo*, Yale Univ. Press, New Haven, pp. 44 – 66.
- Tripepi S., Rossi F., and Peluso G. (1998), "Embryonic development of the newt *Triturus italicus* in relation to temperature," *Amphibia-Reptilia*, **19**, 345 – 355.

CONSERVATION OF RESOURCES OF REPTILES IN ASTRAKHAN' OBLAST' (RUSSIA). ASTRAKHAN' OBLAST' REPTILIAN RESOURCES CONSERVATION (EXPERIENCE OF REGIONAL REALIZATION)

A. T. Bozhansky¹

Keywords: Conservation, Reptilian resources, Astrakhan' Oblast'.

Reptiles is a group of the vertebrate animals which can be used as a perfect sensitive indicator of environment state. Astrakhan' Oblast' is the unique region of the south-eastern European Russia. The territory's physiographic characteristics determine high variety of reptilian habitats and fauna. The region is situated at the border of several zoogeographic provinces: Kazakhstan deserts of the northern type adjoin the European arid steppes and wormwood-gramineous semideserts, true feather-grasses and gramineous steppes alternate the poplar and gallery forests along the Volga delta branches and spot oak woods of Volga – Akhtuba rivers country. Areas of the salt-domic relief of the Baskunchak Lake and the Bol'shoi Bogdo mountain outskirts play a significant role for habitat preferences of reptiles. It is important that State Bogdo-Baskunchak Nature Reserve is functioning within this area. A distinctive feature of the region is that almost all of its territory lies in the Caspian lowland (on average 30 – 40 m below the sea level) with Bol'shoi Bogdo mount dominating over this lowland at 135 m.

Astrakhan' Oblast' is strongly transformed by human activity. Agricultural holdings (melon plantation and pastures) occupy more than 70% of the territory. In addition, the transport network is dense in the region because the branchy inland water routes are easily available even to the remote places. Finally oil, gas and chemical industry as Aksarai gaseous condensate complex and Tengiz – Novorossiisk oil-pipe line (the largest in Europe) are functioning there.

The variety of landscapes and the southern location of Astrakhan' Oblast' determine the herpetofauna diversity and its representatives' abundance. There are 17 species of reptiles in the region. Tortoises are represented by single species (*Emys orbicularis*). One species of geckos (*Alsophylax pipiens*) lives here, in its type locality. Other lizards are represented by the following species *Eremias velox*,

Eremias arguta, *Lacerta agilis*, *Phrynocephalus mystaceus*, *Phr. guttata*, *Phr. helioscopus*. Snake fauna of the region is also quite diverse: *Eryx miliaris*, *Natrix natrix*, *N. tessellata*, *Elaphe sauromates*, *Elaphe dione*, *Coluber caspius*, *Malpolon monspessulanus*, *Vipera renardi*.

Unique characters of Astrakhan' Oblast' having high reptile species diversity, ecotope variety and originality as well as very strong economic developing make the problem of conservation of Astrakhan' Oblast' herpetocomplexes especially actual and urgent. Obviously, the conserving and maintaining the sustainable existing of reptiles need elaboration of coordinated actions which realization is possible within the framework of a regional program.

The particularized regional program on reptilian resources conservation has been realized by us in 1991 – 1996 and included three stages.

The first stage means the fauna inventory all over the region for survey of the species diversity. During this work records of all species are registered and cadastral maps are charted. This stage of the regional program resulted in the model of reptile atlas (Bozhansky and Nikerov, 1993) and in cadastral evaluation of reptilian resources (*Astrakhan' Oblast' Fauna Cadastre*, VNIIPriroda, 1992 [in Russian]). Data acquired in this stage allow to estimate reptile numbers for annotated list of rare and endangered species (Bozhansky and Poluinova, 1998).

The second stage of the program represents the elaboration of zoning of the territory on the basis of the data on reptilian fauna and density. We have zoned only sand-desert sections of the region, steppe and flood-land ecotopes have not been covered (see Table 1). It resulted in allocation of richest ecotopes both in the number of individuals and species diversity. We have noted the Berly sands section, where sand deserts border upon arid steppes, and both desert and steppe species are present. The desert species are the following: *Eremias velox*, *Phrynocephalus guttata*, *Phrynocephalus mystaceus*; intrazonal species: *Eremias arguta*; steppe species: *Vipera renardi*, *Elaphe*

¹ Department on Ecology and Wildlife Management, Russian State Agriculture External University, Fuchika str. 1, Balashikha-8, 143900 Moscow Oblast', Russia; E-mail: mirr@ok.ru, marina@rcmc.ru.

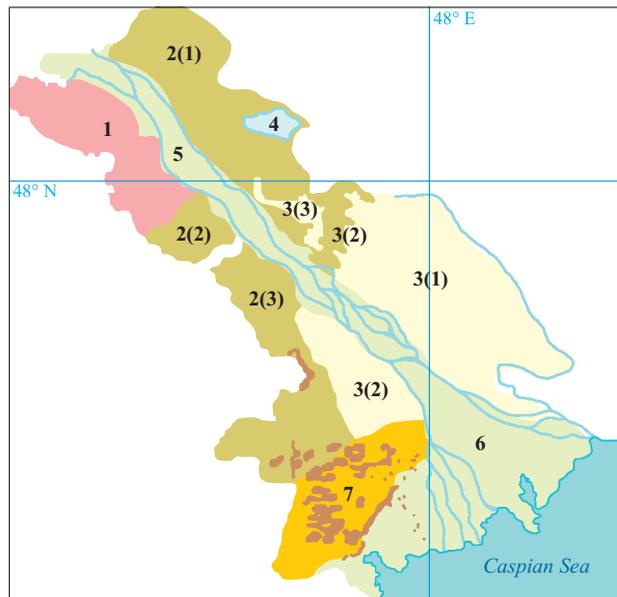


Fig. 1. Landscape and herpetology areas of Astrakhan' Oblast': 1, clay semidesert; 2(1), Sand-clay desert on the left bank of the Akhtuba river; 2(2), sand and sand-clay desert areas; 2(3), sand-clay desert on the right bank of the Volga river; 3(1), Volga-Ural sand desert; 3(2), Volga sand desert; 4, Bogdo-Baskunchak area; 5, flood-lands country between river Volga and Akhtuba; 6, delta; 7, Podstepnye Il'meni.

dione, *Lacerta agilis*. Those places show very high numbers of reptiles.

Except Berly sands of great importance for reptilian diversity conservation are the sands around Malyi Aral settlement and small steppe ponds in Balkhuni settlement outskirts in Akhtubinskii Raion where mass accumulations of *Bufo viridis* and *Pelobates fuscus* occur as they

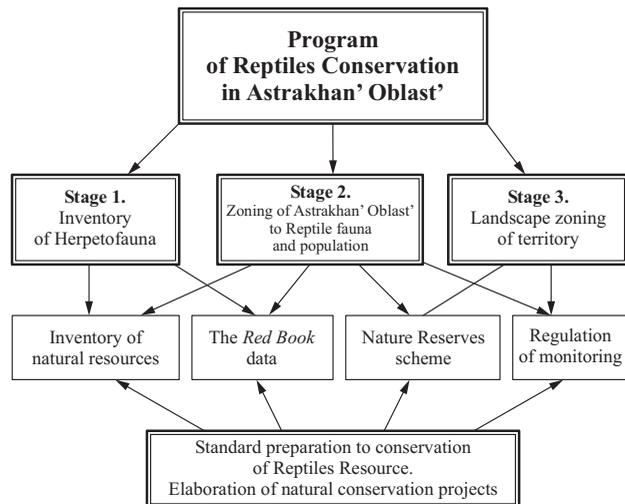


Fig. 2. Structure of action plan for regional reptiles conservation in Astrakhan' Oblast'.

mate and spawn there. In second stage of the program the high variety and originality of Bogdo-Baskunchak reserve ecotopes was refined (Bozhansky and Polynova, 1997).

It would be expedient to complete the second stage with elaboration of documents for particularized regional reserves' projection. So, according to our investigations the Berly Sands reserve was founded in 1998, situated in Kharabali Raion (500 hectares). The Malyi Aral herpetological reserve is under development now. Preproject surveys for complex zoological reserve near Balkhuni settlement were also carried out.

The third stage of the program is concerned with the development of special measures for the territorial protec-

TABLE 1. Reptile Populations of Desert and Semidesert Landscapes in Astrakhan' Oblast' (in specimens/ha)

Species	Landscapes (see Fig. 1)								
	1	2	2(1)	2(2)	2(3)	3	3(1)	3(2)	4
<i>Eremias arguta</i>	0.77	28.5	16.2	53.1	18.9	56.3	45.5	16.9	68.3
<i>Eremias velox</i>	—	0.33	—	7.5	16.2	0.7	4.9	3.2	1.6
<i>Lacerta agilis</i>	43.5	+	5.5	—	5.7	—	6.7	—	+
<i>Phrynocephalus guttata</i>	—	—	—	0.5	—	3.7	3.7	0.95	0.5
<i>Phrynocephalus helioscopus</i>	—	—	1.1	2.6	—	+	—	—	—
<i>Phrynocephalus mystaceus</i>	—	—	—	1.25	—	11.1	—	+	—
<i>Eryx miliaris</i>	—	—	—	+	—	0.33	+	0.03	—
<i>Elaphe dione</i>	+	+	0.56	+	0.3	0.63	+	+	+
<i>Elaphe sauromates</i>	+	—	+	—	—	+	—	—	0.33
<i>Coluber caspius</i>	—	—	—	—	+	+	+	0.97	0.81
<i>Natrix natrix</i>	—	—	—	—	—	—	—	—	1.44
<i>Vipera renardi</i>	0.66	+	1.93	—	+	1.0	2.4	+	+
<i>Alsophylax pipiens</i>	—	—	—	—	—	—	—	—	4.0

Note. —, give a definition; +, give a definition.

tion of individual species and reptile communities, and also with the improvement of legislative acts and by-laws supporting these measures. They are: regional *Red Data Book* regulations, annotated lists of rare species, “For Regional Herpetological Reserves” standard regulations, standard passport of a regional herpetological reserve. At present these standards have been already worked out and are being considered by regional administration.

Thus, the regional program of reptilian diversity conservation foresees:

— intraspecific level of conservation — keeping a certain diversity inside a species;

— biocenosis level — conservation actions for preventing population declining and preserving the territories richest in reptile diversity;

— landscape — preserving the places richest in reptile ecotopes diversity.

See Table 1 and Fig. 2 for formalized layout of the regional reptile conservation program.

Acknowledgment. The job on regional program realization was supported by J. and K. McArthurs’ fund and ISAR fund.

REFERENCES

- Bozhansky A. and Nikerov Y.** (1994), “Materialy k atlasu Astrakhanskoi oblasti [Materials for atlas of Astrakhan’ Oblast’],” *Ékol. Astrakh. Obl.*, Issue 3, 30 – 49 [in Russian].
- Bozhansky A., Polynova G., and Orlova V.** (1998), “Strategicheskie zadachi sokhraneniya gerpetokompleksov peschanykh pustyn’ Astrakhanskoi oblasti [Strategical tasks of herpetofauna conservation of sand deserts in Astrakhan’ oblast’],” in: *Problemy Sokhraneniya Bioraznoobraziya Aridnykh Regionov Rossii [Problems of Conservation of Biodiversity of Arid Regions of Russia]*, Volgograd, pp. 115 – 117 [in Russian].
- Bozhansky A. and Polynova G.** (1998), “Proekt regional’nogo spiska reptilii Krasnoi Knigi Astrakhanskoi oblasti [Project of regional reptiles list for Red Data Book of Astrakhan’ Oblast’],” in: *Problemy Sokhraneniya Bioraznoobraziya Aridnykh Regionov Rossii [Problems of Conservation of Biodiversity of Arid Regions of Russia]*, Volgograd, pp. 57 – 58 [in Russian].

CONFLICTS BETWEEN URBAN GROWTH AND SPECIES PROTECTION: CAN MIDWIFE TOADS (*Alytes obstetricans*) RESIST THE PRESSURE?

M. J. Castro,¹ J. M. Oliveira,^{2,3} and A. Tari^{3,4}

Urban areas are expanding on a global scale. Thus, conflicts between species protection and urban growth are increasingly common. Biodiversity conservation is traditionally associated with pristine habitats. Nevertheless, urban areas may show high levels of species diversity, particularly regarding herpetofauna, which should deserve especial attention. In the city of Coimbra (Portugal) occurs a population of midwife toads (*Alytes obstetricans*) restricted to the surrounding area of the football field in Santa Cruz Park. This Park became engulfed in the urban area, therefore originating an isolated population of midwife toads. These amphibians are threatened by habitat destruction due to construction works that will take place in the football field. Species conservation efforts may be especially rewarded in the particular case of the amphibians because certain populations, such as this particular population of midwife toads, may require simple protection measures. Our 2.5 year study on this population shows that midwife toads' habitat requirements are: permanent waterbody, accessibility of refuge areas and vegetation to ensure prey availability. According to these data, midwife toads' protection and urban development seem compatible. Our study indicates that midwife toads may resist urban growth pressure if: 1) habitat requirements are carefully studied to ensure population survival; 2) protection measures are designed in accordance to habitat requirements and urban development; 3) interdisciplinary cooperation is promoted among conservation biology, architecture, landscape architecture, engineering; 4) discussion occurs among key actors. Achievement of these principles in urban areas provides, beside conservation purposes, benefits for local people including recreational and wildlife oriented activities and environmental education.

Keywords: conservation, biodiversity, urban ecology, *Alytes obstetricans*, urban growth, amphibians.

INTRODUCTION

Urban areas are increasingly becoming larger. It is expected that in 2007 about 50% of human population will live in urban areas (UNPD DESA, 2002). The expansion of global geographic area of urbanized areas will lead to increased conflicts between land development and biodiversity conservation (Balmford et al., 2001). In spite of the traditional association of nature conservation to pristine habitats, urban areas may present important levels of biodiversity. For example, in only two parks in the city of Coimbra (Portugal), up to 9 species of amphibians may be found (representing 53% of Portuguese amphibians) (personal observations). For groups like amphibians and reptiles, a positive correlation between human population density and species richness has been recorded (Araújo, 2003). Thus, biodiversity of urban areas should be considered, especially regarding herpetofauna and mostly if we consider urban areas located within the biodiversity hot-

spots, such as the southern part of Portugal (Myers et al., 2000). Moreover, it may be possible to harmonize species protection and urban development (Beatley, 1994).

In Coimbra, an isolated population of midwife toads (*Alytes obstetricans*) occurs around a football field in a city park (Castro and Oliveira, 2002). Midwife toads are present only surrounding the football field and not elsewhere in the park. In 2001, this population became threatened by construction works. Therefore, a conservation plan had to be undertaken to harmonize urban growth and midwife toads' survival. This plan coincides with "The Alytes Project — study and conservation of an endangered population of midwife toads *Alytes obstetricans*" (Castro and Oliveira, 2002; Oliveira and Castro 2002a, 2002b).

THE PLAN

Conservation plan consists of the following phases: (1) Before construction works: a) study of the species and its habitat requirements; b) creation of temporary housing for toads (protected from works impact); c) translocation of population to temporary housing and d) definition of architectural friendly adaptations for construction works; (2) During works: a) monitoring of translocated population and b) ecological restoration of the football field area; (3) After works: population re-translocation to original

¹ Vasco da Gama University School, Quinta de S. Jorge, Estrada da Conraria, 3040-714 Castelo Viegas, Coimbra, Portugal; E-mail: mariajcastro@hotmail.com.

² ICN — National Institute for Nature Conservation, Mata Nacional do Choupal, 3000 Coimbra, Portugal; E-mail: jmcbo@mail.pt.

³ IMAR — Instituto do Mar, a/c Departamento de Zoologia, Fac. Ciências e Tecnologia, University of Coimbra, 3004-517 Coimbra, Portugal.

⁴ University of Debrecen, Egyetem Tér 1, 4032 Debrecen, Hungary; E-mail: taripanni@freemail.hu.

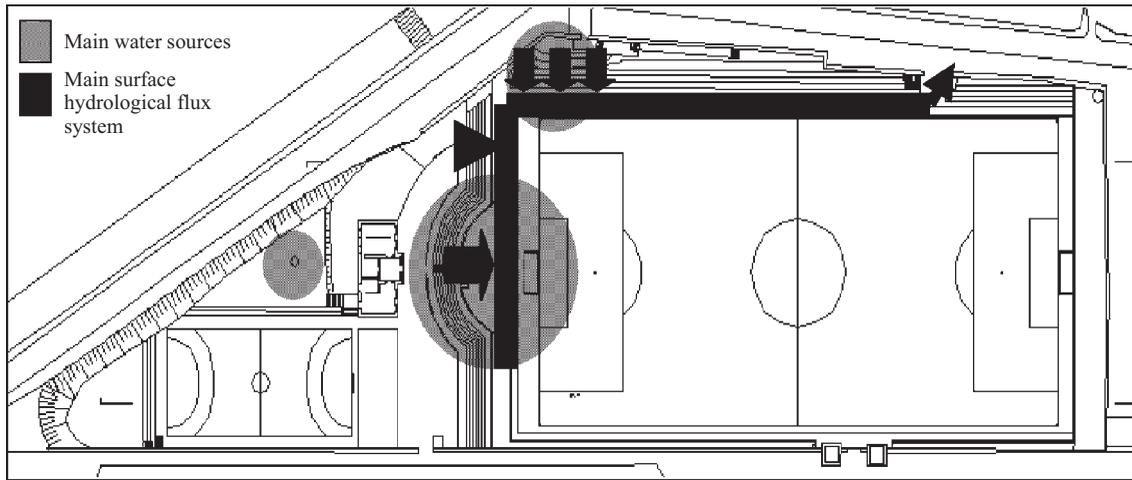


Fig. 1. Location of main water sources and main surface hydrological flux system used by midwife toads *Alytes obstetricans* to breed in the football field of Santa Cruz Park, Coimbra.

habitat. All phases include actions to raise environmental awareness: edition of publicizing material and discussion groups with key actors. According to this conservation plan, midwife toads' protection and urban development seem compatible. We propose the following four items framework as an approach to deal with emergent conflicts between urban growth and species protection:

1. Habitat requirements carefully studied to ensure population survival. First step to conserving any species or population is to know their ecology (Jones, 2002). Our 2.5 year study of weekly field work (comprising environmental parameters, population dynamics and reproductive biology) shows that midwife toads' habitat requirements are: permanent water body, accessibility of

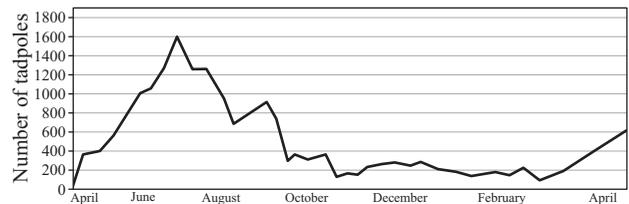


Fig. 2. Number of tadpoles of *Alytes obstetricans* present (April 2001 – April 2002) in the water line surrounding the football field of Santa Cruz Park, Coimbra.

refugia and vegetation. Midwife toads use a water line surrounding the football field to breed (Fig. 1) and tadpoles are present all over the year (Fig. 2). Refuges (and terrestrial habitat) of toads are located around the football field,

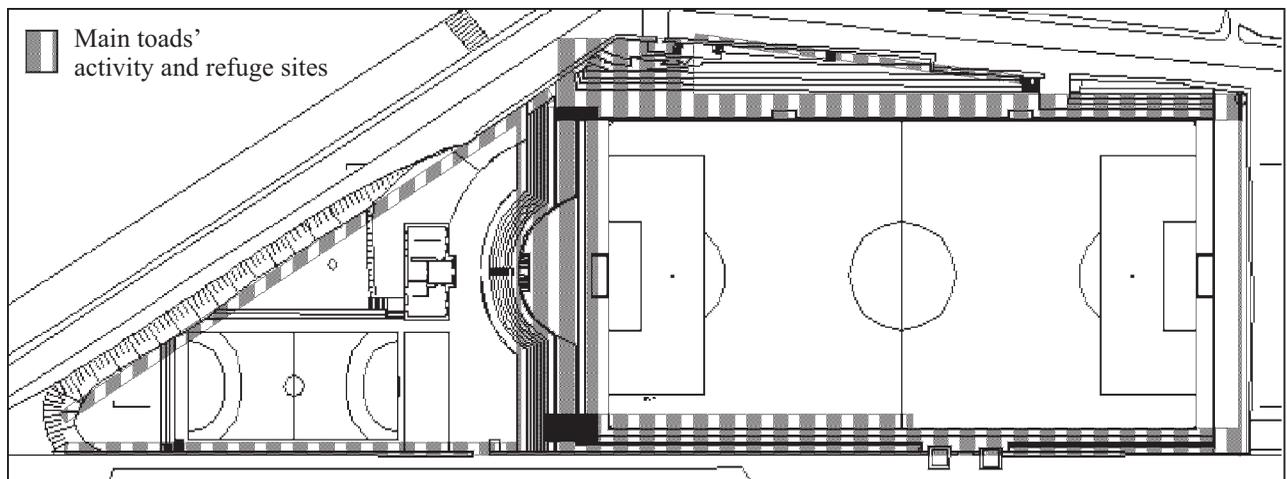


Fig. 3. Main refuge areas (and terrestrial habitat) of midwife toads *Alytes obstetricans* in the football field of Santa Cruz Park, Coimbra.

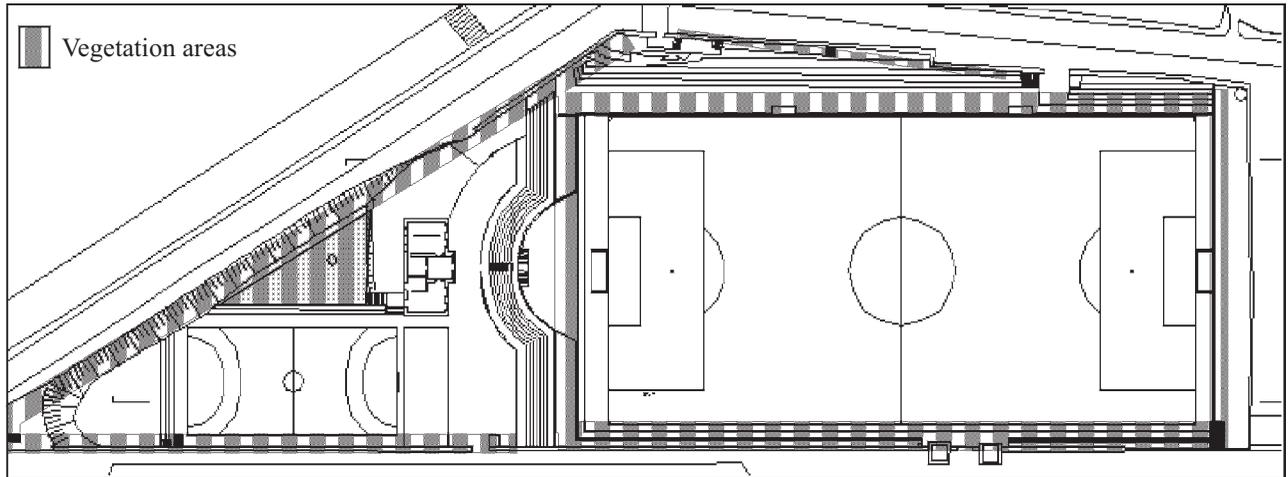


Fig. 4. Main vegetation areas of midwife toads *Alytes obstetricans* in the football field of Santa Cruz Park, Coimbra.

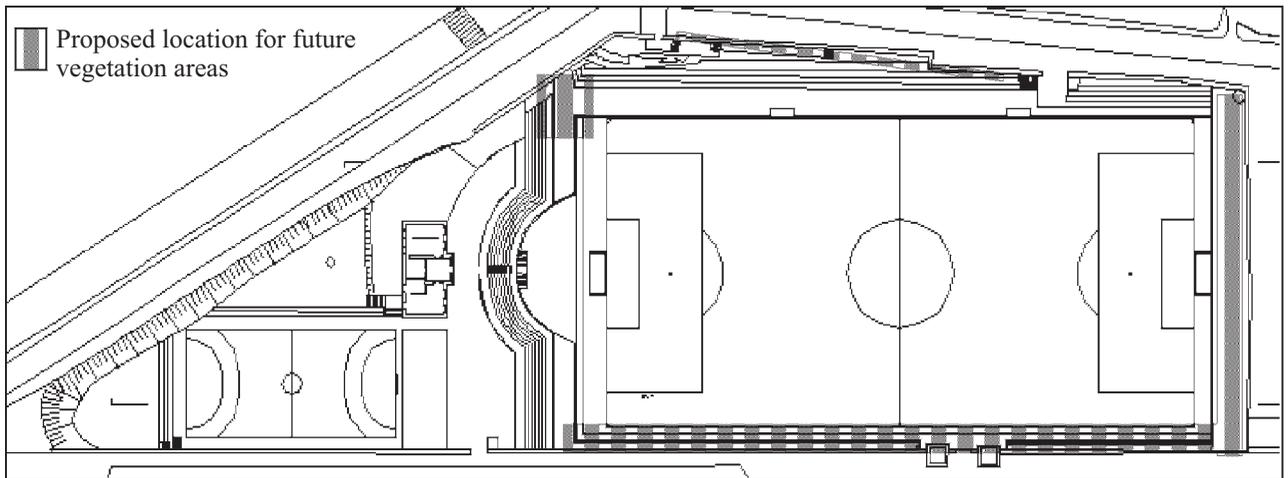


Fig. 5. Proposed location for future vegetation areas for midwife toads *Alytes obstetricans* in the football field of Santa Cruz Park, Coimbra.

in crevices in stony walls and benches (Fig. 3). Vegetation areas surrounding the football field (Fig. 4) are essential for maintaining invertebrate population, thus ensuring prey availability.

2. Protection measures designed in accordance with habitat requirements and urban development. The following protection measures to preserve and restore the ecosystem were defined according to habitat requisites for this population and the construction project: 2.1. Ecosystem preservation: 2.1.1. Terrestrial habitat: refuges preservation to assure connectivity among different microhabitats and toads' mobility through feeding, breeding and refugia. 2.1.2. Aquatic habitat: maintenance of water with adequate bio-physicochemical parameters. 2.2. Ecosystem restoration: 2.2.1. Terrestrial habitat: vegetation and refu-

gia will be created (Figs. 5 and 6). 2.2.2. Aquatic habitat: a new water line and superficial water collector will be provided. A linkage of the water springs to the water line must be assured. The water line proposed should be opened, with low water loss and speed, with trapezoidal cross-section (30×10 cm); margins should have slope less than 45° , made of non-slippery material.

3. Interdisciplinary cooperation promotion among intervening subjects. Making species protection and urban development compatible will only be possible with interdisciplinary cooperation and different backgrounds' knowledge integration (Weddel, 2002). Only through cooperation may the protection measures be properly defined (gathering conservation biology, architecture and landscape architecture) and practical solutions imple-

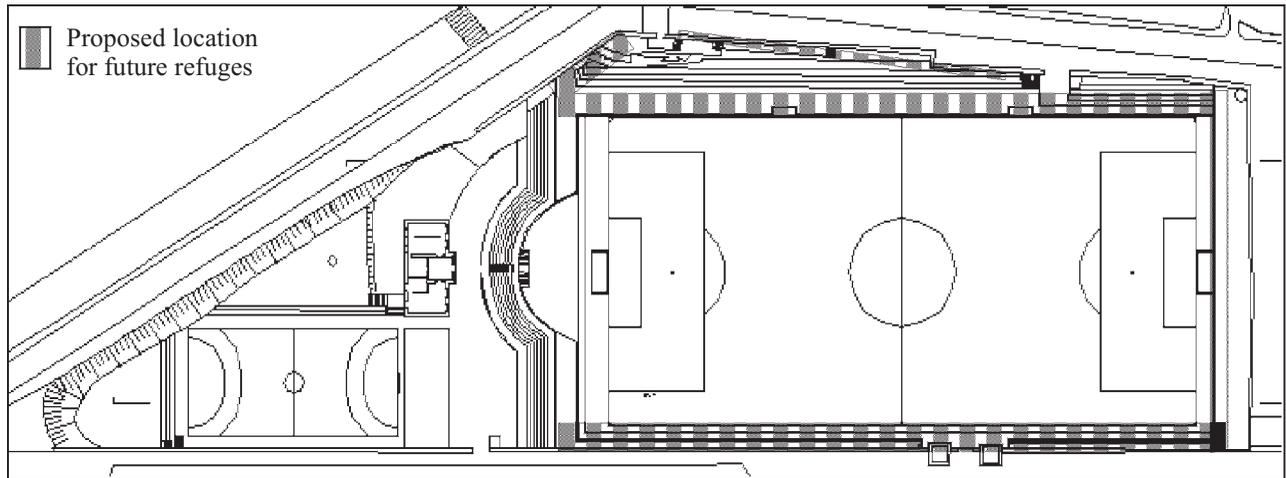


Fig. 6. Proposed location for future refuge areas for midwife toads *Alytes obstetricans* in the football field of Santa Cruz Park, Coimbra.

mented (gathering architecture, civil engineering and workers).

4. Discussion among key actors. Harmonizing species protection and urban development implies the conciliation of different, sometimes opposing, interests (Beatley, 1994). This particular case of midwife toads protection and urban development gathers conservationist (environmentalists and some locals), political (City Hall) and utilitarian (Associação Académica de Coimbra — landowner) points of view. Fulfillment of the whole conservation strategy relies on continuous discussions among the key actors involved, capable of leading to resolution of emergent conflicts and to the definition of practical solutions. The capacity to achieve solutions through discussions may be the driving force to overtake the drawbacks we face, including unnecessary conflicts between conservation and political issues, lack of cooperation by some key actors and ignorance with respect to nature conservation issues.

Acknowledgments. Financial support was provided by a Conservation and Environmental Grant from Ford Motor Company (2002). We thank Richard Bowker, Rafael Márquez, Mário Reis, José Pedro do Amaral, and Humberto Rosa for project discussion. Isabel Ramos, Francisco Cabral, Céline Verissimo, Sónia Francisco, Rui Costa, Paulo Morgado helped with the conservation plan implementation. Support was also provided by IMAR — University of Coimbra, Centro de Estudos de Arquitectura, Ambiente e Ordenamento do Território — Escola Universitária Vasco da Gama, City Hall of Coimbra, Instituto da Conservação da Natureza, Divisão do Litoral e da Conservação da Natureza, and da Comissão de Coordenação e Desenvolvimento Regional do Centro.

REFERENCES

- Araújo M. B. (2003), "The coincidence of people and biodiversity in Europe," *Global Ecol. Biogeogr.*, **12**, 5 – 12.
- Balmford A., Moore J. L., Brooks T., Burgess N., Hansen L. A., Williams P., and Rahbek C. (2001), "Conservation conflicts across Africa," *Science*, **291**, 2616 – 2619.
- Beatley T. (1996), *Habitat Conservation Planning — Endangered Species and Urban Growth*, Univ. of Texas Press, Austin.
- Castro, M. J. and Oliveira J. M. (2002), "Projecto *Alytes* — a conservação de uma população ameaçada de sapos-parteiros (*Alytes obstetricans*) (Amphibia: Discoglossidae)," *Folha Herpetol.*, **16**, 1 – 2.
- Jones C. G. (2002), "Reptiles and Amphibians," in: Perrow M. R. and Davy A. J. (eds.), *Handbook of Ecological Restoration — Principles of Restoration. Chapter 18*, Cambridge Univ. Press, Cambridge, pp. 355 – 375.
- Myers N., Mittermeier R. A., Mittermeier C. G., da Fonseca G. A., and Kent J. (2000), "Biodiversity hotspots for conservation priorities," *Nature*, **403**, 853 – 858.
- Oliveira J. M. and Castro M. J. (2002a), "The *Alytes* Project — The midwife toads (*Alytes obstetricans*) of the Santa Cruz Park Football field in Coimbra: characterization of a threatened population and its habitat," *Poster Presented at "VII Congresso Luso-Espanhol de Herpetologia"*, Évora (Portugal).
- Oliveira J. M. and Castro M. J. (2002b), "The *Alytes* Project — Conservation Plans to Protect midwife toads (*Alytes obstetricans*) (Amphibia: Discoglossidae) in an Urban Area," *Presentation at "VII Congresso Luso-Espanhol de Herpetologia"*, Évora (Portugal).
- UNDP DESA (2002), *World Urbanization Prospects: The 2001 Revision, Data Tables and Highlights*, United Nations Population Division — Department of Economic and Social Affairs, United Nations Secretariat (ESA/P/WP.173), New York. Available at: www.un.org/esa/population/publications/wup2001/wup2001dh.pdf
- Weddel B. J. (2002), *Conserving Living Natural Resources in the Context of a Changing World*, Cambridge Univ. Press, Cambridge.

THE EFFECTS OF DENSITY ON MORTALITY AND DEVELOPMENT OF THE *Bufo bufo* EGGS AND TADPOLES

E. Dmitrieva¹

Keywords: amphibians, toad, embryogenesis, egg density, mortality, tadpole, *Bufo bufo*.

INTRODUCTION

Some authors who studied the tadpole effect of density in different amphibian species have shown that the excreting of substances produced by tadpoles into the water delays tadpole's growth and development (Brockelman, 1969; Pjastolova and Ivanova, 1981; Rous and Rous, 1964; Schvarts et al., 1976). Other authors supposed that the effect of density is based on the behavioral mechanisms (Wassersug, 1974). The high density inhibits growth of tadpoles (Brockelman, 1969; Brady and Griffiths, 2000; Pjastolova and Ivanova, 1981; Reading and Clarke, 1999; Rous and Rous, 1964; Schvarts et al., 1976; Wassersug, 1974). Tadpoles developing under the high density will be smaller at the metamorphosis stage than the "low density" tadpoles (Brockelman, 1969; Brady and Griffiths, 2000; Rous and Rous, 1964; Schvarts et al., 1976). It was shown also that common toad tadpole mortality may be density-dependent (Brady and Griffiths, 2000; Reading and Clarke, 1999).

It is known that density at the tadpole stages influences not only metamorphosis and post- metamorphosis

stages of amphibians' development, but their pubescence as well (Pjastolova and Ivanova, 1981), i.e., "the effect of memory" of developmental conditions was noted. Some authors suppose that we can observe indirect effects of density inhibiting the growth rate and elongating the period of larval development (Rous and Rous, 1964) rather than direct effects of density on the survival of tadpoles.

Unfortunately, information on the influence of amphibian egg density on the developmental and growth rate is not sufficient. Therefore, the purpose of this work was to study the influence of egg density on the mortality and development of embryos and on the growth and development of tadpoles in the common toad (*Bufo bufo*).

MATERIAL AND METHODS

The pairs of common toad (*Bufo bufo*) were collected during mass oviposition in May 2002. The parents were placed into separate aquariums for spawning. Right after the termination of spawning two egg clutches were used in the following experiments:

Experiment 1. The parts of the clutches of the first pair were placed into the identical aquariums (diameter of a bottom was 75 mm, and height of a water column was 30 mm, 0.133 liter of water per aquarium) with different egg density. The initial egg densities were 30, 60, 120, 240, 480, and 960 eggs per aquarium (Fig. 1).

Experiment 2. Other parts of the same clutches were placed into the similar aquariums, 480 and 120 eggs per aquarium. All dead eggs had been removed from aquariums with the help of surgical tools and pipettes. These manipulations were carried out daily at the same time.

Experiment 3. Eggs from the clutches of the second pair were placed into the 90 identical aquariums (diameter of a bottom was 30 mm, and height of a water column was 30 mm, about of 0.02 liter of water), 1 egg per aquarium. In addition, 480 eggs from the same clutch were placed into the aquarium with 0.265 liter of water.



Fig. 1. The aquarium for experiments 1 and 2 with 240 eggs.

¹ Faculty of Biology, M. V. Lomonosov Moscow State University, Leninskie Gory 1/12, 119899 Moscow, Russia; E-mail: dmitrieva@aport.ru.

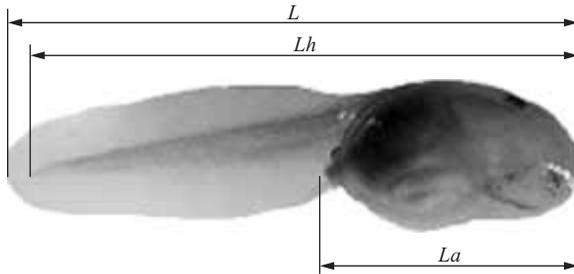


Fig. 2. The measurements of tadpoles.

In the 30 aquariums with singly reared embryos (SRE) a half of water was replaced with the same volume of water taken from the aquarium with 480 eggs. Water was replaced every 8 h (“Test”).

In the other 30 aquariums with SRE water was mixed thoroughly every 8 h without any replacement (“Control 1”). The rest part of SRE (30 aquariums) developed without any special influence until the hatching (“Control 2”).

In all these experiments the numbers of dead eggs were counted, and also the developmental stage of each embryo had been estimated (Caubar and Gipouloux, 1956). “Development rate index” was introduced for the estimation of developmental rate of egg group. For each egg group this index was calculated by the formula:

$$X(t) = \frac{1}{M(t)} \sum_{i=1}^J [K(i)n(i, t)],$$

where $X(t)$ is the development rate index of egg group at the time t , $M(t)$ is the number of survive eggs in the group at the time t , J is the number of developmental stage, $n(i, t)$ is the number of embryos at the developmental stage i at the time t ; $K(i)$, the correction coefficient for each developmental stage (for example, cleavage, 0, gastrula, 1, and so one to hatching, 6). t -Test for independent samples (Statistica 6.0 for Windows) has been used for the comparison of mortality and development rate indexes in the different egg groups (the 95% confidence intervals).

Experiment 4. This experiment was performed on tadpoles. The Tadpoles hatched in the experiments 1 and 3 were placed into the same aquariums (height of a water column was 60 mm) with 0.265 liters of water (2 and 5 tadpoles per aquarium). Other conditions of tadpoles rearing were identical. The experiment was finished on the 25th day of development, after the formation of “trowelform” hind-legs (developmental stage, IV₇(3)). At the end of experiment the L , Lh , and La were measured for each tadpole (Fig. 2). t -Test for independent samples was used for the comparison of tadpole groups.

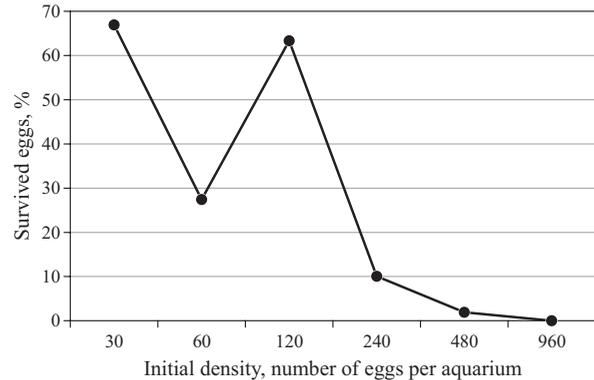


Fig. 3. The dependence of survived eggs percent from initial density.

The temperature of water in all experiments was $17.6 \pm 0.3^\circ\text{C}$.

RESULTS AND DISCUSSION

The main result of experiment 1: the maximum percentage of surviving embryos was observed at the densities of 30 and 120 eggs per aquarium (Fig. 3). An increase of mortality rate was observed at the densities higher than 120 eggs per aquarium. This effect also appeared at the intermediate density (60 eggs per aquarium). The mass mortality of eggs began at the highest density (480 and 960 eggs) since earlier stages of embryogenesis than at low densities. The development rate was highest at the densities less than the 240 eggs per aquarium (Fig. 4). The inhibition of development (development was stopped at the gastrula stage) was observed at the highest density (960 eggs).

The correlation between the survival of embryos and initial density in the experiments with removing of dead eggs is shown in Fig. 5. The mortality at the density of 480 eggs was higher than at the density of 120 eggs. The rate of development at the density 480 was lower than at the 120 eggs. These results are similar to the results of the experiment 1 (without removing of dead eggs). Therefore, an influence of manipulations associated with the removing of dead eggs levels expected positive effect of the absence of dead embryos and leads to the increase of death rate and slight delay of development.

In the experiment 3 (development of single eggs) the maximum mortality was observed in the Test (16.67%), and the minimum mortality in the Control 2 (3.34%). Thus, the best conditions were in the aquariums with eggs developed without any influence. Water mixing without replacement produced more unfavorable conditions. The

worst conditions were observed in the experiments with water replacement. These experimental treatments caused no appreciable effect on the development rates. The development rates of single eggs were higher, than in the clutch (480 eggs per aquarium). The hatching of single eggs came much quicker, than in the clutch. The high synchronization of developmental rate of single eggs was observed.

Tadpoles, reared at a density of 2 tadpoles per aquarium, differed significantly ($p < 0.05$) from the tadpoles, reared at a density of 5 (experiment 4). The means of *Lh* significantly differed in all cases and the same tendency was observed for *La* and *L*. The tadpoles from density 2 had longer body than tadpoles from density 5 (Table 1). Density had no influence on the developmental rate of tadpoles. Essential distinctions in tadpole mortality were not observed also.

“The effect of memory” of embryonic development was observed at the high density of tadpoles as an effect of

the initial density of eggs (Table 1). The tadpoles reared under the different initial egg density significantly ($p < 0.05$) differ by mean values of *La*. This tendency was observed for *Lh* and *L* as well, but distinctions were not always significant because of small number of animals.

The tadpoles reared under the egg density 30 were significantly larger compared with tadpoles under egg density 120 (Table 1). Tadpoles developed from the SRE Test and the SRE Control 2 differed significantly ($p < 0.05$) (Table 1). The best way to show this result is the comparison of *La* means. The tadpoles under the Test had a larger *La*, than the tadpoles under the Control 2 (Table 1). This tendency was observed for *Lh* and *L* as well, but the distinctions were not always significant because of small sampling size. These differences occurs at the low (2 tadpoles) and at the high (5 tadpoles) density. Thus, “the effect of memory” of embryonic development was observed, the tadpoles passed embryogenesis without any special influences were larger than the tadpoles that developed from the eggs reared under the water replacement.

Thus, dependence of mortality and developmental rate from the egg density has a non-monotone segment. At the density of 120 eggs the survival and the developmental rate appeared to be much higher than one could expect,

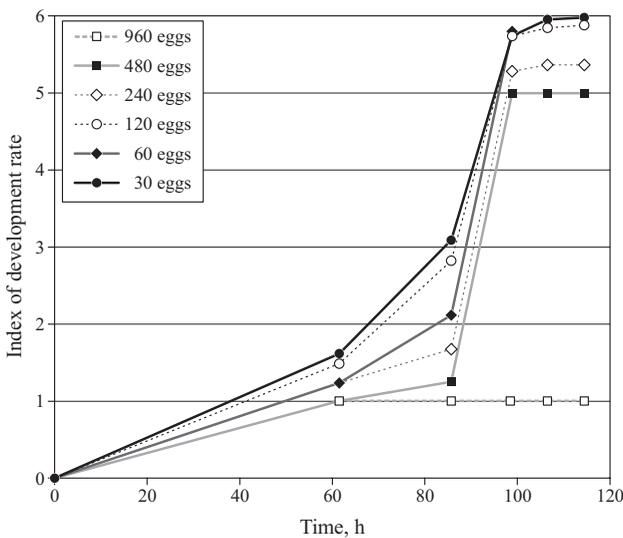


Fig. 4. The time-dependence of the development rate index at different density.

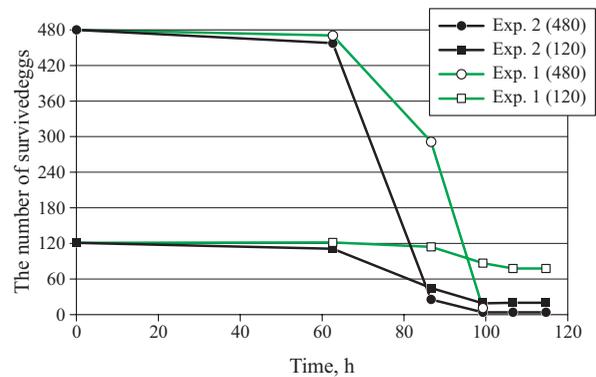


Fig. 5. The dependence of survival rate of eggs on density at removal of dead eggs.

TABLE 1. Measured Characteristics of Tadpoles at Different Density

Initial density of eggs	Density of tadpoles					
	<i>L</i>		<i>Lh</i>		<i>La</i>	
	2	5	2	5	2	5
120	13.37 ± 0.55*	11.66 ± 0.30*	12.94 ± 0.54*	11.24 ± 0.29*	5.89 ± 0.23*	5.37 ± 0.13*
30	13.91 ± 0.10*	12.13 ± 0.16*	13.46 ± 0.10*	11.72 ± 0.16*	6.23 ± 0.06*	5.69 ± 0.09*
SRE Test	13.91 ± 0.18	13.33 ± 0.22	13.46 ± 0.17*	12.84 ± 0.21*	6.31 ± 0.09	6.17 ± 0.11
SRE Control 2	13.55 ± 0.31	12.75 ± 0.12	13.14 ± 0.30*	12.32 ± 0.12*	5.86 ± 0.12	5.71 ± 0.05

SRE, the singly reared embryos from experiment 3.

*, indicates that the distinction is significant at $p < 0.05$.

and were similar to the survival and developmental rate of animals at a low density (30 eggs). By contrast, at a density of 60 eggs the survival appeared to be lower than expected. This effect was described for the first time. In the experiments with eggs, the inhibition of development was observed and mass mortality began since the earliest stages of embryogenesis at the highest egg density. The tadpoles under the high initial egg density were smaller than the tadpoles under the low initial egg density. However, the density had no effect on the developmental rate of tadpoles, which is consistent with the data of other authors (Brady and Griffiths, 20001).

Acknowledgments. This research was supported by the programs “Leading Scientific School” (grant No. SSch-1825.2003.4) and RFBR (grant No. 02-04-49230).

REFERENCES

- Brady L. D. and Griffiths R. A.** (2000), “Developmental responses to pond desiccation in tadpoles of the British anuran amphibians (*Bufo bufo*, *B. calamita* and *Rana temporaria*),” *J. Zool.*, **252**, 61 – 69.
- Brockelman W. J. Y.** (1969), “An analysis of density effect and predation in *Bufo americanus* tadpoles,” *Ecologia*, **50**(4), 632 – 644.
- Caubar R. and Gipouloux J.** (1956), “Table gchronologique du development embryonnaire et larvatre du crapaud commun *Bufo bufo* R.,” *Bull. Biol. Fr. Belg.*, **XC**, F2.
- Pjastolova O. A. and Ivanova N. L.** (1981), “Effect of tadpoles model population density on sexual maturation in the Oriental fire-bellied toad, *Bombina orientalis* (Blgr.),” in: *Herpetological Investigations in Siberia and the Far East*, Izd. ZIN AN USSR, Leningrad, pp. 87 – 91 [in Russian].
- Reading C. J. and Clarke R. T.** (1999), “Impacts of climate and density on the duration of the tadpole stage of the common toad *Bufo bufo*,” *Oecologia*, **121**(3), 310 – 315.
- Rous S. and Rous F.** (1964), “The excretion of tadpoles the substances, which delay their grow,” in: *Mechanisms of Biological Competition*, Moscow, pp. 263 – 276 [in Russian].
- Schvarts S. S., Pjastolova O. A., Dobrinskaya L. A., and Runkova G. G.** (1976), *The Effect of Group in the Populations of Water Animals and Chemical Ecology*, Nauka, Moscow [in Russian].
- Wassersug R. J.** (1974), “Aspects of social behavior in anuran larvae,” in: Blair W. F. (ed.), *Evolutionary Biology of the Anurans*, Acad. Press, New York, pp. 274 – 290.

THE AMPHIBIAN DECLINE IN NORWAY – REASONS AND REMEDY (CASE: ACIDIC PRECIPITATION)

D. Dolmen¹

Earlier studies indicate, but do not prove, that amphibian declines in Scandinavia in part are due to anthropogenic acidification. For instance, amphibians are very rare in the acidified region of Southern Norway. In this study, a total of 58 lakelets (pH range: $4.4 - 6.9$; mean \pm S.D.: 5.0 ± 0.5) were investigated in 1996 and 1997. Only a very few amphibian sites were registered. In early springs 1998 – 2001, liming was then carried out in 4 of the lakelets that lacked amphibians, but lay within reasonable migration distance. They were all strongly acidified before liming (pH in spring 1997: 4.7 ± 0.1). The pH values rose (1998: 5.1 ± 0.2 , 1999: 5.5 ± 0.8 , 2000: 6.1 ± 0.4 , and 2001: 6.5 ± 0.6). All four years, amphibians of all three species for the region (*Rana temporaria*, *Bufo bufo*, *Triturus vulgaris*) started to show up, and two of them were spawning in increasing numbers. Conclusions: Acidification is the main reason for an amphibian decline in Southern Norway — and liming of lakes is a remedy.

Keywords: amphibian decline, *Rana temporaria*, *Bufo bufo*, *Triturus vulgaris*, acidification, liming, Norway.

INTRODUCTION

Declining amphibian populations have been observed world-wide in recent decades (e.g., Phillips, 1990; Houlihan et al., 2000). This is also the case in Scandinavia. Dolmen et al. (2004) indicated that acidification due to long-transported pollution has caused the regional extinction of amphibians over an area of several hundred km² of Southern Norway, as it has of fish (cf. Rodhe et al., 1995; Hesthagen et al., 1999).

Unfortunately, the distribution of amphibians was not studied prior to the anthropogenic acidification, and it may still be questioned whether the almost total absence of amphibians from large parts of Southern Norway is really due to the acidification or to more subtle environmental factors.

If acidification is the main culprit, we can predict that a) liming ponds and lakes (which raises the pH level) will improve the reproductive success where it is poor today, and b) within migrating distance from amphibian refugia (ponds and lakes with better water quality), liming will lead to the (re-)establishment of amphibians at the site. A project on amphibian distribution and liming of acidified lakes therefore began in 1996.

METHODS

A total of 58 bog lakelets (small lakes) and pools in Tovdalen, a valley in the acidified region of Southern Norway, were investigated for amphibians. Only a few am-

phibian refugia were discovered, and these had populations of *Rana temporaria* L., 1758, *Bufo bufo* (L., 1758), and/or *Triturus vulgaris* (L., 1758). Four of the many acidified lakelets were chosen for the liming experiment (Ogge B, Ogge D, Øynaheia A, and Bås D). They all lacked amphibians, or amphibian reproduction had failed, but were within a reasonable migrating distance from a breeding locality, i.e., 100 – 1500 m away. None had fish. Another two lakelets (Ogge A and Bås C) were chosen as reference localities, but they will not be considered much here.

During 1996 (June, July, September) and 1997 (May, June, August), the lakelets were thoroughly examined using two methods: a) z-sweep sampling with a net in the water at ten potentially suitable amphibian sites near the shore (Dolmen, 1991), and b) observations from the shore using polarizing spectacles and spending ~1 h at each locality; the number of individuals observed was added to the number taken during the sampling. Any amphibians caught were then released. Egg clumps of *R. temporaria* were always counted in early May. Only the number of tadpoles (0+) counted in June are considered, i.e., those which had survived the first few weeks of life. [Adults and juveniles (>0+) are not considered or quantified here.] For *T. vulgaris*, the highest counts of adults or larvae on one day have been used, regardless of the month.

Lime was spread on the ice in winter or early spring in 1998 – 2001. About 25 – 400 kg of powdered limestone (85% CaCO₃) were used at each locality, most at the larger ones, and successively more each year. New investigations were carried out in May, June, and September.

¹ NTNU University Museum, NO-7491 Trondheim, Norway;
E-mail: dag.dolmen@vm.ntnu.no.

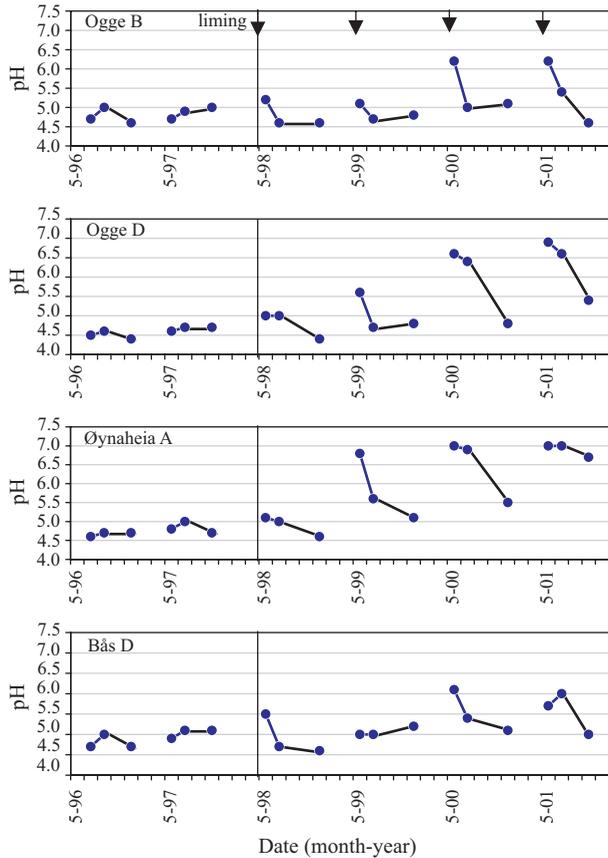


Fig. 1. The course of the pH in the experimental lakelets before and after liming. Arrows indicate liming.

RESULTS AND DISCUSSION

Before liming, in 1996 and 1997, the lowest pH values were normally recorded in May and September. After liming, May usually had the highest pH; after which the values fell. On average (mean ± S.D.), after liming, the values in the limed lakelets in May rose from 4.7 ± 0.1 (in 1997) to 5.1 ± 0.2 (1998), 5.5 ± 0.8 (1999), 6.1 ± 0.4 (2000), and 6.5 ± 0.6 (2001) (Fig. 1). The highest value (pH 7.0) was obtained at Øynaheia A in 2000 and 2001.

Where the liming brought the pH up to 5.0 or more for some periods, *R. temporaria* began successful breeding in the first or second year of liming (Fig. 2). The increase in the number of egg clumps (1998 – 1999) was statistically significant ($P < 0.001 - 0.01$; χ^2 test) at Ogge B, Ogge D and Bås D. Juvenile (>0+) and adult *R. temporaria* were also often seen from 1998 and 1999, especially at Ogge D (not shown in Fig. 2). In addition, *T. vulgaris* was observed for the first time in two of the lakelets; breeding was recorded in 2000 in one of them, and in a third in 2000 and 2001. No further increase of *R. temporaria* was seen

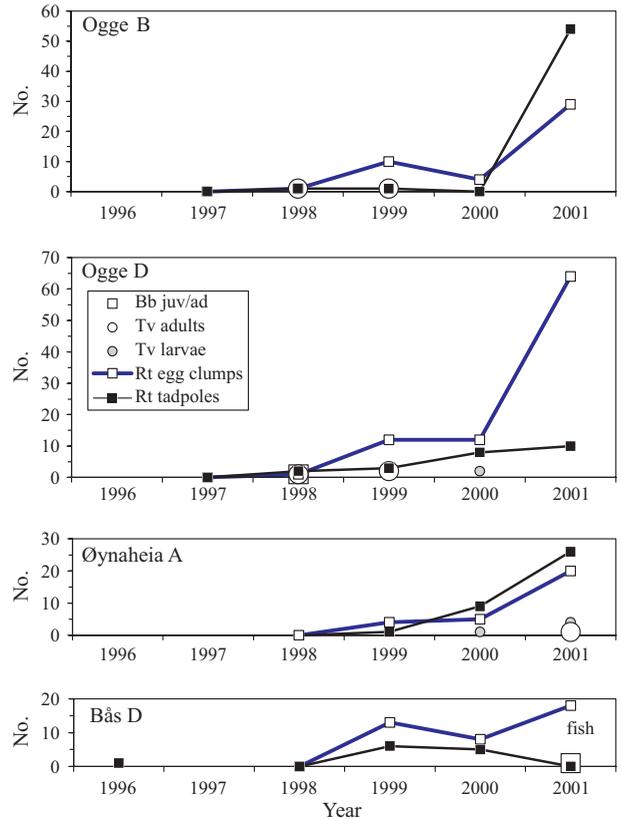


Fig. 2. The number of amphibians in the experimental lakelets before and after liming: Tv, *Triturus vulgaris*; Rt, *Rana temporaria*; Bb, *Bufo bufo*.

in 2000, but the reference localities (not shown in Fig. 2) showed that this was a very unfavorable year for amphibian breeding. However, there was a new, very strong and significant ($P < 0.001 - 0.05$) increase in 2001.

A juvenile and an adult *B. bufo* were observed at Ogge D (1998) and Bås D (2001), respectively, but reproduction was not proved. In 2001, fish *Perca fluviatilis* were recorded for the first time at the Bås locality. Although as many as 18 *R. temporaria* egg clumps were counted in May, no tadpoles were recorded that year.

The appearance of *R. temporaria* and the clear increase in the number of egg clumps and tadpoles in all four limed lakelets show the beneficial effect on amphibians of raising the pH in acidic water bodies. Although *R. temporaria* responded most quickly, *T. vulgaris* also appeared at three localities and reproduced at two of them; even *B. bufo* seemed to be attracted by the improved water quality. The fact that no *R. temporaria* tadpoles were recorded at Bås in 2001 is easily explained by the predatory fish which had invaded the lakelet.

Under normal circumstances, and as was also seen in the present study in 1996 – 1997, the lowest pH values are usually measured in spring. This is first of all due to the easy-melting acidic components of the snow. The values then rise through the summer, in part because of the photosynthesis effect, and decrease in autumn (cf. Hagen and Langeland, 1975). However, liming on the ice (1998 – 2001) radically changed the course of the pH curve by elevating the pH in spring (especially) and early summer (Fig. 1). The pH state thus became more or less ideal for the most sensitive amphibian development stages, i.e., the eggs, embryos, and tiny larvae (cf. Clark and LaZerte, 1985).

Dolmen et al. (2004) found that, in acidified districts, *R. temporaria* sometimes reproduced when the pH was as low as approximately 4.6 (May – early June measurements) if the calcium content in the water was high enough. [The aluminum (Al_a) concentration was usually below 150 $\mu\text{g/liter}$.] A somewhat higher pH seemed necessary when the calcium content was very low (0.5 – 1 mg Ca^{2+} /liter). However, although eggs hatch and many larvae grow up, even a pH level as high as 5.0 (and a high Ca^{2+} level) has demonstrably negative effects on egg and larval viability and fitness (Andrén et al., 1988). Liming should therefore probably aim at bringing the pH up to 6.0 or more for at least one month. True enough, the pH then falls again in the course of the summer, because of more acidified rain and the fact that lime is washed out of the lakelets (cf. Fig. 1). However, in the most critical period and also later, the pH may still be high enough for a healthy development of eggs and larvae.

The time perspective for when amphibians, if ever, will appear at a newly restored lakelet, depends on the distance from other amphibian localities, the migration capacity of the species, individual vagility and other factors. Over time, in accordance with this investigation, a couple of kilometers seems to be no big problem for any of the three species in question.

It is unclear, however, whether the amphibians' new-establishment should be explained solely as the statistical result of the by chance discovery of a new locality, by the few more or less vagile individuals spread around in the landscape. A lake of good water quality in an otherwise relatively inhospitable environment may possibly act as a "trap" for such "casual passers-by."

However, the statistical probability of finding a new lakelet diminishes quickly, at a geometrical rate, with the distance (see, e.g., Udvardy, 1969) — unless special senses of orientation are in action. Which senses, if any, and to which degree, have been debated. Savage (1961) pointed out that *R. temporaria* seemed to recognize the

smell of its "home pond" or of certain plants growing there, and that this could be of help in orientation back for breeding. Pasanen and Sorjonen (1995) found that *R. temporaria*, after having been translocated, was able to orientate towards its hibernation pond from a distance of at least 500 m, probably by use of the sense of smell. It seems therefore possible that the establishment of amphibians in new localities — or localities that have been made suitable through liming — is not only by chance.

CONCLUSIONS AND PERSPECTIVES

In the acidified region of Southern Norway, it has been shown that a) where the reproductive success of amphibians was poor, liming improved it, and b) within migrating distance from amphibian refugia, liming led to the (re-)establishment of amphibians.

The main reason for the absence of amphibians in the region is therefore the acidification, and one remedy is to lime ponds and lakes.

POSTSCRIPT

It should be born in mind that liming is not the solution to "bio-diversity problems" in all acidic lakes, because many of them are not anthropogenically acidified, but naturally acidic and have a natural, acidophilic fauna, that should not be limed to extinction. And another important aspect is, that because of fish predation, liming for fish can often be worse off for the amphibians than not liming at all.

REFERENCES

- Andrén C., Henrikson L., Olsson M., and Nilson G. (1988), "Effects of pH and aluminum on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina*," *Holarctic Ecol.*, **1**, 127 – 135.
- Clark K. L. and LaZerte B. D. (1985), "A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles," *Can. J. Fish. Aquat. Sci.*, **42**, 1544 – 1551.
- Dolmen D. (1991), "Dammer i kulturlandskapet — makroinvertebrater, fisk og amfibier i 31 dammer i Østfold," *NINA Forskn. rapp.*, **20**, 1 – 63 [in Norwegian].
- Dolmen D., Blakar I., and Skei J. K. (2004), "The distribution of *Rana temporaria* L. (Amphibia) in an acidified and a non-acidified region of Norway," *Fauna Norv.*, **24**, 19 – 29.
- Hagen A. and Langeland A. (1973), "Polluted snow in southern Norway and the effect of the meltwater on freshwater and aquatic organisms," *Environ. Pollut.*, **5**, 45 – 57.

- Hesthagen T., Sevaldrud I. H., and Berger H. M.** (1999), "Assessment of damage to fish populations in Norwegian lakes due to acidification," *Ambio*, **28**, 112 – 117.
- Houlahan J. E., Findlay C. S., Schmidt B. R., Meyer A. H., and Kuzmin S. L.** (2000), "Quantitative evidence for global amphibian population declines," *Nature*, **404**, 752 – 755.
- Pasanen S. and Sorjonen J.** (1995), "An orientation experiment conducted during autumn migration of the common frog (*Rana temporaria*)," *Mem. Soc. Fauna Flora Fenn.*, **71**, 113 – 117.
- Phillips K.** (1990), "Where have all the frogs and toads gone?," *BioScience*, **40**, 422 – 424.
- Rodhe H., Langner J., Gallardo L., and Kjellström E.** (1995), "Global scale transport of acidifying pollutants," *Water Air Soil Pollut.*, **85**, 37 – 50.
- Savage R. M.** (1961), *The Ecology and Life History of the Common Frog* (*Rana temporaria temporaria*), Pitman, London.
- Udvardy M. D. F.** (1969), *Dynamic Zoogeography with Special Reference to Land Animals*, Van Nostrand Reinhold, New York.

THE EUROPEAN TREE FROG REINTRODUCTION IN LATVIA

I. Duncce¹ and J. Zvirgzds¹

Keywords: *Hyla arborea*, reintroduction, captive breeding.

INTRODUCTION

Wildlife reintroduction, along with restoration and protection of the essential habitats is a vital aspect of species conservation, as it is stressed by the Rio de Janeiro Convention of Biodiversity, the Bern Convention on the Conservation of European Wildlife and Natural Habitats as well as the World Zoo Conservation Strategy.

The European tree frog (*Hyla arborea*) is included in IUCN Red List of Threatened Animals as a near threatened species (LRnt), in Appendix II of the Bern Convention, in Appendix IV of the European Union's Habitat and Bird Directive 92/43/EEC, and in Latvian Red List as 1st Category species. *Hyla arborea* is protected in Latvia by the Law of Protected Species and Biotopes since 16 March 2000.

In 1988, the tree frog was considered extinct in Latvia already for several decades. Data on its former distribution are rather incomplete. Several zoologists (Fischer, Seidlitz, Schweder) have mentioned the species as present in Latvia in the 18 – 19th centuries. During the 20th century there were several additional reports, especially in the first half of the century (Silins and Lamsters, 1934).

We consider that there are no theoretical grounds to oppose the claim that, in recent past, the northern border of tree frog's range included Latvia.

The crucial factor influencing the decline of the tree frog population in Latvia could have been a rapid deterioration of wetlands, caused by the increasing intensity of agriculture at the end of the 19th century. This, in turn, led to destruction of the natural landscape.

The second factor causing the deterioration of wetlands was extinction of the beaver (*Castor fiber*) in Latvia. According to literature sources, the last Beaver in Latvia was shot in 1871 (Rupeiks, 1936). In 20th century the beaver population in Latvia was restored. According to our observations, dikes, canals or trails in dense vegetation of overgrown ponds as well as the subsequent changes in the water regime caused by beaver, are providing and maintaining the habitats suitable for the tree frog.

MATERIAL AND METHODS

The reintroduction was planned with captive-bred tree frog young in their first year of life. The considerations were as follows:

- 1) the translocation of larger amount of adult specimens from other natural populations could place the donor population at risk, even if the population is considerably stable;
- 2) the youngsters would have a considerably higher ability to adapt to wild conditions than adults, if captive specimens are to be released into wild.

The adult specimens for captive breeding were caught in Southern Belarus' (near the confluence of Goryn' and Pripyat' Rivers). The adults were kept in outdoors terraria and fed with artificially bred food insects (*Musca domestica*, *Gryllus* sp., *Galleria mellonella* larvae) as well as Caliphoridae and Sarcophagidae flies collected in the wild.

Breeding was stimulated with hormone injections, using Surphagon, a synthetic analogue of Luliberin (produced by Bapex Co., Latvia). During the first year of breeding effort the hormone treatment was given in the beginning of May, in other years — already in the beginning of March. In both cases the results were virtually identical. Two males and one female were usually placed in a 35-liter aquarium with a water level of about 5 cm and several water plants.

Each female produced 200 – 1000 or even more eggs. Hatching usually started on the 8 – 10th day of development.

The larvae were placed into aquariums with aerated water. The larvae density never exceeded 2 – 3 larvae per liter. The natural photoperiod was simulated using luminescent lamps (40 W). Temperature range was 24 – 27°C at day, 20 – 23°C at night. Tadpoles were fed *ad libitum* with dried and boiled nettles, meat, aquarium fish food (Tetra) and pollen.

The metamorphosis took 30 – 60 days (in the wild it usually takes 90 days). Froglets were fed with wild-caught insects (Diptera) and laboratory-bred food insects (*Drosophila*, *Gryllus* and, later, also *Musca domestica*). 2 – 6 weeks after full metamorphosis the froglets were ready to be taken to the reintroduction site.

¹ Department of Amphibians, Riga Zoological Garden, Meza av. 1., LV 1014 Riga, Latvia; E-mail: ilzeduncce@hotmail.com.

The release site was chosen in SW Latvia (Liepaja District, ca. 56°30' N 21°42' E), where a protected area ("Lake Blazgis") was established with total area of 350 ha. The area accommodates a large number of natural ponds and abandoned artificial fish ponds. Beavers have considerably changed most of them.

During 1988 – 1992 a total of 4110 juveniles, progeny from 14 – 17 breeding pairs, were released. All releases were conducted in one locality, enabling accurate further monitoring of population dispersal.

The distribution of the newly created population was monitored mainly on the basis of the spring mating calls. All new-recorded localities were registered by GPS and mapped (the map scale 1:50000).

RESULTS

Under laboratory conditions the breeding can be effected to happen earlier in the season than in the wild, and, furthermore, in the laboratory the larvae develop faster, reaching the metamorphosis in a shorter period of time. Thus, the released froglets have more time in their first summer to adapt to natural conditions as well as for feeding and growing. We hypothesize that it could result in a much higher survival rate during the released froglets' first winter as well.

Our data show that the released froglets can mature and breed successfully in the wild. The first vocalizations of adult tree frog male were recorded in 1990 — two years since the start of our reintroduction program. That confirms that under particular conditions males can reach sexual maturity in 2 years. The first tadpoles in the wild were found in 1991, at the release site. Thus, under particular conditions, females can reach sexual maturity in 3 years.

Our monitoring data show also that tree frogs hibernate well under the climatic conditions of Latvia. The tree frog population was not affected by several severe winters

when temperature frequently stayed below -20°C for a longer period, with little snow cover. In all cases the tree frog population survived and started to increase.

Tree frogs are spreading around the initial release site, gradually colonizing new breeding localities. The first calling males outside the release site were recorded in 1993. The further distribution progressed even faster. Up to 2002 the tree frogs were recorded already in more than 110 localities. The breeding is confirmed in at least 10 different ponds.

14 years after initiating the reintroduction program, our monitoring data show that the stable and regenerating population is established, with already 5 – 6 generations developed naturally. The total area of population dispersal covers already ca. 800 – 900 km². The area extends ca. 35 km in S – N direction, and ca. 39 km in W – E direction.

Our successful reintroduction program could serve as an example of native amphibian population re-establishment through release of captive-bred specimens. Riga Zoo's Laboratory of Ecology (named Amphibian Department now) continues its work in developing breeding technologies for native species, as well as for about 20 threatened tropical amphibian species.

REFERENCES

- The World Zoo Conservation Strategy* (1993), Chicago Zool. Soc., Chicago, pp. 61 – 66.
- Convention on the Conservation of European Wildlife and Natural Habitats* (1992), Strasbourg.
- 1996 IUCN Red List of Threatened Animals* (1996), IUCN, Gland, Switzerland; Conservation International, Washington, p. 166.
- Silins V. and Lamsters V.** (1934), *Latvijas rapuli un abinieki*, A/S Valters un Rapa, Riga, pp. 72 – 73 [in Latvian].
- Rupeiks F.** (1936), *Latvijas zveri*, A/S Valters un Rapa, Riga, p. 50.

INFLUENCE OF HYDROPERIOD, SUN EXPOSURE AND FISH PRESENCE ON AMPHIBIAN COMMUNITIES IN A HUMAN DOMINATED LANDSCAPE

G. F. Ficetola¹ and F. De Bernardi¹

Keywords: amphibian ecology, wetland features, newts, fish introduction, agriculture.

INTRODUCTION

Many studies outlined the importance of wetland features in determining amphibian distributions. However, habitat features can interact among them or with processes active in the landscape: in human dominated landscapes, human activities strongly modify the habitat features, and relationship between habitat features. These interactions can therefore influence the distribution of species living in the landscape, like the amphibians.

For example, water permanence should have at least partially a positive effect on community richness. Some amphibian species require long time for larval development: the effects of pond drying can be dramatic for these species, especially if the wetlands dry during the breeding season: only the species with fast growing tadpoles should prefer temporary wetlands for breeding (Skelly et al., 1999). However, many studies recognized the negative effects of fish presence on amphibians, since they predate larval stages of many amphibian species, and only few species can survive in fish inhabited wetlands. As a consequence, wetlands with fish frequently have very poor amphibian communities (Hecnar and McClosely, 1997). In human dominated landscapes, humans frequently introduce fish for sportive fishing also in semi-permanent, fish-free wetlands. Thus, it is possible that communities living in temporary wetlands are richer than those living in the permanent ones, since short-hydroperiod wetlands are the only ones without fish.

Again, human exploitation of landscape for agriculture can be negative for amphibians, since it decreases the terrestrial habitat available (Joly et al., 2001). However, the decrease of canopy cover can improve the sun exposure of wetlands, and therefore it could favor the abundance of thermophile species (Werner and Glenmeier, 1999).

Aim of this study was to investigate how hydroperiod, fish presence, sun exposure and agricultural use of land-

scape factors influence amphibian communities in a landscape strongly modified by humans. To better evaluate the ecological meaning of the relationship between these factors and amphibian presence, we focused our attention on the effects of human activities on these factors and on the relationship between factors.

STUDY AREA AND METHODS

We investigated a surface area of 520 km² in the river Po floodplain, (Lombardy region, Northern Italy). This area surrounds the city of Milan and is one of the European areas with the largest agricultural and industrial development. The landscape is dominated by the presence of urban suburbs and agriculture. Only a few little wooded fragments still exist and the wooded surface is less than 5% of the landscape. We studied amphibian distribution in 84 wetlands (ponds, temporary pools and ditches). Each wetland was surveyed after dusk at least once every 3 weeks, during late winter, spring and early summer (February/June 2002). In each survey we detected adult presence, calling males, tadpoles, and spawn. We deep-netted each wetland for tadpoles in May, sampling banks and bottom. We recorded sun exposure as the percentage of wetland surface directly exposed to the sunshine between 11.00 a.m. and 1.00 p.m. (UTM) in May. We considered a wetland temporary if it dried up during the amphibian breeding season (February – June). We recorded fish as present if we observed them at least once during our surveys. We also recorded percentage of crop surrounding each wetland in a 250 m array surface on the basis of field surveys and of 1:10000 technical regional map, using a Geographic Information System.

Likelihood ratio test of logistic regression was used to evaluate the relationship between wetland features and the presence/absence of each species; linear regression and analysis of variance were used to evaluate the effects of habitat features on community richness.

¹ Dipartimento di Biologia, Università degli Studi di Milano, via Celoria 26, I-20133 Milano, Italy; E-mail: francesco.ficetola@unimi.it.

RESULTS

Seven taxa of amphibians live in the study area: the Italian crested newt *Triturus carnifex*, the smooth newt *Triturus vulgaris*, the common toad *Bufo bufo*, the green toad *Bufo viridis*, the Italian tree frog *Hyla intermedia*, the Italian agile frog *Rana latastei* and the pool frog *Rana synklepton esculenta*. Since we observed *B. bufo* and *B. viridis*, respectively, only in one and three wetlands, we excluded them from some analysis. The average species richness per wetland is 1.3. The analyzed wetland features have a strong effect on the composition and richness of amphibian communities (Table 1). Two species (*H. intermedia* and *R. s. esculenta*) live mainly in sunny wetlands; newts are associated to temporary wetlands; fish presence seems to have a negative effect on the distribution of *T. carnifex*, *T. vulgaris*, and *H. intermedia*; only *R. latastei* distribution seems to be negatively affected by the abundance of surrounding crops. The richest communities live in sunny wetlands, in temporary wetlands and in wetlands without fish. We did not find a significant relationship between crop percentage and community richness (Table 1).

However, the relationship between some of these factors are strong, and they can not be considered independent. Fish presence is associated with permanent wetlands (likelihood ratio: $\chi_1^2 = 9.830$, $P = 0.0017$); wetlands surrounded by high crop percentage are those with the higher sun exposure (linear regression: $F_{1,82} = 7.683$, $P = 0.007$). Therefore, we used the residuals of the relationship between sun exposure and surrounding crop percentage as an independent variable and species richness as a dependent variable. Species richness strongly depend on the residuals of the relationship crop % — sun exposure (linear regression: $F_{1,82} = 12.607$, $P = 0.0006$): we found the richest communities in the wetlands with high sun exposure but with relatively low percentage of surrounding crops.

The other pairwise relationships (sun exposure/crop % vs. fish presence/water permanence) are not significant ($P > 0.05$).

DISCUSSION

The analyzed wetland features strongly influenced amphibians communities. However, our results show a different pattern from studies performed in more natural landscapes (e.g., Skelly et al., 1999). Newt presence and community richness are strongly associated with temporary water. This pattern can be explained by the strong association between fish presence and water permanence: almost all the wetlands that do not dry annually are occupied by fish, since in this landscape fish are frequently released in almost all the wetlands for sportive fishing. The abundance of fish in this landscape is likely one of the causes of the low average species richness (Hecnar and McCloskey, 1997). Moreover, amphibians have to breed in temporary wetlands: this habitat is unpredictable, quickly evolving and frequently not protected by law. The conservation of a network of ponds with different hydroperiod, and possibly avoiding fish introduction, should be an important action for amphibian protection in agricultural landscape (Beja and Alcazar, 2003).

The most striking result of our study is the lack of relationship between agricultural exploitation of terrestrial habitat and amphibian presence: the percentage of surrounding crop seems to have negative effects only on *R. latastei*, a red-listed frog living in lowland broadleaf woods. The positive effect of sun exposure on amphibians communities can explain this pattern. Sun exposure is positively related to percentage of crop since woods are cut to increase the availability of land for agriculture. Higher sun

TABLE 1. Effects of Sun Exposure, Water Permanence, Fish Presence, and Percentage of Surrounding Cultivated Fields on Amphibian Communities

	Sun exposure		Water permanence		Fish presence		% surrounding crop	
	Effects on the presence/absence of five amphibian species (likelihood-ratio test)							
	χ_1^2	<i>P</i>	χ_1^2	<i>P</i>	χ_1^2	<i>P</i>	χ_1^2	<i>P</i>
<i>Triturus carnifex</i>	0.939	0.333	9.994	0.0016 (–)	6.258	0.012 (–)	1.475	0.225
<i>Triturus vulgaris</i>	0.518	0.472	3.765	0.05 (–)	7.211	0.0072 (–)	0.311	0.577
<i>Hyla intermedia</i>	28.988	<0.0001 (+)	2.952	0.086	7.241	0.0071 (–)	1.546	0.214
<i>Rana latastei</i>	2.017	0.155	0.445	0.505	2.017	0.155	5.634	0.018 (–)
<i>Rana synklepton esculenta</i>	17.637	<0.0001 (+)	1.852	0.174	2.588	0.108	0.116	0.733
	Effects on the richness of amphibian communities (ANOVA/linear regression)							
	$F_{1,82}$	<i>P</i>	$F_{1,82}$	<i>P</i>	$F_{1,82}$	<i>P</i>	$F_{1,82}$	<i>P</i>
Community richness	11.658	0.001 (+)	6.131	0.015 (–)	6.186	0.015 (–)	0.013	0.908

(+), indicate a significant positive association between the variable value and species presence/community richness; (–), indicate a negative association; in bold, significant results.

exposure causes higher water temperature and more light, and it can enhance tadpole growth rate: many species are thus more abundant in sunny wetlands (Skelly et al., 1999). Therefore, the effect of sun exposure could have masked the negative effects of loss of terrestrial habitat caused by agriculture: the negative effects of habitat loss are evident only after taking into account the strong relationship between sun exposure and crop presence. However, the positive effects of sun exposure do not reduce the concern for amphibian conservation in agricultural landscapes: only two species (*H. intermedia* and *R. s. esculenta*) benefit from high sun exposure, and these thermophile species are the most widespread and adaptable. The other five species, less favored by this factor, are now really rare in this landscape.

In a human modified landscape, the effects of relationship between the habitat features analyzed (hydroperiod, fish presence, sun exposure and terrestrial habitat) are extremely important for a correct interpretation of the distributional pattern of amphibians. In Europe almost all the landscapes have been strongly modified: the analysis of

relationship between habitat features and processes active in the landscape can be extremely important to correctly understand the forces driving the species distribution.

REFERENCES

- Beja P. and Alcazar R.** (2003), "Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians," *Biol. Conserv.*, **114**, 317 – 326.
- Hecnar S. J. and McCloskey R. T.** (1997), "The effects of predatory fish on amphibian species richness and distribution," *Biol. Conserv.*, **79**, 123 – 131.
- Joly P., Miaud C., Lehmann A., and Grolet O.** (2001), "Habitat matrix effects on pond occupancy in newts," *Conserv. Biol.*, **15**, 239 – 248.
- Skelly D. K., Werner E. E., and Cortwright S. A.** (1999), "Long-term distributional dynamics of a Michigan amphibian assemblage," *Ecology*, **80**(7), 2326 – 2337.
- Werner E. E. and Glenmeier K. S.** (1999), "Influence of forest canopy cover on the breeding pond distribution of several amphibian species," *Copeia*, **1999**(1), 1 – 12.

POSTMETAMORPHIC GROWTH AND MOVEMENTS IN YELLOW-BELLIED TOADS, *Bombina variegata*: APPROACHING LIFE-PATH ANALYSIS

G. Gollmann¹ and B. Gollmann¹

Since spring 1996 we study population ecology of yellow-bellied toads, *Bombina variegata*, in a near-natural environment, the nature reserve Lainzer Tiergarten at the western border of Vienna. Toadlets were individually registered by photographing their ventral pattern at or shortly after metamorphosis. Their lifetime movement patterns appear to be strongly influenced by the quality of terrestrial habitats surrounding the breeding sites. Toads emerging from puddles on a wet meadow were more sedentary than those from a pool on a forest clearing with bare loamy banks. Several toads born at the latter site returned as adults to their natal pool for short visits, although some of them also participated in breeding activities in other habitats.

Keywords: Amphibia, Anura, behavior, dispersal, ecology, life history, migration.

INTRODUCTION

The last three decades have brought tremendous progress in amphibian ecology, with respect to both larval and adult life stages. For many species, the juvenile phase — between metamorphosis and maturity — remains the least known part of their life cycle. As adults often show strong fidelity to breeding sites, juveniles have been surmised to be the main stage for dispersal (Gill, 1978), but this role has been demonstrated only for few species (e.g., Breden, 1987; Berven and Grudzien, 1990; Kneitz, 1998).

In 1996 we started long-term observations on yellow-bellied toads, *Bombina variegata* (Linnaeus, 1758), in a nature reserve at the western border of Vienna (Gollmann et al., 1999; Gollmann and Gollmann, 2002). Here we present results on movements and growth of toads registered soon after metamorphosis during the first three years of this study, focusing on a comparison between two breeding areas with different habitat structures. These data suggest that factors operating early in life may have long-lasting effects on movement patterns of individuals (cf. Kenward et al., 2001).

MATERIAL AND METHODS

The investigations were carried out in the northwestern part of Lainzer Tiergarten, a nature reserve in the west of Vienna, which is covered by deciduous forest interspersed with meadows. In the years 1782 – 1787 it was surrounded by a wall to keep wild boars inside this hunting-ground. Current wildlife management still maintains

high densities of game animals, especially wild boars, mouflon and several species of deer.

We visited a core study area of about 1 km² weekly (with some exceptions) during the activity period of *B. variegata* since 1996. Adjacent parts of the study area have been searched for toads at bi-weekly to monthly intervals. At two sites in the core study area metamorphs were regularly produced in the first three study years: a wet meadow with many small puddles (site A) and a pool with mostly bare loamy banks in a forest clearing (site C) (site codes follow Gollmann et al., 1999).

At each capture of a yellow-bellied toad, its snout-vent length (SVL) was measured with callipers (readings to 0.1 mm, rounded to 1 mm for presentation in this paper). Toads were individually registered by making color photographs of their ventral pattern. For identification, live toads or color photographs were compared to earlier photographs (metamorphs) or black-and-white copies (subadults and adults; see Gollmann and Gollmann, 2002: 119).

RESULTS

Dispersal distances recorded through the observation period (5 to 7 years) have markedly different distributions for the two study sites: Most (17 of 26) toads first registered at site A were never caught more than 200 m away from the point of their first capture, whereas most (20 of 28) toads originating from site C traveled at least 400 m (median 600 m, maximum 1700 m) (Fig. 1).

Analysis of individual life paths shows that many toads reached the location most distant from their birth place in the second or third year after metamorphosis (Fig. 2). Several toads from site C dispersed to various parts of the study area, but returned occasionally to their

¹ Department für Evolutionsbiologie, Universität Wien, Althanstr. 14, A-1090 Wien, Austria;
E-mail: guenter.gollmann@univie.ac.at, birgitgollmann@yahoo.com.

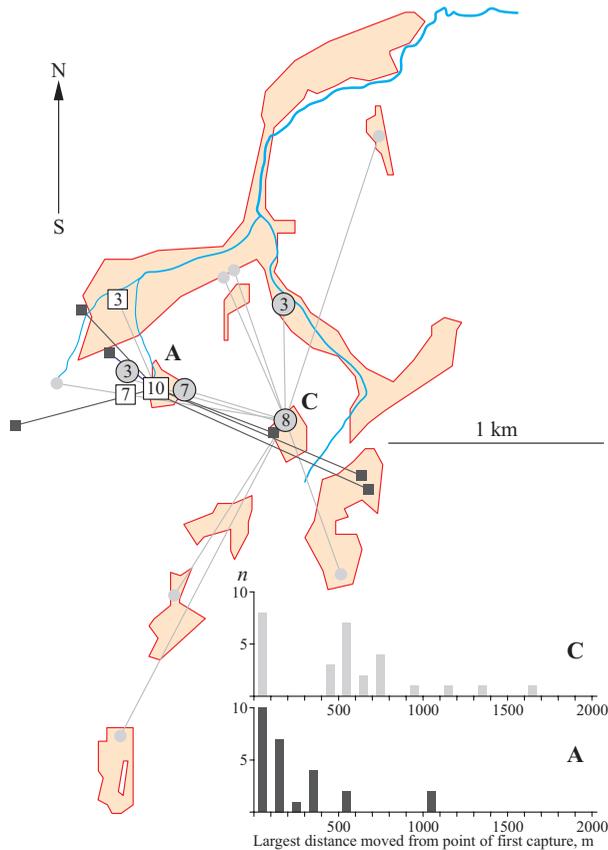


Fig. 1. Dispersal of metamorphs registered at study sites A and C in the years 1996 – 1998. For each individual recaptured after first hibernation, the most distant observation point reached until 2003 is indicated both in a schematic map of the study area (shaded: open areas, white: forest, light grey lines: streams, squares: origin at site A, circles: origin at site C) and in a histogram.

birth place. Two males from the 1996 cohort (σ^7 244, σ^7 280, Fig. 2) moved to site A, but regularly visited site C in May or June from their third year on; similar migration patterns were also developed by toads from later cohorts (data not shown). One male first observed at site A (σ^7 306) also migrated to site C in several years (Fig. 2); first registration of this toad was made late in the year (September 15, 1996) at a fairly large size (SVL 25 mm) together with a known immigrant (σ^7 280) with a similar throat pattern.

Postmetamorphic growth trajectories were similar for both sexes, but showed considerable individual variation (Fig. 3). In our example, the 1998 cohort from site A, great differences in size at metamorphosis were present (Fig. 3; Gollmann and Gollmann, 2002: 75f). Owing to variable growth, size ranks changed over time, in particular among males. After five years, these toads had reached SVLs ranging from 44 to 49 mm.

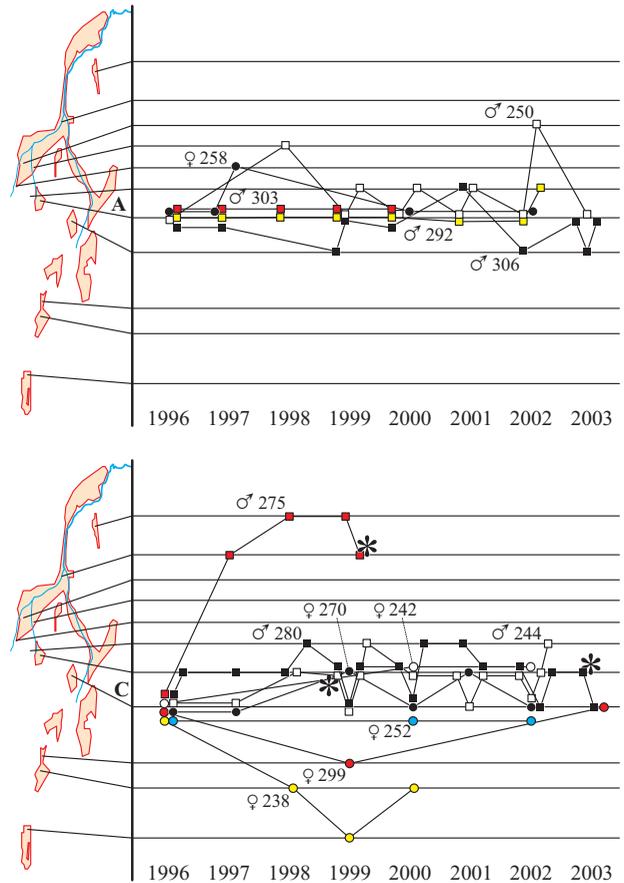


Fig. 2. Individual life paths of *B. variegata* individuals registered shortly after metamorphosis in 1996 (schematic map on the left not to scale, see Fig. 1). For the sake of clarity, only toads recaptured at least three years later are included, repeated recaptures at the same site during a year are not shown. Asterisks indicate observations in amplexus.

DISCUSSION

Dissimilar patterns of dispersal from the two breeding sites (Figs. 1 and 2) were probably caused by differences in habitat quality. The wet meadow (site A) provides constant moisture and dense vegetation, ensuring shelter and food supply, whereas the dry open ground surrounding pool C presents unfavorable conditions for *B. variegata*. For toads metamorphosing in a benign environment the costs of dispersing may outweigh potential benefits of leaving their birth area, whereas for toads entering their terrestrial life phase on inhospitable terrain departure is more clearly advantageous. We suggest that one of the benefits of early dispersal may be gaining knowledge of the landscape, which enables the toads to use different habitats to optimize growth or reproduction. On another wet meadow in the study area we observed mainly juveniles and subadults; some of those toads migrated for breeding

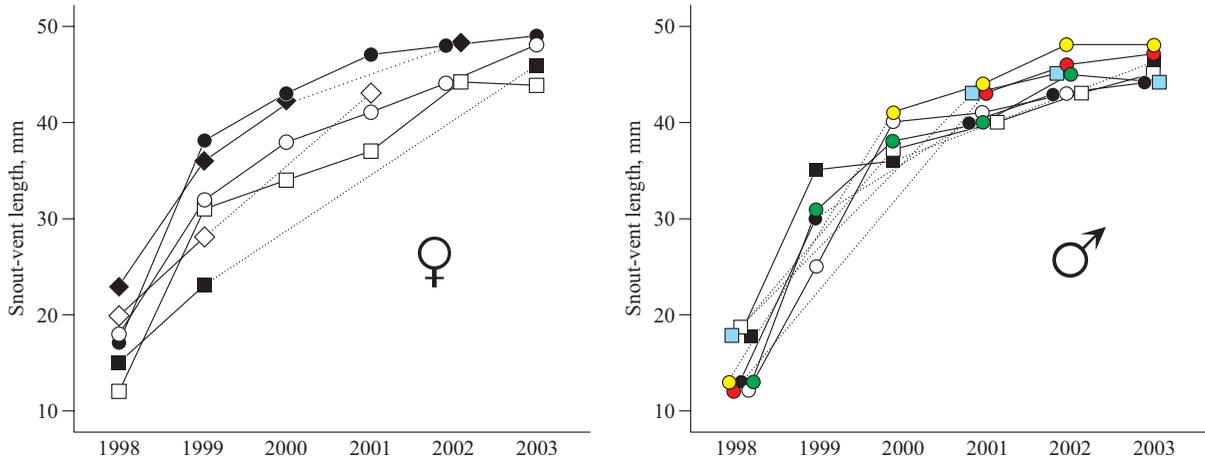


Fig. 3. Growth of *B. variegata* individuals metamorphosing at site A in 1998. For each toad, the first snout-vent length value measured after metamorphosis and the highest values recorded in subsequent years are shown (without regard to time in the year).

to sites they had occupied in earlier years (e.g., ♂ 275, Fig. 2; Gollmann et al., 2000).

Several toads who had dispersed from their birth place repeatedly returned to their natal pool in later years (Fig. 2). Similar recurrent migrations have also been observed in older adult toads (Gollmann and Gollmann, 2002: 80f). These migration patterns appear to be more regular for males than for females. As males were on average captured more often (Gollmann and Gollmann, 2000), this difference is perhaps more a matter of observation than of actual fact. Of the toads first registered in the year of metamorphosis, only individuals born at site C moved from the wet meadow (site A) across the forest to that site during the breeding season (circumstantial evidence presented above suggests that this may also be true for the only apparent exception, ♂ 306).

It is important to note that toads who returned to their birthplace also attempted to reproduce at other sites in the same years (Fig. 2). Reproduction of *B. variegata* follows a risk-spreading strategy: females are able to produce several clutches within a year, what is regarded as an adaptation to spawning in water bodies with a high risk of desiccation (Buschmann, 2002). In their prolonged breeding period (Gollmann et al., 1999; Gollmann and Gollmann, 2000), at least some yellow-bellied toads in our study area distributed their reproductive effort not only in time, but also in space.

Acknowledgments. We thank Christian Baumgartner for help with data collection in the early years of this study. The conservation department of the municipality of Vienna permitted field work in the nature reserve (Magistratsabteilung 22, Wien, Bescheid 2752/96).

REFERENCES

- Berven K. A. and Grudzien T. A. (1990), "Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure," *Evolution*, **44**, 2047 – 2056.
- Breden F. (1987), "The effect of post-metamorphic dispersal on the population genetic structure of Fowler's toad," *Copeia*, **1987**, 386 – 395.
- Buschmann H. (2002), "Fecundity of yellow-bellied toads *Bombina variegata* under free-range conditions: an indication of risk-spreading strategy," *Amphibia-Reptilia*, **23**, 362 – 369.
- Gill D. E. (1978), "The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque)," *Ecol. Monogr.*, **48**, 145 – 166.
- Gollmann B. and Gollmann G. (2002), *Die Gelbbauchunke: von der Suhle zur Radspur*, Laurenti Verlag, Bielefeld.
- Gollmann B., Gollmann G., and Miesler M. (2000), "Habitatnutzung und Wanderungen in einer Gelbbauchunke-Population (*Bombina v. variegata*)," *Z. Feldherpetol.*, **7**, 1 – 16.
- Gollmann G. and Gollmann B. (2000), "Wiederfangstudie an einer Gelbbauchunkepopulation: Überlebensraten und Ausbreitung," *Beitr. Ökol.*, **4**, 75 – 82.
- Gollmann G., Gollmann B., and Baumgartner C. (1999), "Oviposition of yellow-bellied toads, *Bombina variegata*, in contrasting water bodies," in: Miaud C. and Guyétant R. (eds.), *Current Studies in Herpetology*, Soc. Eur. Herpetol., Le Bourget du Lac, pp. 139 – 145.
- Kenward R. E., Walls S. S., and Hodder K. H. (2001), "Life path analysis: scaling indicates priming effects of social and habitat factors on dispersal distances," *J. Anim. Ecol.*, **70**, 1 – 13.
- Kneitz S. (1998), *Untersuchungen zur Populationsdynamik und zum Ausbreitungsverhalten von Amphibien in der Agrarlandschaft*, Laurenti Verlag, Bochum.

RETURN RATES AND LONG-TERM CAPTURE HISTORY OF AMPHIBIANS IN AN AGRICULTURAL LANDSCAPE NEAR BONN (GERMANY)

M. Hachtel,¹ D. Ortman,¹ A. Kupfer,¹ U. Sander,¹ P. Schmidt,¹ and K. Weddeling¹

Keywords: amphibians, long-term study, return rates, minimum survival rates, minimum age, capture history, *Rana dalmatina*, *R. temporaria*, *Triturus cristatus*, Germany

INTRODUCTION

In the context of the long-term study “development of amphibian habitats in an agricultural landscape” amphibian populations of seven native species in an arable landscape near Bonn (Germany) were monitored during eleven years (1989 – 1995, 2000 – 2003, cf. Schäfer, 1993; Kneitz, 1997). Data presented here focus on return rates and minimum ages of long-lived individuals in the field of two frog and one newt species. These parameters influence maintenance and stability of amphibian populations and thus are important concerning ecology and conservation of species.

STUDY AREA AND METHODS

The study focuses on a pond system with two natural and three artificial waterbodies. Using permanent drift fences with pitfall and funnel traps it was possible to catch

and record most of the specimens that reached and quit the ponds throughout the whole year. One aim was to estimate minimum survival rates of the species by capture-mark-recapture techniques. For the anuran species — agile frog *Rana dalmatina* and common frog *R. temporaria* — two individual marking methods were applied: implantation of passive integrated transponders (PIT) and toe-clipping. The great crested newts *Triturus cristatus* were registered by photo identification of the belly pattern. Analyzing data of individually marked specimens we were able to estimate recapture rates for adults from spawning period 2001 to 2003.

RESULTS

Return Rates

***Rana dalmatina* — agile frog.** In 2001 the population of the agile frog consisted of 939 adult specimens (340 females, 599 males). Return rates from one year to the next were about 50% in both periods (Fig. 1). Return rates of males ranged from 55 to 58% and were significantly higher than these of females with 38 to 42% ($\chi^2 = 15.8$,

¹ Zoologisches Forschungsinstitut und Museum Alexander Koenig, Sektion Herpetologie, Adenauerallee 160, D-53113 Bonn, Germany; E-mail: m.hachtel.zfmk@uni-bonn.de; www.amphibien-projekt.de

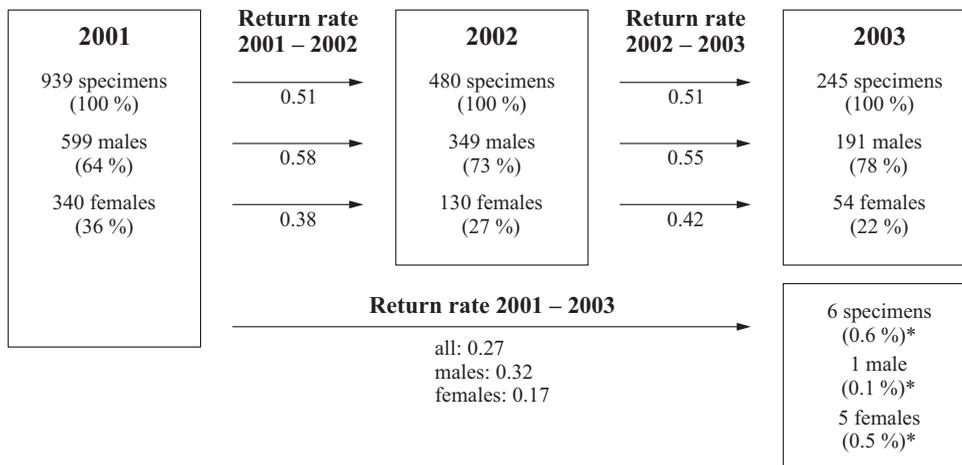


Fig. 1. Return rates of *Rana dalmatina* males and females 2001 – 2003. * Percentage of recaptured specimens from 2001.

$p < 0.001$). Consequently sex ratio within our sample increased from 64% males in 2001 up to 78% in 2003. Nearly all specimens returned to their breeding ponds every year; only 0.6% left out the spawning period 2002. At least 27% of all frogs (males: 32%, females: 17%) took part in reproduction over all three years.

***Rana temporaria* — common frog.** In 2001 a total of 423 individuals (202 females, 221 males) of *R. temporaria* were captured. Return rates ranged from 17 to 30% (Fig. 2) and were thus much lower than those of the agile frog ($\chi^2 = 45.7, p < 0.001$). Only a minority of the population (8%) reproduced all three years. Just six specimens (1.4%) overleaped the breeding season in 2002, i.e., came to their spawning site only in 2001 and 2003. Sex ratio was more balanced than in *R. dalmatina* populations (Fig. 2). No significant differences between return rates of males and females were found (χ^2 test).

***Triturus cristatus* — great crested newt.** In 2001 altogether 170 adult specimens of the great crested newt (101 females, 69 males) were registered individually. 21% returned to their breeding pond in the following year and 11% of them were observed again in the last year (Fig. 3). Differences between return rates in 2002 and 2003 were not significant. Only 3% (6% males, only 1% females) took part in the reproduction over all three years. Because of the small sample size interpretations concerning different return rates of the sexes are not possible.

Long-Term Capture Histories

***Rana dalmatina* — agile frog.** Twelve *Rana dalmatina* adults (nine males, three females) individually marked as metamorphs between 1993 and 1995 were captured again from 2001 to 2003 (Table 1). Minimum ages range from six to ten years. One male that metamorphosed in 1993 at least came back in all three seasons from 2001

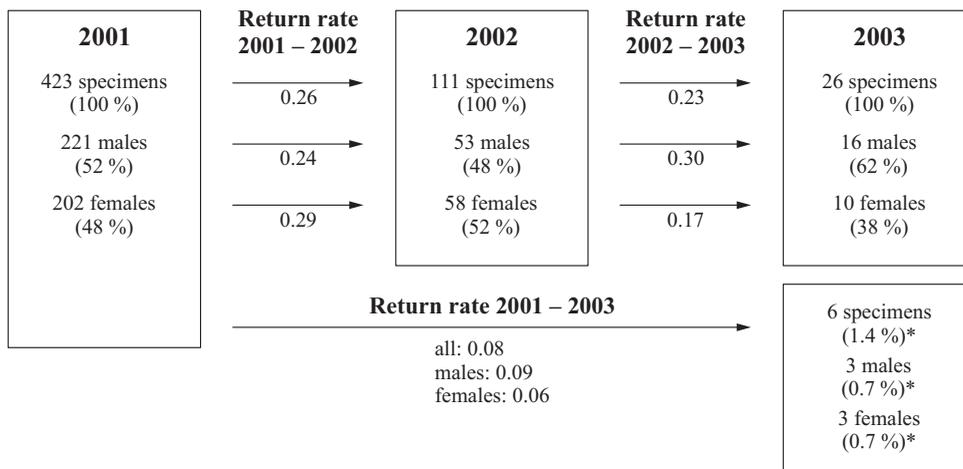


Fig. 2. Return rates of *Rana temporaria* males and females 2001 -2003. *, Percentage of recaptured specimens from 2001.

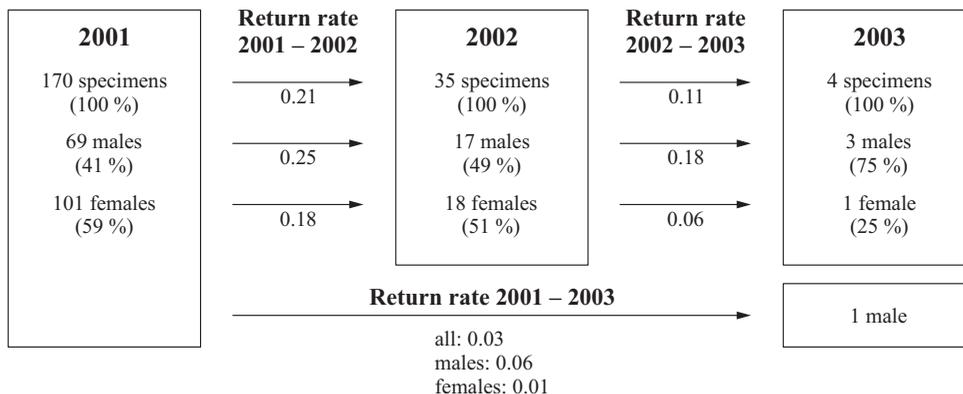


Fig. 3. Return rates of *Triturus cristatus* males and females 2001 – 2003.

to 2003 and thus reached an age of ten years. Most of these old specimens (10 animals) were observed in the same pond in which they hatched many years ago. Only two individuals were found in other water bodies within distances of a few hundred meters from their pond of hatch.

***Rana temporaria* — common frog.** Eight specimens of the common frog (two males, six females) marked as metamorphs between 1991 and 1995 could be observed again between 2001 and 2003 (Table 2). Consequently ages range 7 – 11 and 6 – 10 years for females and males, respectively. Four individuals changed between hatching site and spawning pond.

***Triturus cristatus* — great crested newt.** Two females and one male of *Triturus cristatus* first captured between 1995 and 1997 as adults could be recognized again in 2001 – 2003 (Table 3). During this period the population grew from 40 to 170 individuals. Those two females belonged to the first great crested newts, which colonized

six respectively eight years after the completion the artificial ponds as adults. Minimum ages — estimated by individual recognition of adults — range between eight and ten years.

DISCUSSION

Return Rates

Because in all three species only a small minority of individuals left out the spawning period 2002 (0.6% for *Rana dalmatina* and 1.4% for *R. temporaria*, and only one individual of *Triturus cristatus*) one can assume that adult animals usually return to their spawning site every year. Additionally, transience between spawning waters — even over a long time period — seems to be rare and the adult amphibians reveal a high spawning fidelity (cf. Kneitz, 1997). Taking into account that capture probability with the applied methods does not reach 100% the true survival rates are definitely higher than our observed return rates. In consequence one can equate return rates at least with minimum survival rates.

Common frog and great crested newt show a similar return rate of about 20% from one year to another. Compared to literature these data are extremely low for both species: especially for *Triturus cristatus*, which is considered as a k-strategist, published annual return rates range between 31 and 100% and thus are at least 10% higher than in our results (summary in Arntzen and Teunis, 1993; Baker, 1999). Because our population in the same period was expanding constantly, this indicates a relatively high recruitment rate but disadvantageous adult survival conditions. That idea is supported by the remarkable low body sizes of the adults compared to literature, which suggests an age structure of the population with a high proportion of young animals (Ortmann, unpublished).

Similarly, return rates of *Rana temporaria* are at the lower end of published data, for example Ryser (1986) and Gibbons and McCarthy (1984) with return rates ranging from 36 to 50%. Otherwise Elmberg (1990) shows similar average annual return rates of 31% for males (min. 16%, max. 51%) and 16% for females (min. 5%, max. 33%). It

TABLE 1. Recaptured *Rana dalmatina* from the Period 1993 – 1997

Number of specimens	Sex	Year of marking*	Year(s) of recapture	Age at last capture, years	Recapture place
1	♂	1993	2001 – 2003	10	same pond
1	♂	1993	2001 – 2002	9	same pond
1	♂	1994	2003	9	same pond
3	♀	1994	2001	8	same pond
1	♂	1994	2002	8	same pond
2	♂	1994	2001	7	same pond
1	♂	1995	2002	7	other pond (920 m distance)
1	♂	1995	2001	6	other pond (800 m distance)
1	♂	1995	2001	6	same pond

* Marked as metamorph.

TABLE 2. Recaptured *Rana temporaria* from the Period 1993 – 1997

Number of specimens	Sex	Year of marking*	Year(s) of recapture	Age at last capture, years	Recapture place
2	♀	1991	2002	11	same pond
1	♀	1991	2001	10	other pond (825 m distance)
1	♂	1991	2001	10	other pond (925 m distance)
1	♀	1995	2001 – 2003	8	other pond (860 m distance)
1	♀	1995	2001, 2002	7	same pond
1	♀	1995	2002	7	same pond
1	♂	1995	2001	6	other pond (1275 m distance)

* Marked as metamorph.

TABLE 3. Recaptured *Triturus cristatus* from the Period 1993 – 1997

Number of specimens	Sex	First observation as adult	Year(s) of recapture	Age at last capture, years	Recapture place
1	♀	1995	2001 – 2003	10	same pond
1	♀	1997	2001	8	same pond
1	♂	1995	2001, 2002	8	same pond

is likely that those data differ from population to population depending on climate, structures of spawning and terrestrial habitats.

Exceptionally the agile frog shows a high return probability of about 50%, which was in the years 2000 – 2003 nearly similar to those of the former project phase (Kneitz, 1997). Depending on year, pond and sex Kneitz (1997) acquired rates between 21% and 53% in the same ponds 8 – 10 years earlier. These data indicate good survival conditions for adult agile frogs in our study area.

Only in *Rana dalmatina* we found statistically significant differences between sexes concerning return probability. Because Kneitz (1997) as well had continuous lower return rates of females in all ponds and both study years (1993, 1994) this result seems to be valid for a long time at least in our pond system. In contrast no differences between sexes occur for *R. temporaria* and *T. cristatus*. Baker (1999) indicates no sex-specific differences for *Triturus cristatus*, as well as Gibbons and McCarthy (1984) and Elmberg (1990) for *Rana temporaria*. Thus, similar return rates of both sexes seem to be a frequent phenomenon for these species.

Long-Term Capture Histories

Individual recognition by transponders, toe-clipping, and photo identification, applied over a long period, enables us to determine minimum ages of long-lived individuals in the field. Observed ages belong to the highest recorded in field studies in Europe. Those old individuals show that at least a few specimens can reach very high ages in the field and they are still able to take part in reproduction regularly. They also suggest a high pond fidelity for many years. In most cases hatching pond and spawning water are identical.

Compared with return rates over three years (Figs. 1 – 3) mortality of old animals seems to be much lower than that of younger. This suggests at least two different strategies: The bigger part of a population spawns only a few times, but a minority group of few individuals takes part in reproduction over many years. Baker (1999) as well demonstrates for *Triturus cristatus*, that young individuals experience significant lower survival than older newts. Elmberg (1990) shows for *Rana temporaria* that return rates

increase with every successful previous hibernation, which indicates an increasing survival rate with age. Especially these old specimens might be important for maintenance of populations under temporary bad habitat conditions for reproduction and periods with low recruitment rates (e.g., pond drying, cf. Baker, 1999).

Acknowledgments. This project was financially supported as a “testing and development project” by the Federal Agency for Nature Conservation (BfN) and the Federal Environment Ministry (BMU). Many thanks to our colleagues Gregor Bosbach, Regine Damaschek, Anja Dissanayake, Ruth Rottscheidt, Anja Sampels, Dr. David Tarkhishvili, and Meike Thomas for their work in the field and discussions. Prof. Dr. Wolfgang Böhme was kindly leading the project. Data from 1991 till 1995 are courtesy of Dr. Stephan Kneitz.

REFERENCES

- Arntzen J. W. and Teunis S. F. M. (1993), “A six years study on the population dynamics of the crested newt (*Triturus cristatus*) following the Colonization of a newly created pond,” *Herpetol. J.*, **3**, 99 – 110.
- Baker J. M. R. (1999), “Abundance and Survival Rates of great crested newts (*Triturus cristatus*) at a pond in Central England: monitoring individuals,” *Herpetol. J.*, **9**, 1 – 8.
- Gibbons M. M. and McCarthy T. K. (1984), “Growth, maturation and survival of frogs *Rana temporaria*,” *Hol. Ecol.*, **7**, 419 – 427.
- Elmberg J. (1990), “Long-term survival, length of breeding season, and operational sex ratio on a boreal population of common frogs, *Rana temporaria* L.,” *Can. J. Zool.*, **68**, 121 – 127.
- Kneitz S. (1997), “Langzeituntersuchungen zur Populationsdynamik und zum Wanderverhalten des Springfrosches im Drachenfelder Ländchen bei Bonn,” *Rana Sonderheft*, **2**, 231 – 241.
- Kuhn J. and Schmidt-Sibeth J. (1998), “Zur Biologie und Populationsökologie des Springfroschs (*Rana dalmatina*): Langzeitbeobachtungen aus Bayern,” *Z. Feldherpetol.*, **5**, 115 – 137.
- Ryser J. (1986), “Altersstruktur, Geschlechterverhältnis und Dynamik einer Grasfrosch-Population (*Rana temporaria* L.) aus der Schweiz,” *Zool. Anz.*, **217**, 234 – 251.
- Schäfer H.-J. (1993), *Ausbreitung und Entwicklung von Amphibien-Populationen in der Agrarlandschaft. Ph. D. Thesis*, Univ. Bonn, Bonn.

ELEVEN YEARS OF MONITORING: AMPHIBIAN POPULATIONS IN AN AGRICULTURAL LANDSCAPE NEAR BONN (GERMANY)

M. Hachtel,¹ P. Schmidt,¹ U. Sander,¹ D. Tarkhnishvili,¹ K. Weddeling,¹ and W. Böhme¹

Keywords: Amphibians, monitoring, long-term study, population dynamics, *Bufo bufo*, *Rana dalmatina*, *Triturus alpestris*.

INTRODUCTION

Within the project “development of amphibian habitats in an agricultural landscape“ population dynamics of seven amphibian species in an agricultural landscape near Bonn (Northrhine-Westphalia, Germany) have been surveyed during eleven years (1989 – 1995, 2000 – 2003). Here, we present preliminary results on the population dynamics of three species at two ponds from 1989 till 2003, including and continuing the results of Schäfer (1993) and Kneitz (1998).

Central aims of the project are:

- Monitoring of the amphibian fauna in a typical middle-European landscape;
- Knowledge on population ecology and population genetics of native amphibian species (population sizes and dynamics including sex ratios and reproductive output, return rates, long-term captures and migration based on genetic data);
- Scientific contributions for species and nature conservation in Germany.

Major questions are:

- What do the long-term population dynamics look like?
- Which conditions have to be met for persisting and self-maintaining populations?
- Are there clear differences between natural and artificial ponds?

STUDY SITE AND METHODS

The study area is located 20 km south of Bonn (Germany). It represents a typical middle-European agricultural area with acres, meadows, small forests and villages. In this landscape five breeding ponds were examined, which were situated in distances between 300 and 1800 m. Two of them have a natural origin, three waterbodies (with

waterproofing foil) have been build artificially to support the amphibian populations in this cultural influenced area. These five breeding ponds were enclosed by permanent drift fences with pitfall traps in order to record abundance and migration of all occurring amphibians throughout the whole year.

To mark the specimen, we used two different methods: Either toe-clipping or implantation of passive integrated transponders (PIT) for the anurans and only toe-clipping for newts.

RESULTS

Triturus alpestris colonized the new ponds within a few years and was present in all five study ponds up to the last study year 2003. In the beginning, the number of adult individuals in the whole pond system ranged 455 specimens (1989), increased till 1995 up to 3706 individuals and stagnated in the last three years between 2755 and 4625 animals. In the artificial ponds the population developed from 14 specimens more or less continuously to 3326 specimens till the year 2001, but stagnated as well in 2002 (1866 individuals) and 2003 (1656 individuals). Figure 1 for example shows the dynamics in pond 1. In the same

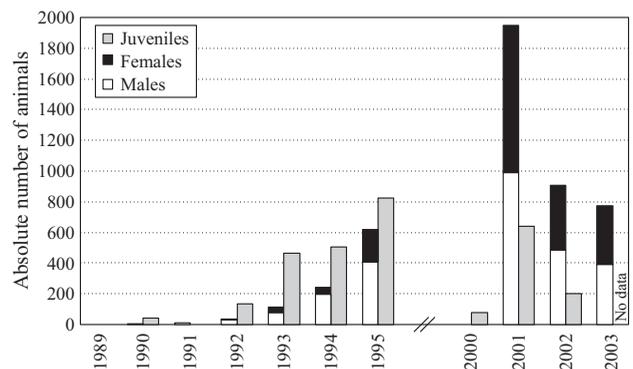


Fig. 1. Development of breeding population of *Triturus alpestris* at pond 1.

¹ Zoologisches Forschungsinstitut und Museum Alexander Koenig, Sektion Herpetologie, Adenauerallee 160, D-53113 Bonn, Germany; E-mail: w.boehme.zfmk@uni-bonn.de; www.amphibien-projekt.de.

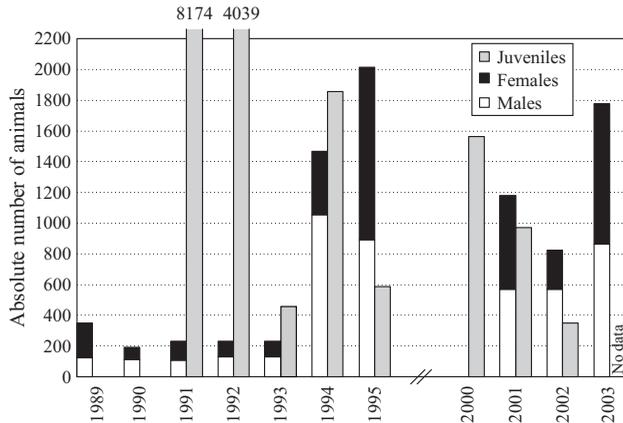


Fig. 2. Development of breeding population of *Triturus alpestris* at pond 3.

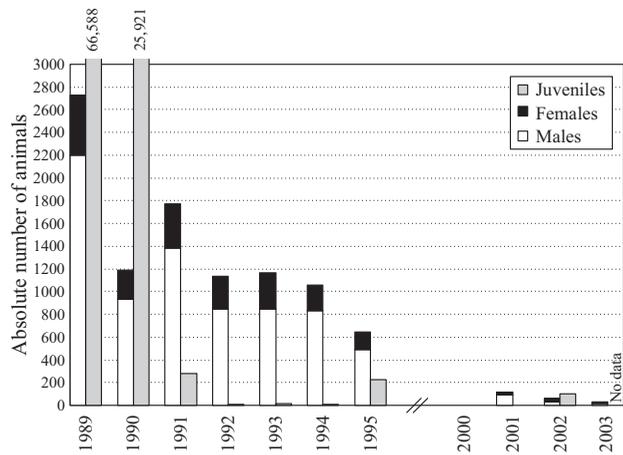


Fig. 3. Development of breeding population of *Bufo bufo* at pond 3.

period annual reproductive success ranged from 0 up to 8174 (year 1991 in pond 3, see Fig. 2).

Bufo bufo in contrast settled only temporarily in all waterbodies; in our study area it showed the most irregular dynamics of all species under concern. The breeding population at pond 3 consisted of 2200 males and 530 females in 1989 (Fig. 3). Reproduction in that year was very successful with 66,000 juveniles. Due to changes in habitat conditions (pond drying, strong decrease of fish population, increasing populations of newts and other potential predators) there were heavy losses in population size of the common toad. Currently, also the artificial pond 4 was regularly used as spawning site with remarkable reproduction, however its success was irregular (Fig. 4). Meanwhile pond 3, representing the former main breeding site, had lost importance. Adult numbers decreased extremely to 15 males and 19 females in 2003. Even in the whole

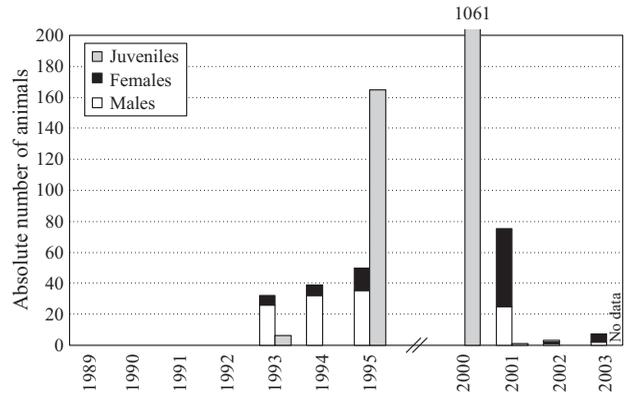


Fig. 4. Development of breeding population of *Bufo bufo* at pond 4.

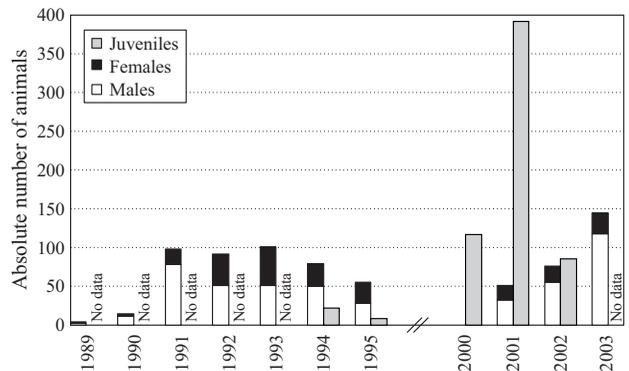


Fig. 5. Development of breeding population of *Rana dalmatina* at pond 2.

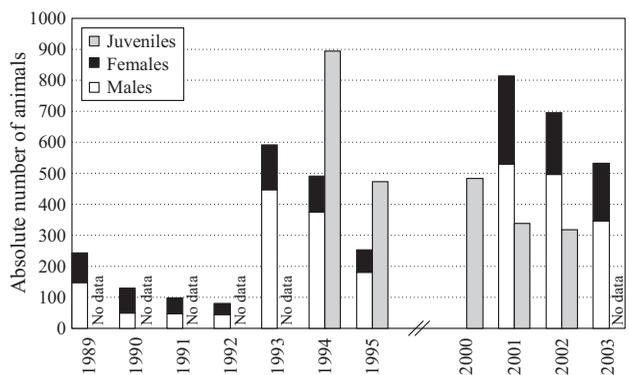


Fig. 6. Development of breeding population of *Rana dalmatina* at pond 3.

study area population size of adults during breeding period in 2003 did not exceed 37 males and 32 females.

Rana dalmatina occurred in all ponds but showed remarkable fluctuations. After colonization of the artificial ponds the spawning populations increased within four

years from 4 up to 138 adults in these waterbodies (for example pond 2 in Fig. 5). Nevertheless, since the year 2000 only two ponds (pond 2 at the edge of the wood and pond 3 situated in arable land; see Fig. 6) showed higher numbers of adults (i.e., far more than ten specimens per sex) and regular reproductive output. In three ponds only single individuals (25 or less per year) could be observed in the last three years, but in some years high reproductive output took place anyway.

In all species no positive correlation between number of adults and juveniles could be observed.

CONCLUSIONS

Especially for newts the population size in the pond system grew according to the creation of additional ponds. In contrast, for *Bufo bufo* the water system seemed to be only temporary convenient whereas concurrence between species (especially newts) and/or predation may be important negative factors. Even though *Rana dalmatina* adopted only some of the new ponds, its population was strengthened and enlarged by their creation.

General conclusions for amphibian populations are:

- Species composition, population size and reproductive output can change within a few years.
- Reproductive success and size of the spawning population are not correlated; a phenomenon, which is characteristic for animal species with large numbers of eggs.

- Population dynamics differ between species. Given the total time of 14 years in our study, the patterns rather look like an irregular developmental process than periodical dynamics which can not yet be recognized.

- There are no obvious differences between natural and artificial ponds with respect to species composition and reproduction rates.

- Artificial ponds in an agricultural landscape can be inhabited by amphibians for long time spans. They are useful to strengthen and interconnect amphibian populations even throughout intensive arable landscapes.

Acknowledgments. This project is financially supported as a “testing and development project” by the Federal Agency for Nature Conservation (BfN) and the Federal Environment Ministry (BMU). Many thanks we would like to say to all people, who helped recording masses of data in the field for all the years. Especially we thank our colleagues Gregor Bosbach, Regine Damaschek, Anja Dissanayake, Ruth Rottscheidt, Anja Sampels, Dr. David Tarkhnishvili, and Meike Thomas. Also we thank Dr. Stephan Kneitz for giving us data from 1991 till 1995.

REFERENCES

- Kneitz S.** (1998), *Untersuchungen zur Populationsdynamik und zum Ausbreitungsverhalten von Amphibien in der Agrarlandschaft*, Laurenti, Bochum.
- Schäfer H.-J.** (1993), *Ausbreitung und Entwicklung von Amphibien-Populationen in der Agrarlandschaft. Ph. D. Thesis*, Univ. of Bonn, Bonn.

GROWTH OF BROWN FROGS OF FAUNA OF RUSSIA: SOME PROBLEMS OF STUDY OF GROWTH IN AMPHIBIANS

V. G. Ishchenko¹

The growth of some brown frogs (*Rana temporaria*, *R. arvalis*, *R. macrocnemis*, *R. dalmatina*, *R. asiatica*, *R. amurensis*, *R. dybowskii*, *R. pirica*) was studied using skeletochronological method. Comparison of correlations between body size and absolute age evaluated as numbers of wintering has revealed different situations. The curves of growth in the populations of different geographical zones can cross “chaotically,” without any obvious geographical regularity: either they coincide, or the growth curves in a population with the greater life time can be a prolongation of a growth curve of population with a shorter lifetime. In some cases growth curves can be parallel or congruent. The interpretation of the results in many cases is difficult as the duration of the period of activity in different populations may differ sharply or, on contrary, may vary and may be overlapped significantly. Therefore, the comparison of growth curves, based on age in months is preferable and it allows obtaining unusual results. The usage of biocoenotic data appears to be more interesting. The comparative analysis of species-specific differences in growth, taking into account geographical and especially various intrapopulation variation is necessary for the assessment of the role of the different factors determining growth in amphibians.

Keywords: amphibians, frogs, growth, skeletochronology.

INTRODUCTION

It is well-known that a study of the growth is of essential interest because many other important traits of life cycles are connected with growth (cost of reproduction, rate of maturity, life-span and, finally, reproductive success of an individual and a population as a whole). The characters of growth are, therefore, among the main traits of a population (Ebenman and Persson, 1988). At present, it is possible to postulate some principal propositions related to population ecology of amphibians on the basis of the study of their growth. Firstly, a higher growth rate is most preferable because it can determine maturation at larger body size and, hence, a comparatively higher fecundity. Secondly, the larger body size provides higher competitiveness, because larger individuals have the wider spectrum of prey.

Till recently, studies of growth and aging of amphibians were carried out in laboratory conditions (Hota, 1994; Kara, 1994), and data on longevity were accumulated by skeletochronological studies. Now there are comparatively few data on the growth character of amphibians in nature, first of all owing to a difficulty of the task itself. From an available arsenal of tools, two are regarded to be the most correct, the individual marking and skeletochronological research (Halliday and Verrell, 1988). These are precisely the methods, which ensure a reliable age deter-

mination at the level of an individual and, hence, a possibility of study of growth. Unfortunately an individual marking can result in “additional” mortality rate and, even without that, a low recapture value, owing to migrations and large mortality, from metamorphosis to maturity and alder age. Therefore, skeletochronological studies are more preferable and more widely spread for descriptions of amphibian growth. However, in many cases researchers have dealt with one-time series, and problems arise in interpretation of results. This paper discusses some aspects of the problems.

MATERIAL AND METHODS

This work is based on studying of series the frogs collected in different time. Samples of eight species of brown frogs of Russia and adjacent territories were studied: *Rana arvalis* Nilsson 1842; *R. temporaria* Linnaeus 1758; *R. dalmatina* Bonaparte, 1840; *R. macrocnemis* Boulenger, 1885; *R. asiatica* Bedriaga, 1898; *R. amurensis* Boulenger, 1886; *R. dybowskii* Günther 1876; and *R. pirica* Matsui, 1991. All specimens were fixed in formalin and kept in formalin or ethanol. The specimens were sexed and snout-vent length was measured nearest to 0.1 mm. Age of animals was determined by examination of microscopic cross-sections of the second phalanx of the fourth toe of right hind leg. Data on age structure of all series were published earlier (Ishchenko, 1996). On the basis of data obtained the relations between mean size and age have

¹ Institute of Plant and Animal Ecology, Ural Branch of Russian Academy of Sciences, 8 March Street 202, 620144 Yekaterinburg, Russia;
E-mail: vgi@ipae.uran.ru.

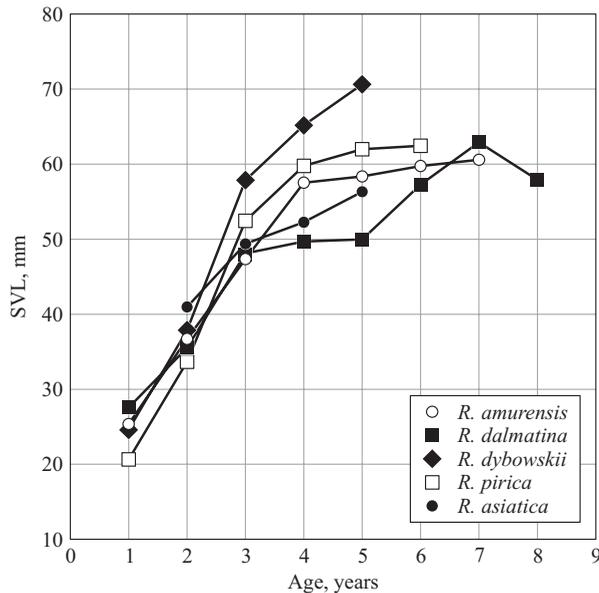


Fig. 1. Relationship SVL – age in some species of brown frogs (*Rana*).

been compared. Total numbers of specimens under study counted were 5573 from 41 populations of 8 species. I used statistical software STATISTICA 6.0 and SPSS 11.5.

RESULTS AND DISCUSSION

The results can be considered at some levels, namely, species-specific, population, and intrapopulation. Plotting of the growth curves describing species-specificity in different species of amphibians that can characterize specificity of species is extremely difficult if possible at all. For example, my data can be used for characterization (description) of relations between size and age in some species of frogs (Fig. 1). This result has been obtained on the basis of four samples of *R. amurensis*, two samples of *R. asiatica* and one sample for each of three other species. Therefore it is very difficult to speak in this case of species-specificity. It is possible only to contend that size differences between species at the age of three years contributes of about 10 mm and at the age of five years, 20 mm. It is necessary to remember that in many cases researchers measure SVL nearest to 1 mm, especially in case with living specimens. Therefore, even significant differences in the mean size of 1.5 – 2 mm in many cases can not be regarded as valid.

Intraspecific comparisons of growth curves are much more appropriate and informative.

At the present time, the comparisons of such kind are usual in amphibians and my data permit to suggest various

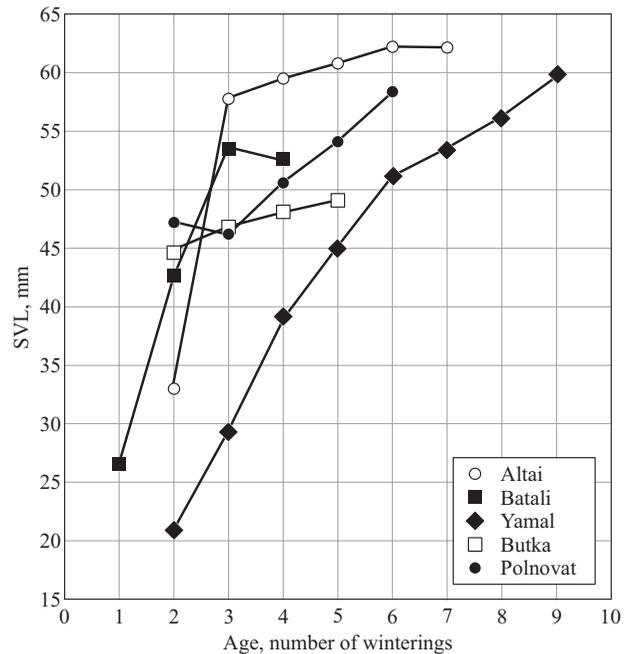


Fig. 2. Relationship SVL – age in some populations of *Rana arvalis*.

kinds of geographical variability of growth. The comparison of five populations of *R. arvalis* (Fig. 2) based on the series from Altai [560 m above sea level (a.s.l.)], forest-tundra zone (Yamal peninsula), middle taiga of Western Siberia (Polnovat), and forest-steppe of Middle and South Urals (Butka and Batali) does not display a regular (cline) geographical variability in age-size dependencies. In other cases regular geographical differences in growth rates were determined (Hemelaar, 1986). According to our data on age and size in different populations of *R. macrocnemis*, the differences in size-age dependencies can be observed by comparison of mountain and lowland populations (Fig. 3). Nevertheless, it is necessary to keep in mind that conclusions about such differences have some limitations.

It is known, the duration of the period of frog activity is very important for attaining of certain size (Licht, 1975) but in some cases this duration is unknown. At least, it is unknown usually whether the duration of period of activity of frogs coincides with a duration of period of growth or not. Therefore, interpretation of observed differences is often difficult, but in some cases it is quite possible. Comparison of populations of *R. temporaria* can be an example (Fig. 4). It is known that populations of amphibians inhabiting the subarctic zone, in particular, at the Polar Urals, are active usually during two months and rarely, during 2.5 months (Schwarz and Ishchenko, 1971), and all phenological events go on with large intensity. In southern popula-

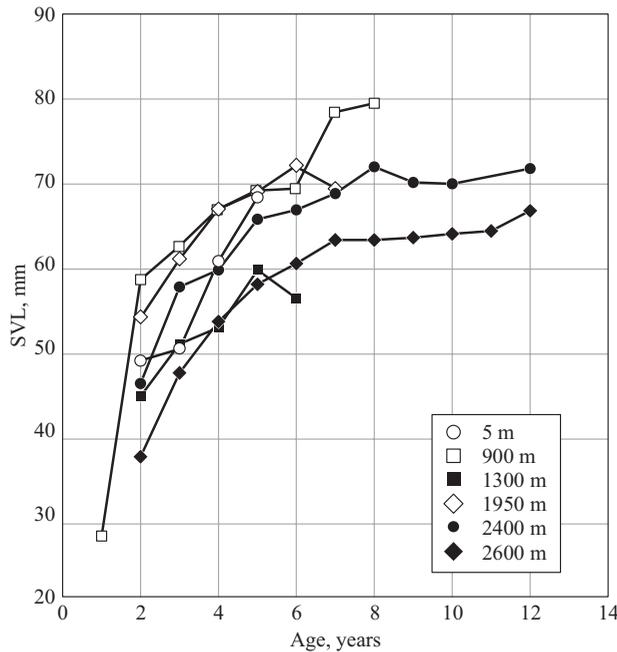


Fig. 3. Relationship SVL – age in some populations (elevations) of *Rana macrocnemis*.

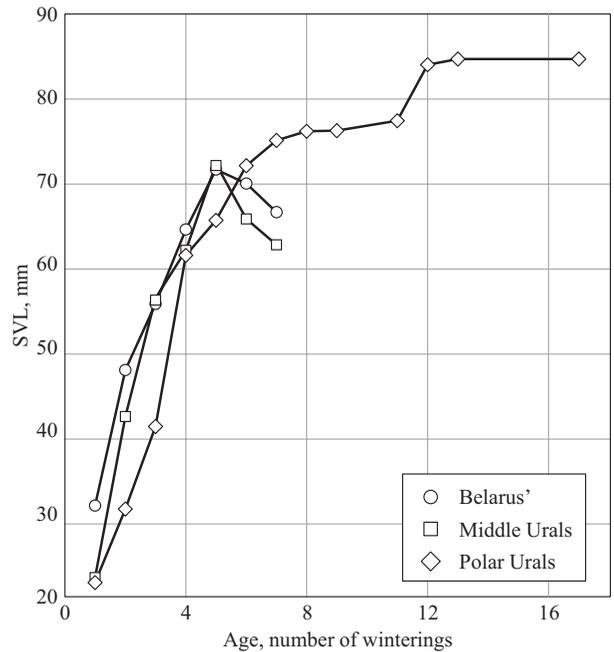


Fig. 4. Relationship SVL – age (number of winterings) in *Rana temporaria*.

tions of the temperate zone duration of the period of activity and potential growth is not less than 4 months and usually it is equal to 5 months. It is quite possible to estimate age of animals in number of months of active life but not in number of winterings. Results obtained in such way for *R. temporaria* (Fig. 5) permit to conclude that northern common frogs are not characterized by comparatively larger life-span, but they are characterized by a more intensive growth. The reasons of this phenomenon seem to be unknown but some explanations can be given. Firstly, long photoperiod (in summer) in the Subarctic can result in increasing of growth rate of juvenile specimens (Richards and Lehman, 1980) and this specificity may be retained at older age. Secondly, according to the data of Olschwang (1992), total biomasses of aboveground invertebrates produced in summer in Polar Urals and Middle Urals are almost equal, therefore middle daily abundance and availability of potential food in northern amphibians is at least twice as large as that in frogs from southern populations. Growth of amphibians is often determined by conditions of feeding (Claussen and Layne, 1983; Seale, 1987).

A similar situation was observed at comparison of populations of *R. macrocnemis* living at different altitudes in various localities of the Caucasus. It is easy to see (Fig. 3) significant distinctions in growth of frogs from various populations and they are retained at comparison

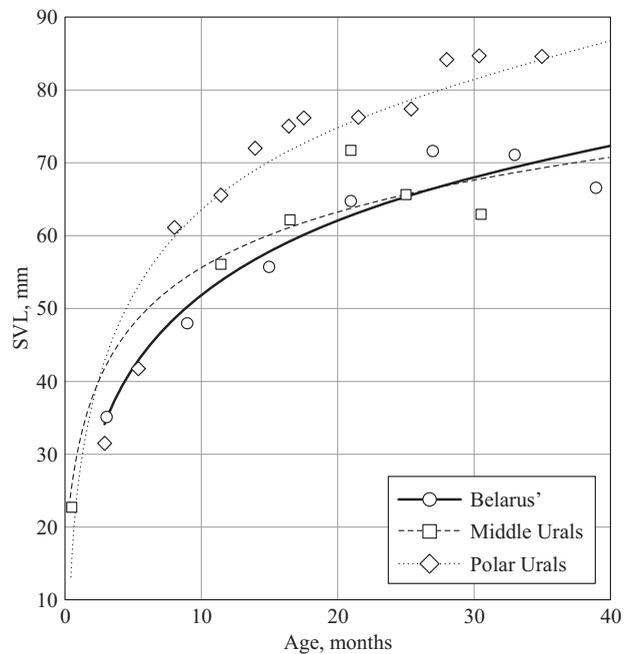


Fig. 5. Relationship SVL – age in *Rana temporaria*.

when their age is expressed in months. High-mountain frogs grow more slowly than those from moderate altitudes. However, it is interesting to note, that the frogs in

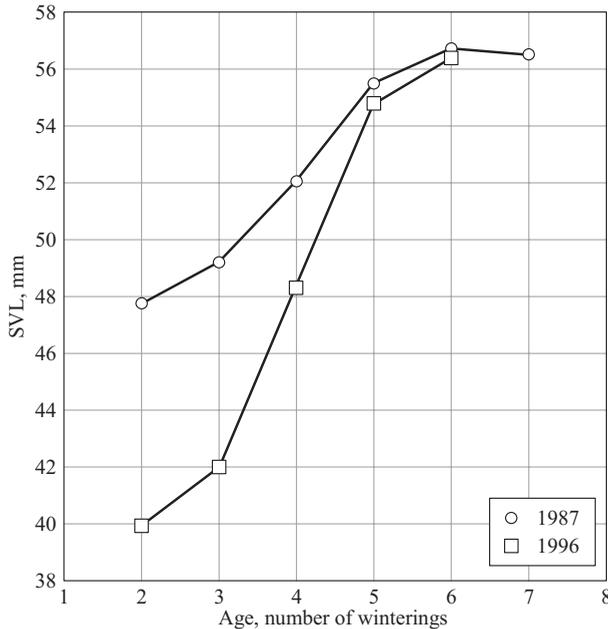


Fig. 6. Relationship SVL – age in *Rana arvalis* in various years.

Kolkhida lowland (5 m a.s.l.) are characterized by the slower growth and minimum body size. In spite of the fact that a period of activity and potential growth in this population is maximum (6.5 – 7 months), growth rate is evidently insignificant. The reasons of this can be presumably biocoenotic. In this site the total biomass of aboveground invertebrates produced through a season of vegetation is similar to it in the Polar Urals, however, in each time unit it is very small. In the habitats of frogs (mainly boggy small-leaved woods) an abundance of invertebrates is always lower than anywhere else. Moreover, a plenty of days with intensive precipitation, typical of humid subtropics zone, make a feeding of frogs complicated, i.e., the factor of actual availability of food resource takes place. It is difficult to prove these assumptions, but it is possible to speculate about them. Moreover, relatively small size of northern *R. arvalis* (Fig. 2) does not confirm to this hypothesis. On the other hand one must not exclude an influence of the size at metamorphosis that can determine size at maturity and at older age (Camp et al., 2000). Additionally, there is one other factor, which is almost impossible to measure comparing populations of frogs. This factor is a certain “phylogenetic variable” determining evolutionary specificity of populations. Any compared populations can differ, but the exact reasons of these differences cannot be comprehended.

There are some more circumstances, which do not permit us to study amphibians’ growth more accurately.

Firstly, it is necessary to remember about replications of results at sampling of animals for a study. They can be obtained at long-term studies, which results cannot be always satisfactory. For example, at comparison of dependence of the body size on age in moor frogs collected in the same population in different years (Fig. 6) we notice a great difference.

I have got some explanations. Summer of 1995 was very dry season, and the most part of frogs lived in a shore zone of non-dried shallow ponds in conditions of high density (5 – 7 specimens per 1 m²), thus failing to feed successfully. Therefore the growth of young individuals was strongly inhibited. In addition, the differences of such kind in dependencies between size and age of animals collected in various parts of large spatially structured population can be observed, too. Further, zoologists usually diligently overlook that relations between body size and the age are not growth *sensu stricto*, because a growth is a vectorial change of the size or mass of an individual or a group of individuals of the same time of birth in time continuum. Usually, when we compare the average body size in different age groups of the same population, we are dealing not only with individuals of different age, but also with individuals of different generations born in different years and this can be a good reason for differences in size (Ishchenko, 1989). Differences between generations can be determined by conditions of larval growth, and genetically, and by the fact that in different years a population can be invaded by immigrants from the other neighbor populations, which can differ in growth (Augert and Joly, 1993). Many of the factors listed above practically were not defined in descriptions of the growth of amphibians in nature unless their marking was done. Thus, we usually do not know an impact of information noise on the results of our studies. There are ways of studying the growth allowing to avoid some restrictions. Besides of being used for individual marking, skeletochronological studies can be highly promising. Similar problems are resolved in dendrochronology at studying of tree growth. Dendrochronologists do not measure the height of trees for studying growth, but use diameter and width of annual rings. Amphibians appear to be a beneficial object in this aspect because of an insignificant increase of length can be imperceptible, because of natural errors of measurement; however studying cross-sections of a bone (and phalanx) permits registering any growth precisely. Moreover, it is well known the correlations between body size and diameter of a phalanx are quite often high and the calculation of body length is available. Thus one can work with this material and can easily find out an individual variability of growth. However, this method has some minor restrictions. As a

matter of fact a line of the first wintering is often resorbed, and the animals keeping this line can be characterized by specific growth type, in comparison with individuals with resorption. Nevertheless, studies on variability of curves of growth obtained on the basis of analysis of microscope sections of bones are very promising. It is difficult, however, to imagine a mathematical tool allowing for, e.g., a cluster analysis of many hundreds or thousands of curves. In my opinion, there is one more comprehensible way for studying of variability of growth. It consists of determining the variants components of body size.

Analysis of variance of body length has shown that the most part of variability (63.54%) of 41 populations of 8 species of frogs is determined by age, while species or population specificity plays a smaller role (13.19 and 3.65%, respectively). The part of “sex” factor is about 4.4% of variance of SVL. Thus, distinctions in character of growth can be rather insignificant, and observed differences in it between populations seem to be determined mainly by distinctions in life-span (in years!). This conclusion does not contradict the plots because in the analysis of variance all the individuals have been taken into account. The author use not only average sizes in the plots, but also the intragroup variance of body size. However such an approach can only be used in search analysis.

Now, when skeletochronological data are available for more than 110 species of amphibians (in my database), it is quite possible to do a comparison of various kind between the growth curves (more correctly — curves of dependencies of the size on age). Despite of the abundant evidence available, the studies on growth of amphibians in nature, in my opinion, are only beginning.

Acknowledgment. I am grateful to Dr. Helen Shchupak and Dr. Arkady Ledentsov for help me at field work and Dr. Svetlana Grebennikova for preparing of many microscopic cross-sections of many series of brown frogs. This work was supported by the Russian Fund for Fundamental Research (grant No. 02-04-49451).

REFERENCES

- Augert D. and Joly P.** (1993), “Plasticity of age at maturity between two neighbouring populations of the common frog (*Rana temporaria* L.),” *Can. J. Zool.*, **71**, 26 – 33.
- Camp C. D., Marshall J. L., and Austin R. M.** (2000), “The evolution of adult body size in black-bellied salamanders (*Desmognathus quadramaculatus* complex),” *Can. J. Zool.*, **78**, 1712 – 1722.
- Claussen D. L. and Layne J. R., Jr.** (1983), “Growth and survival of juvenile toads, *Bufo woodhousei* maintained on four different diets,” *J. Herpetol.*, **17**(1), 107 – 112.
- Ebenman B. and Persson L.** (eds.) (1988), *Size-Structured Populations: Ecology and Evolution*, Springer-Verlag, Berlin.
- Halliday T. R. and Verrell P. A.** (1988), “Body size and age in amphibian and reptiles,” *J. Herpetol.*, **22**(2), 253 – 265.
- Hemelaar A. S. M.** (1986), *Demographic Study on Bufo bufo L. (Anura, Amphibia) from Different Climates, by Means of Skeletochronology*. Ph.D. Thesis, University of Nijmegen.
- Hota A. S.** (1994), “Growth in amphibians,” *Gerontology*, **40**, 147 – 160.
- Ishchenko V. G.** (1989), “Population biology of amphibians,” *Soviet Sci. Rev. Ser. F. Physiol.-Gen. Biol.*, **13**, 119 – 151.
- Ishchenko V. G.** (1996), “Problems of demography and declining populations of some euroasiatic brown frogs,” *Russ. J. Herpetol.*, **3**(2), 143 – 151.
- Kara T. C.** (1994), “Aging in amphibians,” *Gerontology*, **40**, 161 – 173.
- Licht L. E.** (1975), “Comparative life history features of the western spotted frog, *Rana pretiosa*, from low- and high-elevation populations,” *Can. J. Zool.*, **54**, 1277 – 1284.
- Richards C. M. and Lehman G. C.** (1980), “Photoperiod stimulation of growth in postmetamorphic *Rana pipiens*,” *Copeia*, **1980**(1), 147 – 149.
- Olshvang V. N.** (1992), *Structure and Dynamics of Populations of Insects of South Yamal*, Nauka, Yekaterinburg [in Russian].
- Schwarz S. S. and Ishchenko V. G.** (1971), *The Ways of Adaptation of Animals to Living Conditions in Subarctic. Vol. 3. Amphibians*, Yekaterinburg [in Russian].
- Seale D. B.** (1987), “Amphibia,” in: Pandian T. J. and Vernberg F. J. (eds.), *Animal Energetics. Vol. 2*, Acad. Press, New York, pp. 468 – 552.
- Smirina E. M.** (1994), “Age determination and longevity in amphibians,” *Gerontology*, **40**, 133 – 146.

**SPERM STORAGE IN TWO SPECIES OF SNAKES:
ASIAN PIT VIPERS *Trimeresurus albolabris* (GRAY, 1842)
AND *Trimeresurus erythrurus* (CANTOR, 1839), BRED
AT THE LENINGRAD ZOO TERRARIUM**

E. R. Kamelin¹ and Yu. A. Lukin¹

Keywords: Viperidae, Serpentes, *Trimeresurus albolabris*, *Trimeresurus erythrurus*, breeding, sperm storage.

INTRODUCTION

Long term sperm storage in the subfamily Crotalinae is known in North American temperate genera such as *Agkistrodon*, *Crotalus*, and *Sistrurus* (Schuett, 1992). Limited data has been published on delayed fertilization in Asian pit vipers such as *Calloselasma* and *Trimeresurus* (Kudryavtsev and Mamet, 2003). Therefore all new information on long term sperm storage is of great interest.

We have documented long-term sperm storage in one female white-lipped pit viper (*Trimeresurus albolabris*) and 3 female red-tailed pit vipers (*Trimeresurus erythrurus*).

MATERIAL AND METHODS

An adult female *Trimeresurus albolabris* caught by Nikolai Orlov in a rocky region of Tam-Dao in Northern Vietnam was brought into the collection of Leningrad Zoo in 1997. Two males *Trimeresurus albolabris* were born in 1998 at the Department of Herpetology in Leningrad Zoo as a result of breeding the above mentioned female. A male and 3 females *Trimeresurus erythrurus* born in 1999 the Moscow Zoo and were received by our department later that year.

These snakes are kept in glass terrariums measuring 500 × 300 × 500 mm. From spring till the middle of autumn, they receive 12 h of daylight. The temperature was kept between 27 – 28°C in the warm side of the terrarium; 20 – 25°C in the cold side during the daytime. During the night time, the temperature was kept between 23 – 25°C in the warm side, and 19°C in the cold side. Humidity was kept between 75 – 85%.

Before hibernation, daylight period was gradually reduced. Snakes were hibernated at the same terrariums where they were kept. The duration of hibernation was 1

month. Temperature was kept between 15 – 18°C, with the average being 16.5°C. In 2002 hibernation began in December, while in 2003 it began in January. In 2003 the red-tailed pit viper were not hibernated, however they were kept at the same laboratory. Therefore there was a 2 – 3°C drop in ambient temperature.

When hibernation was completed, the daylight length was gradually increased. Males were introduced to females when daylight reached 12 h.

Methods for keeping Asian pit vipers were discussed in detail in our previous article (Kamelin and Lukin, 2000).

RESULTS AND DISCUSSION

On August 13, 2002, a female *Trimeresurus albolabris* gave birth to 6 newborn snakes, 2 dead neonates and 2 infertile ova. In 2002 this female had not been prepared for reproduction. This female had given birth annually since 1998, so we decided to give her a year of rest.

On February 2, 2001, a male was introduced to a female and copulation was noted. Then during a period of one month, two males were introduced to this female, one at a time, second copulation was not noted. On 30 July 2001, this female gave birth to 11 newborn snakes, 2 dead neonates, and laid 1 infertile egg. Later she fed normally, was hibernated for a month, and continued to feed after hibernation was completed. During this period, males were never introduced to this female.

From the middle of May 2002, the female went without food for 80 days before delivery. This coincides with a starvation period in other gravid females (Kamelin and Lukin, 2000).

On 13 August, delivery took place. This happened 2 months later than the parturition of a different female, which copulated in 2002 and gave birth on 12 June. The newborn snakes were normally developed, and their weight and dimensions (Table 1) were identical to neo-

¹ Department of Herpetology, Leningrad Zoo, Aleksandrovsky park 1, 197198 St. Petersburg, Russia; E-mail: ekamelin@mail.ru.

TABLE 1. Dimensions of Newborn Asian Pit Vipers

Species	n	m, g		L., mm		L.cd, mm	
		min – max	x	min – max	x	min – max	x
<i>Trimeresurus erythrurus</i>	27	3.0 – 6.8	5.6	156 – 212	193.5	26 – 59	44.1
<i>Trimeresurus albolabris</i>	6	2.5 – 5.5	4.5	173 – 191	188.6	33 – 41	38.2

n, Number of newborn snakes measured; m, weight of measured newborn snakes; x, average value.

TABLE 2. Chronology of *Trimeresurus erythrurus* Breeding

Female number	First copulation	Second copulation	Number of days from copulation till delivery	Number of starvation days	Date of parturition
1	June 18, 2002	August 7, 2002	324 – 274	164	May 7, 2003
2	June 2, 2002	July 19, 2002	367 – 320	151	June 3, 2003
3	June 15, 2002	July 12, 2002	359 – 332	156	June 8, 2003

nates born at the Department of Herpetology earlier from other females (Kamelin and Lukin, 2000).

The average duration of gravidity for *Trimeresurus albolabris* is 146 days according to data we obtained from 1998 to 2002. This means that fertilization occurred approximately at the beginning of March 2002. Therefore sperm was stored in the female for a year.

A *Trimeresurus erythrurus* male was introduced to several females in turn from the beginning of February 2002. However, sexual activity was noted only at the end of May. From June through August, 6 copulations were noted; two copulations took place with each female (Table 2). From the second copulation till delivery, the male was not introduced to the females.

Female No. 1 stopped feeding in November 2002. Before delivery she fed only two times. Females Nos. 2 and 3 refused to feed in January 2003, and never fed till delivery. Breeding results are represented in Table 3.

The average duration of gravidity for *Trimeresurus erythrurus* is approximately 150 days or 5 months (Kudryavtsev et al., 2002). In our case, the period from copulation till delivery was significantly longer, 9 – 12 months (Table 2). Therefore the duration of sperm storage would never been 4 – 7 months.

All living newborn *Trimeresurus erythrurus* were normally developed, and their weight and dimensions (Table 1) coincided with those of newborn specimens from Moscow Zoo (Kudryavtsev et al., 2002).

Long-term sperm storage is known from different species of the subfamily Crotalinae, who inhabit temperate climate zones. It has been noted for species copulating both in spring and autumn (Schuett, 1992).

In his fundamental work concerning this subject, American herpetologist G. Schuett suggested that long-term sperm storage could happen in species of *Trimeresurus* from temperate climate zones. In Tam-Dao, the white-

TABLE 3. Results of *Trimeresurus erythrurus* Breeding

Number of females	Living neonates	Dead neonates	Infertile ova
1	7	1	1
2	10	1*	2
3	10	1	1

*, Underdeveloped.

lipped pit viper inhabits the temperate zone of tropical mountains. During the winter period from the end of November till the beginning of March; the night temperature in Tam-Dao is between +8 – 12°C, day-time temperature is +15 – 18°C. In June the night temperature is +19 – 24°C, while the day-time temperature is from +26 – 31°C (Orlov, 1997). At the Department of Herpetology, white-lipped pit vipers are kept in similar conditions to those found in nature (Kamelin and Lukin, 2000). Therefore breeding of white-lipped pit vipers at Leningrad Zoo as the result of long-term sperm storage provides evidence for sperm storage in temperate species of the genus *Trimeresurus*.

Copulation typically occurs 1 to 3 months earlier than ovulation in the majority of tropical and subtropical species which breed in the spring (Kudryavtsev and Mamet, 2003). However, several cases of longer sperm storage are known (Table 4).

In our case, long-term sperm storage in female *Trimeresurus erythrurus* could be related to being kept at a laboratory where other animals were being hibernated. This may have led to a decrease in ambient temperature in terrariums. Two out three females *Trimeresurus erythrurus* refused to feed beginning in January 2003. This coincides with the beginning of hibernation to other snakes.

According to the data presented, it is reasonable to conclude that long-term sperm storage in Crotalinae from tropical and subtropical climate zones is an adaptation

TABLE 4. Data on Sperm Storage in Different Species of Crotalinae

Species	Sperm storage duration	Sources
<i>Trimeresurus popeiorum</i>	7 months	Nickerson, 1974
<i>Trimeresurus trigonocephalus</i>	7 months and 7 days	De Silva, 1983
<i>Trimeresurus flavomaculatus</i>	9 – 10 months	Kudryavtsev and Mamet, 2003
<i>Trimeresurus erythrurus</i>	6 months	Toriba et al., 1990

mechanism, which helps to preserve the gene pool under unfavorable environmental conditions.

REFERENCES

- Kamelin E. R. and Lukin Yu. A.** (2000), "Keeping and breeding of green tree vipers (*Trimeresurus* Lacepede, Crotalidae) in terrarium," *Nauch. Issled. Zooparkakh SPb.*, **12**, 121 – 126 [in Russian].
- Kudryavtsev S. V., Mamet S. V., and Fedin V. V.** (2002), "Some successful results in husbandry and breeding of the rare and hard-to-keep venomous snakes at the Moscow Zoo (part II: 2000 – 2001 years)," *Nauch. Issled. Zooparkakh Mosk.*, **14**, 197 – 205 [in Russian].
- Kudryavtsev S. V. and Mamet S. V.** (2003), "On possibility of physiological sperm conservation in Philippine Pit Viper *Trimeresurus flavomaculatus* (Grey, 1842)," *Nauch. Issled. Zooparkakh Mosk.*, **16**, 163 – 166 [in Russian].
- De Silva A.** (1983), "Reproductive habits of *Trimeresurus trigonocephalus* (Sonnini et Latreille)," *The Snake*, **15**, 16 – 21.
- Nickerson M. A.** (1974), "Comments on the reproduction of Pope's pit viper (*Trimeresurus popeiorum*)," *Br. J. Herpetol.*, **5**, 451 – 452.
- Orlov N. L.** (1997), "Viperid snakes (Viperidae Bonaparte, 1840) of Tam Dao mountain ridge (Vinh-Phu and Bac-Thai Provinces, Vietnam)," *Russ. J. Herpetol.*, **4**(1), 64 – 74.
- Schuett G. W.** (1992), "Is long-term sperm storage an important component of the reproductive biology of temperate pit-vipers? " in: *Biology of the Pitvipers*, Selva Tyler, Texas, pp. 169 – 184.
- Toriba M., Hirabayashi K., and Khin Aung Cho** (1990), "A case of reproduction of Burmese green pit viper, *Trimeresurus erythrurus* with notes on morphological features," *The Snake*, **22**, 8 – 10.

HABITAT VARIATION IN *Rana arvalis* OF NORTHEASTERN UKRAINE

I. Kotserzhynska¹

Keywords: *Rana arvalis*, short-legged and long-legged forms, habitat differentiation.

INTRODUCTION

The moor frog, *Rana arvalis* Nilsson, 1842 is a widely distributed Eurasian species ranging from eastern France and the Netherlands in the west to Yakutia in the east, and from the Polar Circle, southern Yamal Peninsula and Putoran Plateau in the north to the southern part of the Pannonian Basin, Altai Mountains, and Transbaikalia in the south (Borkin, 1998).

Previously, the frogs of the Pannonian lowlands (including Transcarpathian Ukraine) were assigned to a separate subspecies *R. a. wolterstorffi* Fejérváry, 1919, while frogs from the northern area of the Pannonian Basin were recognized as *R. a. arvalis* Nilsson, 1842. The former subspecies is characterized by slender habitat with longer hind legs and larger body size (Fejérváry, 1919). According to Tarashchuk (1984), the long-legged form inhabits both Transcarpathia and some central and southern regions of Ukraine. Other authors, however, questioned the validity of *R. a. wolterstorffi* (Shcherbak and Shcherban, 1980; Babik and Rafiński, 2000). They suggested that the body shape differences in this species may be resulted from the phenotypic plasticity and clinal variation correlating with local climatic factors. Moreover, genetic divergence between those groups proved to be relatively low (Rafiński and Babik, 2000). Indeed, *R. arvalis* demonstrated obvious clinal variation in the leg length (Toporkova, 1965; Bannikov et al., 1977; Ishchenko, 1978): the southern frogs have longer legs in comparison with that of the northern latitudes.

In northeastern Ukraine, both forms of *R. arvalis* were found in the same territory but in different habitats.

MATERIAL AND METHODS

The studies of *R. arvalis* populations were carried out in 2000 – 2003 in the Desnyansko-Starogutskii National Nature Park and adjacent territories (Sumy Oblast', northeastern Ukraine, the forest zone, Fig. 1), throughout an

area of about 42 × 16 km. Four kinds of habitats were recognized: the coniferous forest, the deciduous forest, bogs, and river meadows. 323 frogs were registered by the transect sampling method. Among them, 110 individuals, including males, females, and juveniles, were taken for morphometric measuring (33 from a pine forest, 35 from river meadows, 27 from oak-and-birch forests, 8 from a wooded river bank, and 7 from a marsh). After treatment, all animals were released to habitats, respectively. For each frog 24 external measurements (in mm) were taken with a calliper (with an accuracy of 0.1 mm): L., L.c., Lt.c., D.r.n., Sp.n., D.r.o., D.n.o., L.o., L.t.y.m., D.t.y.m.o., Sp.oc., Lt.p., Sp.p., L.m., D.p., Lt.m., F., T., C.s., D.p.4, Lt.c.s., D.p.1, C.int., H.int. Eventually, 15 indices were calculated: L./L.c., L.c./Lt.c., L.c./Sp.n., L.c./L.o., L.c./L.t.y.m., L.c./D.r.o., Sp.oc./D.r.o., D.p.1/C.int., T./C.int., L./T., F./T., D.p.1/D.p.4, D.p.4/C.int., L./(F. + T.), C.int./H.int. 44 samples (817 specimens) from Ukraine and 2 samples (46 specimens) from Russia were analyzed with respect to the hind leg length. Standard statistical methods (factor and cluster analyses, *t*-test) were used. Calculations were performed using STATISTICA 5.0.



Fig. 1. Region of investigation.

¹ Schmalhauzen Institute of Zoology, National Academy of Science of Ukraine, Bogdana Khmel'nitskogo 15, 01601 Kiev, Ukraine; E-mail: kotserzh@hotbox.ru.

RESULTS AND DISCUSSION

In northeastern Ukraine, two kinds of *R. arvalis* were identified: the short-legged form (ankle joint reaching the frog's eye) and the long-legged form (ankle joint reaching the nostril or the end of snout). In deciduous forests (birch, alder, and oak) and bogs, all 177 individuals were only short-legged (100%). The majority of 146 frogs from meadows and pine forests located along Desna River were long-legged (78% from meadows and 78.1% from pine forests), some of them had intermediate leg length (12.2 and 14.1%, respectively), and only few individuals were with short legs (9.8 and 7.8%). However, these frogs with short legs (9.8 and 7.8%) differed from short-legged frogs inhabiting deciduous forests by other proportions, and by these proportions they belonged to the long-legged form.

110 specimens taken from various habitats of the region were analyzed morphometrically by cluster and factor analyses. The analysis procedure has divided frogs into two groups: the first one included specimens from decidu-

ous forests and bogs (the short-legged form), whereas the second group contained specimens from pine forests and meadows along Desna River (the long-legged form; Figs. 2 and 3). Differentiation by sex and age did not affect the division. The first group statistically differed from the second one by shorter legs, shorter breadth of wrestle joint and shorter breadth of foot. However, the short-legged frogs had larger head, and eyes, larger distance between nostril and snout, as well as larger distance between eye and snout (Table 1).

Our examination of *R. arvalis* throughout Ukraine (44 samples, 817 specimens) revealed that almost all of Ukrainian territory is occupied by the long-legged form. Only in the northern part of Chernigov and Sumy Oblast's (northeastern Ukraine, the forest zone), all 10 samples studied (96 specimens) belonged to the short-legged form. By the way, 2 samples (46 specimens) from the Russia's forest zone, situated far away north-east from Ukraine, contained the short-legged frogs as well. It should be taken into account, that the most of Ukrainian territory belongs to the

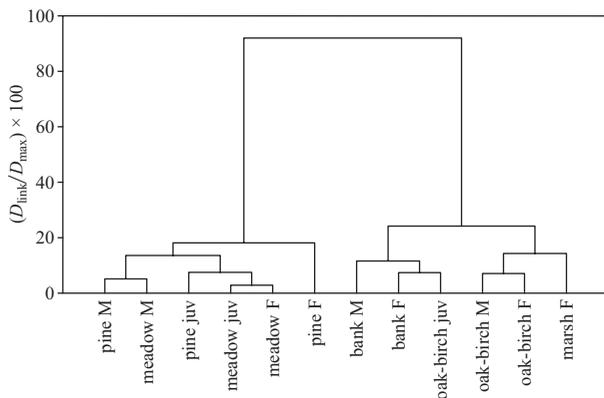


Fig. 2. Dendrogram of 12 samples ($N = 110$) of *Rana arvalis* from different biotops in the North-East of Ukraine obtained from cluster analysis.

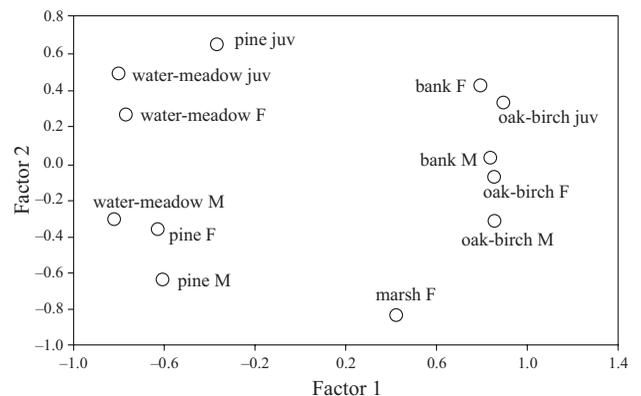


Fig. 3. The distribution of 12 samples ($N = 110$) of *Rana arvalis* from different biotops in the North-East of Ukraine obtained from factor analysis.

TABLE 1. Differentiation of Two Forms of Frogs (t -test $p < 0.05$)

Indices	"Short-legged" form ($N = 52$) (deciduous forests, bogs)		"Long-legged" form ($N = 68$) (pine forest, meadows)	
	mean \pm standard error	range	mean \pm standard error	range
L.c./Lt.c.	1.03 \pm 0.06	0.95 – 1.22	1.07 \pm 0.06	0.93 – 1.2
L.c./L.o.	2.41 \pm 0.17	2.03 – 2.93	2.59 \pm 0.19	2.23 – 3.4
L./T.	1.93 \pm 0.07	1.77 – 2.14	1.84 \pm 0.06	1.66 – 1.98
L./(F. + T.)	0.98 \pm 0.04	0.91 – 1.08	0.94 \pm 0.04	0.85 – 1.05
Parameters (normalized)				
Dr.n.	1.05 \pm 0.07	0.96 – 1.2	0.98 \pm 0.06	0.76 – 1.09
Dr.o.	1.03 \pm 0.05	0.93 – 1.13	0.99 \pm 0.07	0.85 – 1.02
L.o.	1.07 \pm 0.07	0.88 – 1.25	0.97 \pm 0.06	0.76 – 1.02
Lt.p.	1.045 \pm 0.07	0.93 – 1.2	0.98 \pm 0.06	0.85 – 1.13
Lt.m.	0.85 \pm 0.09	0.65 – 1.03	1.07 \pm 0.08	0.93 – 1.34
Lt.cs.	0.94 \pm 0.06	0.79 – 1.08	1.02 \pm 0.05	0.86 – 1.15

steppe and forest-steppe zones, and the forest zone covers the northern and mountain regions only. Our results, therefore, can be regarded as an evidence that, as a rule, the long-legged form of *R. arvalis* inhabits the steppe and forest-steppe zones while the short-legged form occurs in the forest one.

Thus, in northeastern Ukraine, the both forms of the moor frog can be found, however, they inhabit different habitats. It seems likely that the southern long-legged form spreaded across steppe and disturbed areas (including pine forests) near large rivers, while the short-legged form invaded from Russia through native deciduous forests. In the territory under the study, the two forms of *Rana arvalis* are sympatric.

So far it is not possible to assert whether these forms of the moor frog have genetic differences or they are merely ecological races based on epigenetic phenomenon (like some fishes). Appropriate genetic studies are going to be arranged in the nearest future. However, the problem is that the draught of the last two years caused a significant decline in local populations of the moor frog. Further research will only be possible if its populations will renew.

Acknowledgments. I am very grateful to M. A. Kaporulya, the Director of the Desnyansko-Starogutskii National Park, S. Panchenko and G. Gavrish for their help during the field work as well as to E. M. Pisanets (Kiev, Ukraine) for an opportunity to work with museum collections. L. J. Borkin (St. Petersburg, Russia) and L. Luiselli (Rome, Italy) provided valuable, and sometimes, critical comments, and D. Palets (Kiev, Ukraine) made English corrections.

REFERENCES

- Babik W. and Rafiński J.** (2000), “Morphometric differentiation of the moor frog (*Rana arvalis* Nilss.) in Central Europe,” *J. Zool. Syst. Evol. Res.*, **38**, 239 – 247.
- Bannikov A. G., Darevsky I. S., Ishchenko V. G., Rustamov A. K., and Shcherbak N. N.** (1977), *Guide to Amphibians and Reptiles of the USSR [Opredelitel' Zemnovodnykh i Presmykayushchikhsya SSSR]*, Prosveshchenie, Moscow [in Russian].
- Borkin L. J.** (1998), “Amphibians,” in: Ananjeva N. B., Borkin L. J., Darevsky I. S., and Orlov N. L. (eds.), *Amphibians and Reptiles. Encyclopedia of the Nature of Russia [Zemnovodnye i Presmykayushchiesya. Éntsiklopediya Prirody Rossii]*, ABF, Moscow, pp. 19 – 174 [in Russian].
- Fejérváry G. J.** (1919), “On two south-eastern varieties of *Rana arvalis* Nilss.,” *Ann. Mus. Nat. Hung.*, **17**, 178 – 183.
- Ishchenko V. G.** (1978), *Dynamic Polymorphism in Brown Frogs of the USSR Fauna [Dinamicheskii Polimorfizm Burykh Lyagushek fauny SSSR]*, Nauka, Moscow [in Russian].
- Rafiński J. and Babik W.** (2000), “Genetic differentiation among northern and southern populations of the moor frog *Rana arvalis* Nilsson in Central Europe,” *Heredity*, **84**, 610 – 618.
- Shcherbak N. N. and Shcherban M. I.** (1980), *Amphibians and Reptiles of Ukrainian Carpathians [Zemnovodnye i Presmykayushchiesya Ukrainskikh Karpat]*, Naukova Dumka, Kiev [in Russian].
- Tarashchuk S. V.** (1984), “On the variation in *Rana arvalis* in the territory of Ukraine,” *Vestnik Zool. Kiev*, **5**, 80 – 82 [in Russian].
- Toporkova L. J.** (1965), “On the geographic variation of amphibians,” *Dokl. Vyssh. Shkoly. Biol. Nauki*, **1**, 31 – 36 [in Russian].

THE RED DATA BOOK OF UL'YANOVSK OBLAST': AMPHIBIANS AND REPTILES

V. Krivosheev¹

Keywords: Red Data Book, biological diversity, amphibians, reptiles.

INTRODUCTION

The protection of biological diversity as a basis of biosphere functioning is one of the most important problems at present. An ecological-faunistic study of Amphibians and Reptiles in many regions of Russia is a topical issue because of insufficient knowledge and decrease of the numbers. The Ul'yanovsk Oblast' is one of such regions. It is an industrial-agrarian region where natural ecosystems are strongly influenced. The most effective way to protect vertebrates is to establish reserves and national parks. However, there are no such territories in the Ul'yanovsk Oblast', but there are 14 protected areas where Amphibians and Reptiles are included (Krivosheev, 2002). To make recommendations for the conservation of amphibian and reptilian diversity within the regional area, detailed explorations of their distribution and ecology are necessary.

MATERIAL AND METHODS

Materials for the Red Data Book are based on field data collected by the author in 1979 – 1981, 1984 – 2002. The protection status is determined with two characteristics of a species: rarity and changes of the numbers (Ananjeva et al., 2004; Saksonov and Rozenberg, 2000). The first index (rarity scale) characterizes the rarity of a species within the region: 0 — has not been found lately (5 – 10 years); 1, extremely rare; 2, very rare; 3, quite rare; 4, rare; 5, conditionally rare. The second index shows the changes of the numbers: 0, unknown; A, quick decline; B, slow decline; C, annual changes of the numbers; D, stable quantity; E, increase of the numbers.

RESULTS AND DISCUSSION

AMPHIBIANS

Triturus cristatus (Laurenti, 1768).

Order Caudata. Family Salamandridae

Status: Index 4/B. Rare, slow decline of the numbers.
Distribution: Found widely in the Ul'yanovsk Oblast',

though its number is less than that of *Triturus vulgaris*. In the southern raions (Pavlovka, Radishchevo, Staraya Kulatka) it is found mainly in forested gullies and ravines, where it prefers forest habitats. In the northern areas the species is mainly found in deciduous and pine-deciduous forests. **Biology:** In the Ul'yanovsk Oblast' it starts hibernation at the end of October and it appears at the end of April – beginning of May, when the air temperature is +9 – 10°C and that of water is +6°C. Spawning lasts about 2 months; female spawns 80 – 600 eggs, but usually about 100 – 200. Larvae eat crustaceans: *Daphnia*, *Cyclops*, and also larvae of *Diptera*. Metamorphosis finishes after 80 – 100 days; the larvae lose their tail fins and gills and leave the water. Number: The number is not large; there is a decline in the region. In Ul'yanovsk they are found in Vinnovskaya grove and Victory Park (about 80 pairs were registered). As a result of the construction of a new bridge and a connecting road, original habitats were destroyed, the hydrological regime was changed, and the number of newts quickly decreased. **Limiting factors:** Anthropogenic changes of original habitats (melioration, pollution, forest cutting), use of pesticides, collection for trade. **Protection:** Establishment of reserve areas, ban on catching, reduction of anthropogenic influence, purification of water and cleaning of water protection zones.

Rana temporaria Linnaeus, 1758.

Order Anura. Family Ranidae

Status: Index 5/D. Conditionally rare species, stable number. **Distribution:** In the Ul'yanovsk Oblast' the frog is found in all raions in forest and grassland zones. It prefers wet habitats near streams, wet grassy vegetations, banks of marshes, rivers and lakes. In settlements it is found in parks and gardens. **Biology:** Adults are usually single but congregate during the breeding period and hibernation. Hibernation starts at the end of September – beginning of October, and takes place in frost-free small rivers and springs. Appears in the middle of April, when the water temperature is 7 – 8°C. Spawns 670 – 4000 eggs. The development of tadpoles lasts 49 – 90 days; they eat algae and other plants. Young frogs eat *Diptera*, beetles, caterpillars. Number: In the northern raions (Sura, Inza, Karsun, Staraya Maina) the number of frogs is

¹ Faculty of Ecology, Ul'yanovsk State University, L. Tolstoy str. 42, 432970 Ul'yanovsk, Russia; E-mail: krivoshev@list.ru.

45 – 60 per 100 m of route. Changes of the numbers were not investigated. In Ul'yanovsk the frog is found in Victory park, Vinnovskaya grove, Solovei ravine. **Limiting factors:** Anthropogenic destruction of original habitats, drainage, pollution of streams with silt and sewage, road mortality during spring migration towards reservoirs, freezing during cold wet winters. Declining as a result of killing these non-popular animals. Establishment of protected areas, limitation of anthropogenic influence in settlements. The frog is protected in following "hunting" reserves: Sosnovki, Surskii, Bazarnosyzganskii, Novochemshanskii, Sengileevskii, Mainskii, "Surskie holmy," landscape reserve "Shilovskaya lesostep'."

REPTILES

Emys orbicularis (Linnaeus, 1758).

Order Testudines. Family Emididae

Status: Index 2/0. Very rare species, changes of numbers unknown. **Distribution:** In the Ul'yanovsk Oblast' found in Sura, Karsun, Ul'yanovsk, Terenga, Kuzovatovo, Nikolaevka, Novospassk, Pavlovka, Melekess raions. **Biology:** The habitats are formed by rivers, lakes, ponds with silt bottoms. Diurnal and crepuscular activity. Feeding: invertebrates (larvae, dragon-flies, beetles, worms), tadpoles, plants and algae. Hibernates on the bottom from the end of October. Appears after winter at the end of April – beginning of May, when the temperature is 5 – 10°C. Females lay 3 clutches (May, June, July) of 5 – 10 eggs in a hole of 10 cm. Number: the total population within the Ul'yanovsk Oblast' is about 100 – 150 individuals (subpopulations). **Limiting factors:** Anthropogenic destruction of original habitats (pollution with sewage, disturbance of breeding sites). Casualties by netting. Collecting for trade. **Protection:** restriction of anthropogenic influence on habitats, establishment of reserves, bans on the use of nets in their habitats, prohibition of collecting.

Natrix tessellata (Laurenti, 1768).

Order Squamata. Family Colubridae

Status: Index 3/0. Quite rare, number unknown. **Distribution:** in the Ul'yanovsk Oblast' found in Sura, Karsun, Inza, Melekess, Kuzovatovo, Barysh, Sengilei, Cherdakly, Staraya Maina raions. Habitats near lakes and other reservoirs. Prefers ravines, stony slopes of lakes and rivers. Biology: diurnal activity, with tow peaks in the morning and evening. Spends the most part of the daytime in the water, but leaves it for the night. Eats fish, amphibians and small rodents. Hibernates from September – October not far from the water, single or in group conglomeration (sometimes with *Natrix natrix*) in rodent holes, deep clefts, old stumps. Appears at the end of April – beginning

of May. In the breeding period the snakes form groups, up to ten individuals. Females lay 4 – 15 eggs at the end of June – beginning of July. Number: data are absent. **Limiting factors:** Anthropogenic changes of original habitats (forest cutting, pollution with sewage). Use of electric rods. Catching and killing (often confused with *Vipera berus*). **Protection:** Restriction of anthropogenic influence on habitats, establishment of reserve areas to decrease the pressure from recreation. Bans on collecting. Education and information booklets for school children and local people.

Coronella austriaca Laurenti, 1768.

Order Squamata. Family Colubridae

Status: Index 3/0. Quite rare, number unknown. **Distribution:** all raions of the Ul'yanovsk Oblast', more often in Bazarnyi Syzgan, Barysh, Kuzovatovo, Terenga, Sengilei, Melekess raions, but the numbers are small everywhere. Biology: Habitats are pine and pine-deciduous forests, where they usually prefer edges of forests, clearings on hills, especially on sunny slopes. They use holes of rodents and lizards, trunks, clefts in the ground as a refuge. Hibernation at the same places, going to below the frostline, from the end of September trough the beginning of October. Appears at the end of March trough the beginning of April. Females give birth to 2 – 15 young in August. Number: in some raions (Bazarnyi Syzgan, Barysh, Sengilei, Melekess) up to 3 snakes per 5 – 7 km of road can be recorded. In other raions the number of snakes is normally small. **Limiting factors:** Anthropogenic destruction of original habitats (forest cutting, use of pesticides, disturbance). Death on forest roads. Destruction by settlers as a result of misinformation and superstition. **Protection:** Restriction of anthropogenic influence on original habitats; establishment of reserve areas; bans on catching; education and information for local people.

Elaphe dione (Pallas, 1773).

Order Squamata. Family Colubridae

Status: Index 3/0. Quite a rare species, number unknown. **Distribution:** in the Ul'yanovsk Oblast' the species is found in the Radishchevo raion. Prefers ravines, grass slopes. Biology: Hibernates at the end of October in rodent holes and under stones. Appears at the middle of April. Females lay 8 – 15 eggs at the end of June – beginning of July. Young of 220 mm length appear at the end of September. Number: in the Radishchevo raion (Vyazovka village and its surroundings) 1 – 4 snakes per km can be found. **Limiting factors:** Anthropogenic changes of original habitats (ploughing of grass slopes, over-grazing by livestock); catching, killing. **Protection:** Restriction of

anthropogenic influence on original habitats; establishment of a landscape reserve in the Radishchevo raion, 4 km south from Vyazovka village for the conservation of native grasslands. Bans on collecting. Education and information for local people.

Vipera renardi (Christoph, 1861).

Order Squamata. Family Viperidae

Status: Index 4/B. Rare species, slow decrease of the numbers. **Distribution:** In the Ul'yanovsk Oblast' in Radishchevo, Staraya Kulatka, Pavlovka, Kuzovatovo, Terenga, Sengilei, Melekess, Novaya Malykla, Novospassk, and Ul'yanovsk raions. **Biology:** In the Ul'yanovsk Oblast' it inhabits forest-grassland zones, grasslands, dry ravines, slopes. Population density low. Rodent holes, clefts in the ground, spaces between stones are used as refuges. Hibernation at the same places, going to below the frost-line. Appears in March – April. Pregnancy lasts 90 – 130 days. In August females give birth to 5 – 6 young of 120 – 180 mm in length. Number: over the region the number is decreasing. In some raions (Radishchevo, Novospassk) up to 4 snakes per 1 km can be found. **Limiting factors:** Anthropogenic destruction of original habitats

(ploughing of grasslands, use of pesticides and fertilizers). Grazing by livestock. Death on roads during spring and autumn migration. Destruction by settlers as a result of misinformation and superstition. **Protection:** Restriction of anthropogenic influence on original habitats; establishment of reserve areas (Radishchevo, Novospassk, Staraya Kulatka, Nikolaevka, Sengilei raions). Bans on catching, education and information for the local people.

REFERENCES

- Ananjeva N. B., Orlov N. L., Khalikov R. G., Darevsky I. S., Ryabov S. A., and Barabanov A. V. (2004), *Colored Atlas of the Reptiles of North Eurasia (Taxonomic Diversity, Distribution, Conservation Status)*, St. Petersburg [in Russian??].
- Krivosheev V. A. (2002), "Red Data Book of the Ul'yanovsk Oblast': amphibians and reptiles," in: *The Nature of the Simbirsk Volga Region: Scientific Works Collection. Vol. 3*, Ul'yanovsk regional museum, Ul'yanovsk, pp. 157 – 165 [in Russian].
- Saksonov S. V. and Rosenberg G. S. (2000), *The Organization and Methodical Aspects of Red Listing*, Izd. Inst. Ékol. Volzhskogo Bass., Tol'yatti [in Russian].

THE CONTENT OF CHEMICAL ELEMENTS IN THE ORGANISM OF ANURA, AMPHIBIA, AS AN INDICATOR OF THE ENVIRONMENTAL CONDITIONS

V. N. Kuranova,¹ N. V. Baranovskaja,² and L. P. Rikhvanov²

Keywords: Amphibia, chemical elements, bioindication, geochemical monitoring.

INTRODUCTION

The biomonitoring of chemical elements content level in an ecosystem has a great significance. Western Siberia is distinguished by geochemical specificity and particular functioning of industry, which form local zones of pollution, industrial biogeochemical provinces. The element composition of biota on such territories have their specificity (Saet et al., 1990). The role of chemical elements in living organisms are diverse: they are components of enzymes, vitamins and hormones, participants in biochemical processes, and may cause toxic effects on important organism functions. The excess or deficit content of different chemical elements in living organisms may be caused by both industrial pollution and the peculiarity of the environment's geochemistry. A study of regional specificity of accumulation in organs and tissues of biological objects is an urgent problem. Amphibia are the links of trophic chains of land and fresh-water ecosystems and play an important role in the exchange of substance and energy between ecosystems of different biological cycles that promotes their application as the test-objects in ecological monitoring of the environment.

MATERIAL AND METHODS

The study of chemical elements content in the organism of amphibians *Bufo bufo* and *Rana arvalis* (Anura, Amphibia) was carried out in 1992 – 1993 on territories with different industrial burdens: the central part of Tomsk (2 stations: University Grove, Yuzhnoe cemetery) and its northern (6: SCIP, TPW, Kuzovlevo, Malinovka, Oktyabr'skii) and southern (6: Botanical Garden, Radio Plant, Stepanovka, Predtechensk, Kar'er, Timiryazev) suburbs, valleys of the rivers Tom' and Ob' (3: Monatka, Porosino, Kaltai) and Kuznetsk Alatau (2 stations: Lomachevka, Berikul).

The studied territory is characterized by the presence of large enterprises: Tomsk Petrochemical Works (TPW), nuclear fuel cycle enterprises (Siberian Chemical Integrated Plant, SCIP), heat power industry as well as natural anomalies (deposits of zircon-ilmenite ores in Malinovka, gold-sulfide ores in Berikul). The contents of elements was determined by INAA and x-ray micro spectrometry analysis methods. Totally 289 organs and tissues (liver, kidney, ovary, testicle, skin) of 112 specimens of *B. bufo* and *R. arvalis* of both sexes and different ages were processed. Data processing (correlation and cluster analysis) was carried out with STATISTICA, Golden Surfer, and EXCEL programs.

RESULTS AND DISCUSSION

The content of 28 elements (Ca, Zn, Br, Sc, Ce, Na, K, Cr, Fe, Co, Se, Rb, Hg, Ag, Sb, Ba, La, Sm, Eu, Tb, Yb, Lu, Hf, Ta, Au, Th, U, Cs), their distribution, Th/U and La/Yb ratios, ratio of sums of light lanthanides (La, Ce) to heavy (Yb, Lu) ones was revealed in *Rana arvalis* and *Bufo bufo* specimens. According to Bowen (1966), scheme 6 elements (Ag, Au, Cr, Hg, Sb, Zn) are very intensive, 3 (Ba, Ca, Fe) are intensive, 5 (Br, Co, K, Na, Rb) moderate, and 3 (La, Sr, Ta) are weak potential pollutants.

The data obtained show specificity in accumulation of elements by different organs and species. The *R. arvalis* skin reveals high content of Cr, Co, Hg, insignificant excess of Ba, Se, Br, K; liver — insignificant excess of Na, Co, Sb, La, Au. The skin of *B. bufo* accumulates rare — earth elements, Ag, Hf; kidneys — Zn, Br; liver — Tb (Fig. 1).

The differences are concerned with details of a diet and degree of terrestrial mode of life. As a whole, the most sensitive organs, accumulating trace elements, are the skin and the liver. The results confirm conclusions about variations in the element content of Anura amphibians organism depending on species, sex, age of specimens and the concentration affinity of tissues and organs (Misyura, 1985; Leontjeva, 1990; Misyura et al., 2003).

¹ Tomsk State University, Lenina Pr. 36, 634050 Tomsk, Russia; E-mail: Kuranova@bio.tsu.ru; boiga@mail2000.ru.

² Tomsk Polytechnical University, Pugachev Pr. 1-65, 634015 Tomsk, Russia; E-mail: valery@ant.tomsk.ru.

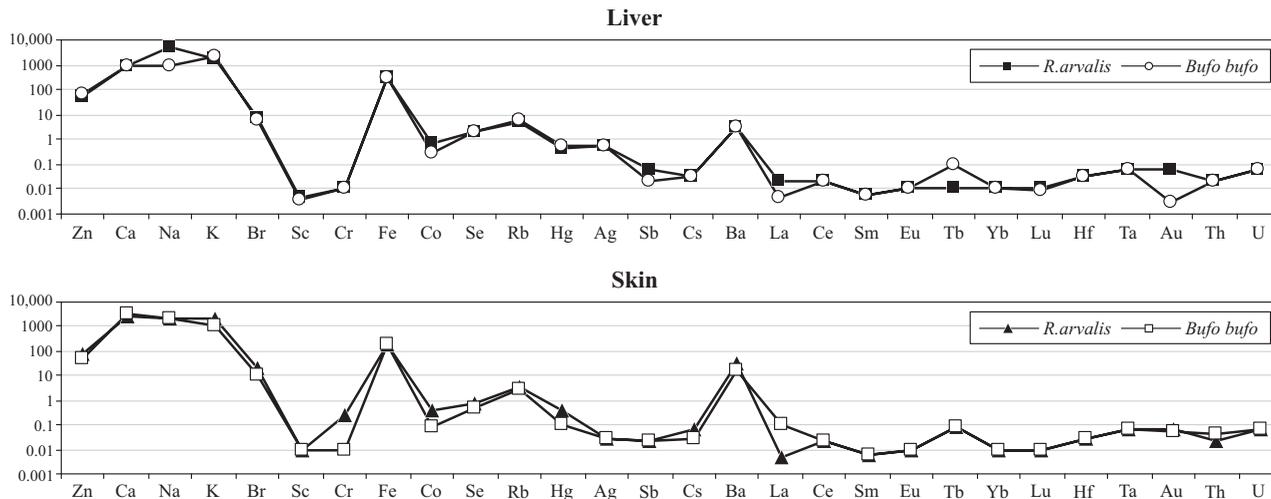


Fig. 1. The contents of chemical elements in different organs of amphibians: abscissa, elements; ordinate, log of concentration, mg/kg.



Fig. 2. The scheme of cobalt distribution of *Rana arvalis* specimens through Tomsk suburb zone.

At the same time, accumulation of chemical elements corresponds with the geochemical situation of the investigated territory (Rikhvanov, 1997).

The chemical composition of *R. arvalis* specimens on the key territories is clearly distinguished by excess accumulation of several elements: in Tomsk — Th, Zn, Rb, Tb, Yb, Se; its northern suburb — Co, Rb, Sb, Cs, Au, Se, Br, Eu, Sm; southern suburb — Th, Tb, Yb; river valleys — Ba. The excess contents of chemical elements in the organs are characteristic for amphibians of the influence zones of Siberian chemical and Tomsk petrochemical integrated plants (northern suburb), where ratio of the sum of light lanthanides (La, Ce) to heavy (Yb, Lu) change

1.5 – 2 times, ratio La/Yb — 10 times the comparatively medium levels on the investigated territory.

High values Th/U are characteristic for *R. arvalis* populations in river valleys, low — for the northern suburb of Tomsk. A number of *R. arvalis* populations (Malinovka, Berikul) reveal a wide spectrum of the element composition, which reflects a natural biogeochemical specificity of the territories and is confirmed in geochemical rows of element concentration. Geochemical rows are constructed by concentration indices relatively klark living substance (Sokolov, 1990) and permit the disclosure of the sources of ingress of elements into the environment and the amphibian organism (Table 1).

The geochemical specificity of amphibian specimens from river Tom' valley (Kaltai) attributed to the substance transgression from the neighboring territories, what is confirmed by works of a number of authors (Adam et al., 1994; Schatilov, 2001). At the same time, accumulation rows in the Kaltai *R. arvalis* population coincides with the same for populations from the territories with natural anomalies (Malinovka, Berikul). The industrial influence on the amphibian organism is reflected in schemes of the element distribution, levels of accumulation, and geochemical associations. The chart of cobalt distribution shows the primary influence of the Northern industrial center on amphibian specimens from northern Tomsk suburb (Fig. 2). The halo coincides with the zone of excess concentrations of Co^{60} in aerosol and dust fallouts in building attics of the settlements (Merkulov et al., 1996); a hair and blood composition of population of the district (Baranovskaja and Rikhvanov, 2002).

In the *R. arvalis* populations from the rivers Tom' (Kaltai, Porosino) and Ob' (Monatka) valleys elements

TABLE 1. Geochemical Rows of Elements Amassing in *R. arvalis* Organism (according to Sokolov, 1990)

Points	Geochemical specter																		
SCIP	Au ²²⁷	Se ¹³⁶	Sb ⁸²	Hg ⁶⁷	Hf ¹⁵	Na ⁸	Co ⁶	Br ⁵	Ag ⁴	Cr ⁴	U ⁴	Zn ²	Rb ¹	Ba ¹	K ¹	Fe ¹	Ca ^{0.2}	Cs ^{0.1}	
TPW	Au ⁸⁶³	Se ³²³	Sb ²⁰⁰	Hg ⁶⁰	Co ¹⁷	Hf ¹⁰	Na ⁸	Cr ⁸	Br ⁴	Ag ⁴	U ⁴	Zn ⁴	Rb ³	Ba ²	Fe ²	K ²	Ca ^{0.6}	Cs ^{0.3}	
Kuzovlevo	Au ¹²⁹⁹	Sb ⁴³³	Se ³⁸⁰	Hg ¹⁴³	Hf ²²	Na ¹¹	Co ¹⁰	Cr ⁹	Rb ⁵	Zn ⁴	U ⁴	Br ⁴	K ²	Fe ²	Ba ²	Ag ¹	Ca ^{0.3}	Cs ^{0.1}	
Kopylovo	Au ⁸⁰⁰	Se ³⁰⁰	Hg ³³	Co ³²	Hf ¹⁰	Na ¹⁰	Br ⁶	U ⁴	Rb ⁴	Zn ³	Fe ²	Ag ²	K ¹	Sb ¹	Ba ^{0.2}	Cr ^{0.2}	Ca ^{0.2}	Cs ^{0.1}	
Malinovka	Au ⁴¹³	Se ³²⁵	Hf ²⁶⁵	Cr ²²⁵	Ag ⁸⁷	Fe ¹⁷	U ¹⁶	Na ¹⁰	Br ⁸	Co ⁶	Ba ³	Zn ²	Ca ^{0.5}						
Oktyabr'skii	Au ³⁷⁶	Se ²⁹⁰	Sb ¹⁶⁴	Hg ¹²⁴	Br ¹³	Na ¹³	Hf ¹⁰	Co ⁷	Cr ⁵	Rb ⁴	U ⁴	Zn ³	Ag ³	K ²	Fe ¹	Ca ^{0.5}	Ba ^{0.5}	Cs ^{0.1}	
University Grove	Se ⁴¹⁵	Au ¹⁰⁰	Sb ⁷⁸	Hg ⁷⁸	Hf ²⁹	Na ¹⁰	Br ⁹	Zn ⁹	Co ⁷	Ag ⁶	U ⁴	Cr ²	Rb ²	K ¹	Fe ¹	Ba ¹	Ca ^{0.1}	Cs ^{0.1}	
Yuzhnoe cemetery	Se ¹²⁰	Sb ⁸³	Hg ⁴³	Ag ¹⁹	Hf ¹⁰	Na ⁹	Br ⁸	Au ⁵	Zn ⁴	U ⁴	Co ³	Fe ²	Ba ²	Rb ¹	K ^{0.7}	Cr ^{0.5}	Ca ^{0.1}	Cs ^{0.1}	
Botanical Garden	Sb ³⁴³	Au ²⁶³	Se ¹¹⁸	Hg ⁵⁵	Hf ³⁴	Ag ⁸	Na ⁷	Br ⁶	Cr ⁶	Zn ⁴	U ⁴	Co ³	Rb ²	Ba ²	Fe ¹	K ^{0.4}	Ca ^{0.1}	Cs ^{0.1}	
TRTC	Hf ³⁰	Na ²⁰	Br ¹⁴	Au ¹⁰	Sb ¹⁰	Zn ¹⁰	U ⁸	Hg ²	Ag ²	K ²	Se ¹	Ba ^{0.3}	Rb ^{0.2}	Cr ^{0.1}	Ca ^{0.02}	Cs ^{0.03}	Co ^{0.01}	Fe ^{0.01}	
Stepanovka	Sb ¹¹⁴	Au ¹⁰⁰	Se ⁷⁴	Hg ⁶⁰	Na ⁹	Br ⁵	U ⁴	Cr ⁴	Zn ³	Rb ³	Hf ³	Ag ³	Co ²	Ba ²	Fe ¹	K ¹	Ca ^{0.1}	Cs ^{0.1}	
Predtechensk	Au ¹⁰⁰	Hg ⁶⁸	Se ⁶⁷	Se ³⁰	Na ⁹	Br ⁸	Cr ⁴	U ⁴	Hf ³	Zn ³	Co ²	K ²	Ag ¹	Rb ^{0.6}	Ba ^{0.4}	Fe ^{0.3}	Ca ^{0.1}	Cs ^{0.1}	
Kar'er	Au ⁷⁷⁷	Sb ²³⁴	Se ¹⁵³	Hg ⁶³	Hf ²⁴	Na ⁷	Br ⁶	Zn ⁵	Ag ⁵	Co ⁴	U ⁴	Rb ²	Cr ²	Fe ¹	Ba ¹	K ^{0.6}	Ca ^{0.1}	Cs ^{0.1}	
Timiryazevo	Au ³⁹³	Se ¹²⁷	Hg ⁶⁷	Sb ³⁴	Ag ²⁹	Hf ¹³	Na ¹³	Br ⁸	U ⁴	Zn ³	Rb ²	Co ²	Ba ¹	Fe ¹	Cr ¹	K ^{0.7}	Ca ^{0.3}	Cs ^{0.1}	
Monatka	Au ⁵⁶³	Se ²⁸⁰	Hg ¹⁸²	Sb ⁵⁰	Ag ¹⁵	Na ¹²	Co ¹²	Hf ¹⁰	Br ⁵	Zn ⁴	U ⁴	Cr ³	Ba ³	Rb ³	K ²	Fe ¹	Ca ^{0.3}	Cs ^{0.2}	
Porosino	Se ²⁵³	Hg ¹⁶⁷	Au ¹⁰⁰	Sb ²⁹	Cr ⁸	Co ⁸	Ba ⁷	Br ⁷	Zn ⁴	U ⁴	Na ³	Hf ³	Fe ²	Rb ¹	Ag ¹	K ^{0.4}	Ca ^{0.1}	Cs ^{0.1}	
Kaltai	Se ²⁷⁰	Cr ²²⁴	Hf ⁸⁵	Au ¹¹⁵	Br ²⁵	Fe ¹³	Na ⁹	Co ⁵	Ag ⁵	U ³	Zn ²	Ba ²	Ca ^{0.5}						
Lomachevka	Au ⁵²⁵	Se ⁹⁰	Sb ⁵³	Hg ⁴⁵	Br ²⁷	Na ¹¹	Hf ¹⁰	Zn ⁴	U ⁴	Rb ²	Co ²	Ba ²	Ag ¹	K ^{0.6}	Fe ^{0.4}	Ca ^{0.3}	Cs ^{0.1}	Cr ^{0.05}	
Berikul	Se ²⁴⁵	Hf ¹⁷⁰	Cr ¹⁶⁵	Au ¹⁰⁹	Ag ⁵⁴	Br ²⁴	K ¹⁷	Fe ⁹	Na ⁷	Co ⁶	Ba ⁴	Zn ²	Ca ^{0.5}						

Black, 100 and higher; dark-gray, from 10 to 100; light-gray from 1 to 10; white; 1 and lower.

The rows show the geochemical specifics of environment the animals live in, influenced by natural and technical factors. This, natural anomalies in Malinovka (the deposit of zircon-ilmenite ore) and Berikul (gold-sulfide deposit) are determined as complexes containing apart from gold such elements as iron, chrome, hafnium, uranium, brome, and the other elements. The high maintenance of zinc in TRTC is induced by the auto road, antimony in different points of investigation appears as a result of the influence of oil plants and petrol stations.

which are characteristic for heat power and nuclear fuel cycle industry discharges are accumulating (Au, Se, Hg, Hf, Co, Cr, Na, Ag).

The analysis of the dendrogram correlation matrix of element composition of amphibian tissues from the studied territory reveals several associations of chemical elements: 1 — Sm, Hf, La, Fe, Ce, Cr, Sc, and U, Ag; 2 — Lu, Na, Zn, Ta, Eu, Tb, K, Sb; 3 — Co, Au, Cs, Rb, Se; 4 — Hg, Yb, Ba (Fig. 3).

The similar element associations are recorded in other habitats of the region (Rikhvanov, 1997). Existence of the associations reflects a complex polyelement influence on the environment, confined to amphibians, of the industry (TPW, SCIP, etc.) and the natural anomalies.

Thus, use of the biogeochemical examination method by trace element composition of a biological objects also amphibians, may be successful for the evaluation of ecosystem pollution and ecological specification of the territory.

REFERENCES

Baranovskaja N. and Rikhvanov L. (2002), "Trace elements in composition of biosubstrates of people living in a district of the nuclear plant of Russia," in: *Macro and Trace Element.*

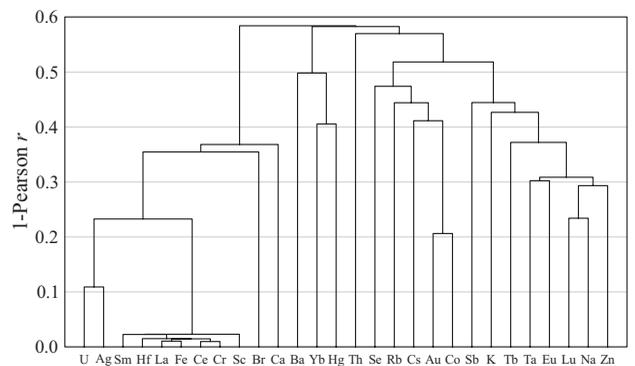


Fig. 3. The dendrogram correlation matrix of the geochemical spectrum of *Rana arvalis* specimens. (1-Pearson $r_{0.5} = 0.5$).

21th Workshop, Friedrich Schiller Univ., Jena, pp. 1266 – 1270.

Bowen N. J. M. (1966), *Trace Elements in Biochemistry*, Acad. Press, London – New York.

Schubert R. (ed.) (1988), *Bioindication of Terrestrial Ecosystems Pollution*, Mir, Moscow [Russian translation].

Saet U. E., Revich B. A., Yanin E. P., et al. (eds.) (1990), *Geochemistry of Environment*, Nedra, Moscow [in Russian].

Leontjeva O. A. (1990), *Anura Amphibia as Bioindices of the Anthropic Alterations in the Moscow District Ecosystems. Author's Abstract of Doctoral Thesis*, Moscow [in Russian].

- Merkulov V. G., Glukhov G. G., and Reschikov V. I.** (1996), "Using of dust and aerosol falls for radiation monitoring of environment," in: *Radioaktivnost' i Radioaktivnye Élementy v Srede Obitaniya Cheloveka [Radioactivity and Radioactive Elements in Environment]*. *Proc. of the Int. Conf., Tomsk, May 22 – 24, 1996*, Izd. TPU, Tomsk, pp. 464 – 467 [in Russian].
- Misyura A. N.** (1985), "The content dynamics of some heavy metals in organs and tissues of lake frog in connection with duration of its presence in sewage," in: *Sovremennye Problemy Éksperimental'noi Biologii i Biotekhnologii [Current Problems of the Experimental Biology and Biotechnology]*, Izd. DGU, Dnepropetrovsk, pp. 111 – 114 [in Russian].
- Misyura A. N., Gasso V. Y., and Marchenkovskaya A. A.** (2003), "Comparative characteristic of the heavy metals body burden in urodele amphibians of Ukraine," in: *Program and Abstrs. of the 12th Ord. Gen. Meet. Soc. Eur. Herpetol.*, Izd. ZIN RAN, St. Petersburg, p. 114.
- Rikhvanov L. P.** (1997), *General and Regional Problems of Radioecology*, Izd. TPU, Tomsk [in Russian].
- Sokolov A. P.** (ed.) (1990), *Reference Book on Geochemical Prospecting of Minerals*, Nedra, Moscow [in Russian].
- Shatilov A. U.** (2001), *Substance Content and Geochemical Characteristics of Dust Atmospheric Falls on Ob' Basin Territory. Author's Abstract of Doctoral Thesis*, Tomsk [in Russian].
- Adam A. M.** (ed.) (1994), *Ecology of Northern Industrial Complex of Tomsk: Problems and Solutions*, Izd. TGU, Tomsk [in Russian].

THE STUDY OF THE ECOLOGICAL NICHE SEGREGATION FOR SYMPATRIC SPECIES OF LIZARDS *Lacerta agilis* AND *Zootoca vivipara*

V. N. Kuranova,¹ S. V. Patrakov,¹ N. A. Bulakhova,¹ and O. A. Krechetova¹

Keywords: Lizards, sympatry, ecological niche, home range.

Sand and viviparous lizards, *Lacerta agilis* and *Zootoca vivipara* belong to Lacertidae family. Both species are widely spread in Palearctic zone. Due to the high number and ability to accumulate a considerable amount of biomass in the forest zone, lizards play an important role in the substance and energy transformation in biocenoses (Gasso, 1987). *L. agilis* and *Z. vivipara* within the bounds of their extensive natural habitat, that includes also the South of Western Siberia are represented both by allopatric and sympatric populations.

The information on the relationship between the sympatric species of reptiles, in particular Lacertidae is of fragmentary character (Strijbosch, 1986; Glandt, 1987). The analysis of the relationship among sympatrics is connected with the concept of “ecological niche” (Ananjeva, 1981; Pianka, 1981; Tuniyev and Beregovaya, 1986; Shenbrot, 1986). The aim of this work is to study the relationship between the sympatric species, *L. agilis* and *Z. vivipara*, in South-West Siberian boreal coniferous forest.

MATERIAL AND METHODS

Researches and gathering of the material were carried out since May up to the end of September 2002 – 2003 in the environs of Tomsk, on two experimental plots (800 m² on the swath and 2100 m² in the pine forest). The first experimental plot was split into 464 squares, the second one into 319 squares (2 × 2 m each), respectively. In each square the numbers of individuals of *L. agilis* and *Z. vivipara* were calculated. Capturing of lizards was done by hands; marking was done by paint from molt to molt and by amputation of digits (Kuranova et al., 1986). Activity was determined by the results of marking and recapturing, chronometry of behavioral acts was also used (Dinesman and Kaletskaya, 1952; Darevsky, 1987). 69 excursions and 8 daily registrations were held. 171 individuals of both sexes and different ages were marked. The analysis of diet ingredients was done on the basis of excrements and using

the method of gastric lavage (Kuranova and Kolbintsev, 1983).

The following indexes were taken for the estimation of the ecological niche occupied by the species:

1. Width of the ecological niche was calculated with help of Simpson index:

$$D = \frac{1}{\sum p_i^2},$$

where p_i is a portion of a resource in the general range of resources used by an individual (Shenbrot, 1986).

2. Degree of overlapping of ecological niche was calculated as a probability of interspecific meetings (Pianka index — C_{ij}):

$$C_{ij} = \frac{\sum p_{ih} p_{jh}}{\sqrt{\sum p_{ih}^2 \times \sum p_{jh}^2}},$$

where p_{ij} and p_{jh} are probabilities of the use of h resource by i and j species, respectively (Shenbrot, 1986).

3. Nutrition spectrum — set of food objects (%), consumed by an individual or a group of individuals (Darevsky, 1987).

Statistical data processing was done according to traditional methods (Rokitsky, 1967; Lakin, 1980), and using of application programs (Excel 7.0, STATISTICA 6.0). The difference between averages was found with the help of a non-parametric criterion of Mann – Whitney (U -test), the degree of correlation in its turn was estimated with the help of Spearman quotient (r_{sp}).

RESULTS AND DISCUSSION

Spatial constituent of the ecological niche. The cohabiting species of lizards differ in the way they use their territory. *L. agilis* has its own home range [an area restricted by the extreme points of the more or less frequent visits of the given individual — for the animals that have

¹ Tomsk State University, pr. Lenina 36, 634050 Tomsk, Russia;
E-mail: zoo@bio.tsu.ru.

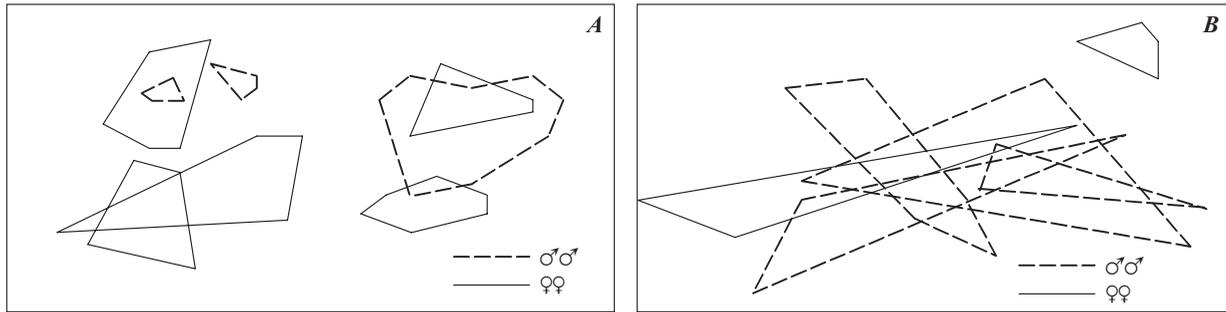


Fig. 1. Individual home ranges of males and females of *Lacerta agilis* (A) and *Zootoca vivipara* (B) (the environs of Tomsk, 2003).

permanent or temporary individual ecotope (Reymers, 1990)], which is conditioned by the strongly expressed territorial behavior typical for this species. Home ranges of adults, especially males, are rarely overlapped (Fig. 1A). Males are more active than females when mating. They can be more frequently met in the overlapping zone of their individual territories with the ones of females (Fig. 1A). These tendencies are marked in the works of different investigators (Dinesman and Kaletskaya, 1952). The average square of an individual home range of *L. agilis* adult males is $133.9 \pm 19.5 \text{ m}^2$ (limit $114.4 - 153.4 \text{ m}^2$), and that of adult females is $56.9 \pm 28.5 \text{ m}^2$ (limit $36.9 - 76.9 \text{ m}^2$), respectively.

The size of home range varies during the whole active season. By the end of July – the beginning of August the length of home range [a maximum length between points of finding the observed individual, which is a relative characteristic (Nerouchev and Kopoustina, 1983)] of adult males decreases to 10 – 15 m. The length of home range of females increases to 3 – 5 m. Females consume much food after laying eggs. An increasing competition exists

between *L. agilis* and *Z. vivipara*. Viviparous lizards compete for food and territory because of their offspring of the current year.

Viviparous lizard tend to live in groups. That affects the whole active period: adults have overlapping home ranges during this period. The size of home range of *Z. vivipara* is difficult to determine because of the lack of recurring marked species. According to the data collected by Pilorge and Xavier (1981) the average size of a male individual home range of *Z. vivipara* is about 540 m^2 . Males are more active in warm days. They can move to 60 m and more. They are sure to come back by the end of the day (Buschinger and Verbeek, 1970). Lizards do not stay within the same home range, the size of which constantly varies (Buschinger and Verbeek, 1970; Pilorge and Xavier, 1981).

The average length of moving (a distance between the first and the second point of meeting) of *Z. vivipara* adult is 1.5 – 2 times more than that of *L. agilis*. It decreases during the active season (Table 1). Decreasing of this average is more typical of *L. agilis* because it possesses its in-

TABLE 1. The Features of Spatial Dispensation of the Sand and Viviparous Lizards, *Lacerta agilis* and *Zootoca vivipara* (the Environs of Tomsk, 2003)

Sex	Age group	Indexes	Length of home range, m		Length of moving, m	
			<i>L. agilis</i>	<i>Z. vivipara</i>	<i>L. agilis</i>	<i>Z. vivipara</i>
Males	Adultus	$\bar{x} \pm m_{sx}$	17.4 ± 8.7	33.0 ± 18.7	11.4 ± 3.6	17.0 ± 9.0
		limit	8.2 – 26.0	14.3 – 51.7	3.2 – 26.0	1.7 – 51.7
		<i>n</i>	5	2	15	10
Females	Adultus	$\bar{x} \pm m_{sx}$	6.0 ± 3.5	12.5 ± 5.1	5.1 ± 2.5	11.3 ± 5.1
		limit	2.0 – 9.0	1.9 – 42.3	2.0 – 9.0	1.9 – 42.3
		<i>n</i>	3	8	11	15
Males	Subadultus	$\bar{x} \pm m_{sx}$	7.5 ± 3.0	8.2 ± 3.7	5.0 ± 1.3	6.1 ± 2.5
		limit	4.0 – 11.0	3.0 – 16.0	1.2 – 11.0	1.6 – 16.0
		<i>n</i>	6	4	14	14
Females	Subadultus	$\bar{x} \pm m_{sx}$	4.0 ± 2.8	4.6 ± 0.6	3.2 ± 1.6	4.1 ± 0.7
		limit	3.0 – 5.0	4.0 – 5.3	2.0 – 5.0	3.0 – 5.3
		<i>n</i>	2	2	7	5

Note. *n*, sample number; limit, limits of values; $\bar{x} \pm m_{sx}$, average and its bias.

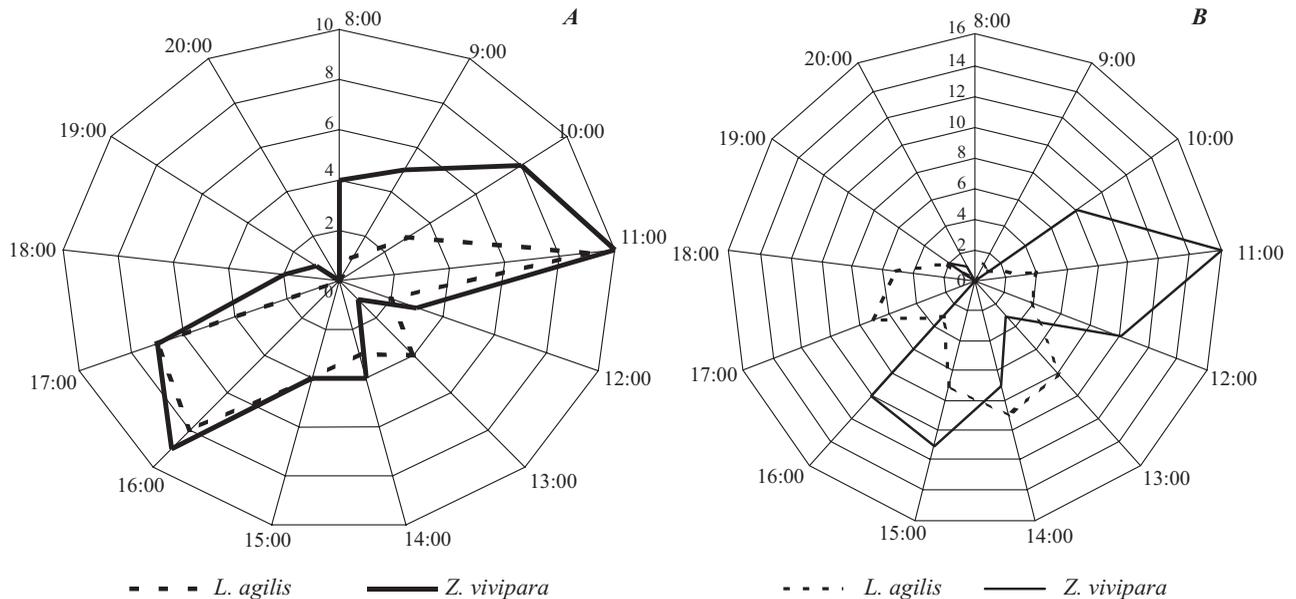


Fig. 2. Chronograms of *Lacerta agilis* and *Zootoca vivipara* activity during the summer time (based on daily census data): **A**, June 20, 2002; **B**, July 27, 2002.

dividual home range and permanent shelters. The difference between the averages of subadults of both species is slight (subadults males $U = 80, p = 0.44$; subadults females $U = 55, p = 0.85$).

The spatial constituent of ecological niche of *L. agilis* in the year 2002 was 1.3 times higher than that of *Z. vivipara*, in the year 2003 the constituent is 1.3 times lower, respectively (Table 2). In 2003 the observations were made on a different experimental plot ($S = 2100 \text{ m}^2$) where microclimatic conditions were better for the viviparous lizard, because there were a lot of shelters under felled trees and fallen leaves. This is supported by other authors' data (Glandt, 1987; Zamolodchikov and Avilova, 1989).

The temporary constituent of ecological niche. Both lizards are active during day time. The largest number of *L. agilis* was noticed on plots under analysis from May till June. *Z. vivipara* in its turn was most active from May till July. The springtime activity is related to the mating season. The number of *Z. vivipara* increased sharply on the experimental plot in 2002 (beginning from July, 17) and in 2003 the increase was related to appearance of the youth of the current year (starting from July, 3). The daily activity of *Z. vivipara* does not change in June – July and has two peaks of intensity — 11 a.m. and 4 p.m. (Fig. 2). Sand lizard has 2 peaks of the most intense activity (11 a.m and 4 p.m.) in June and only 1 in July which shifts to 2 p.m. It reduces the competition between the 2 species as the number of *Z. vivipara* rises considerably because of the youth. By the end of the summer a day active time of

both species shortens, August is the month when the offspring display the highest activity.

The size of *L. agilis* is bigger ($L = 78.8 – 89.9 \text{ mm}$) and it gets warm and cools down slower that is the reason why it becomes active later and remains the same longer. *Z. vivipara* is smaller in size ($L = 48.8 – 72.4 \text{ mm}$), that is why it starts and ceases to be active earlier, this fact is also confirmed by Strijbosch (1986).

TABLE 2. The Characteristics of Ecological Niche of *Lacerta agilis* and *Zootoca vivipara* (the Environs of Tomsk, 2002 – 2003)

Terms of observations	Constituents of ecological niche	The width of constituent of ecological niche, D		The overlapping of constituents, C_{ij}
		<i>Lacerta agilis</i>	<i>Zootoca vivipara</i>	
2002		41.9	31.2	0.16
2003		47.1	62.9	0.36
June 2003	Spatial constituents	39.4	25.8	0.21
July 2003		13.5	55.8	0.15
06/20/2002	Temporary constituents	6.84	8.94	0.85
07/14/2002		3.57	2.67	0.78
07/24/2002		8.40	6.57	0.69
06/25/2003		4.88	4.57	0.59
07/03/2003		1.80	5.35	0.43
08/03/2003		4.48	6.53	0.54
08/24/2003		1.00	6.61	0
2002		Trophic constituents	7.07	6.78

Temperature of air and soil, cloudiness, humidity, and wind influence the character and dynamic of seasonal and daily activity of Lacertidae (Yablokov, 1976; Nuland and Strijbosch, 1981; Kuranova, 1983; Damm et al., 1987). In June – July the fluctuations of temperature are slight, that is why the dependence of lizard activity on the air temperature is minimal. With the cloudiness and humidity rising the activity of the sand lizard goes down ($r_{sp} = -0.65$, $p \leq 0.005$; $r_{sp} = -0.52$, $p \leq 0.005$, respectively) and that of the viviparous lizard goes up ($r_{sp} = 0.65$, $r_{sp} \leq 0.005$; $r_{sp} = 0.52$, $p \leq 0.005$).

The width (D) of the temporary constituent of ecological niche of both species reduces during the activity season: in June the width of the temporary constituent of the sand lizard ecological niche is 1.2 – 1.3 times bigger than that of the viviparous lizard. From July till August the width of the temporary constituent for *Z. vivipara* is bigger than that for *L. agilis* (Fig. 3). The maximal overlapping of temporary constituents typical of sympatriants is observed in June (Table 2), when the peaks of their seasonal and daily activity concur (Fig. 2A).

The competition for food and territory goes down owing to the smaller overlapping (C_{ij}) of temporary niche during the season.

Trophic constituent of ecological niche. The trophic spectrum of *L. agilis* is wider judging by the fact that no representative of Gastropoda class (*Mollusca phylum*) and no adult individual of Lepidoptera (Insecta class, *Arthropoda phylum*) were found in the *Z. vivipara* gaster (Fig. 4). The viviparous lizard shows a certain selective type of eating habits. The width of trophic constituent of ecological niche typical of the sand lizard is not much bigger than the one typical of the viviparous lizard. According to Hutchinson's rule the competition is lessened because of the smaller size of viviparous lizard jaws (big alimental objects consumed by sand lizards are not available) (Giller, 1988).

The maximal overlapping (C_{ij}) 0.85 is equal to the overlapping of temporary constituents of ecological niche, 0.75 is equal to the overlapping of trophic constituents (Strijbosch, 1986). The overlapping of spatial constituents is minimal and is 0.15 to 0.36 (Table 2).

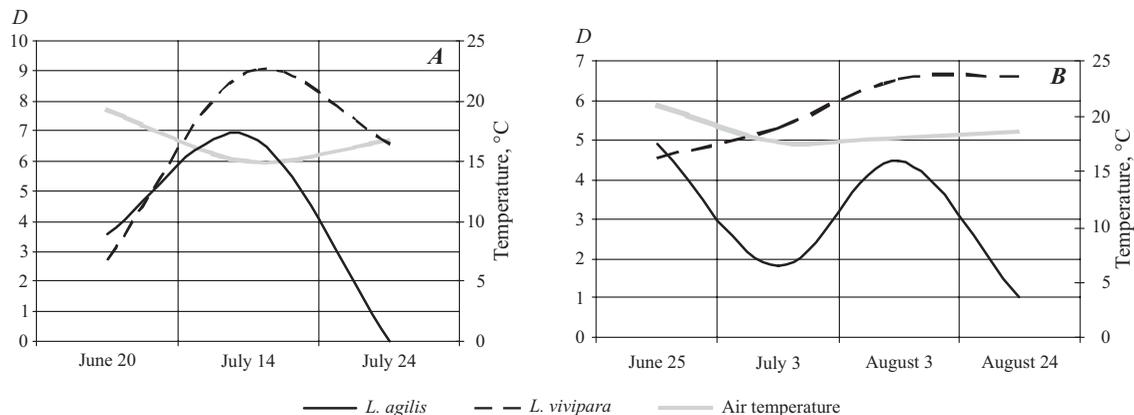


Fig. 3. Seasonal dynamic of width (D) of temporary constituents of *Lacerta agilis* and *Zootoca vivipara* ecological niche: *A*, 2002 year; *B*, 2003 year.

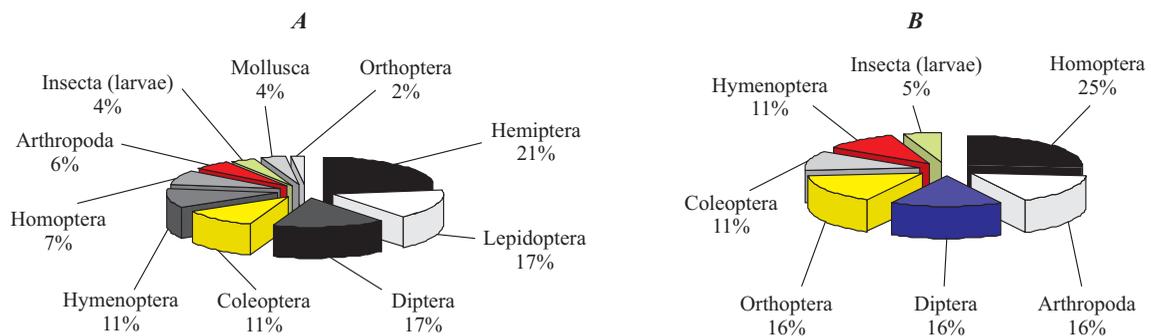


Fig. 4. The ratio of different groups of invertebrates in the diet of *Lacerta agilis* (*A*) and *Zootoca vivipara* (*B*) (the environs of Tomsk, 2002).

To sum up all above-mentioned, differences in body size, strategy of multiplication and territory usage, species peculiarities of daily and seasonal activity, selectivity in the size of alimetal objects contribute to the coexistence and less tense competition of sympatriants *L. agilis* and *Z. vivipara*.

REFERENCES

- Ananjeva N. B.** (1981), "By study of sympatric species of reptiles," in: *Problems of the Newest History of Evolutionary Doctrine*, Nauka, Leningrad, pp. 15 – 22 [in Russian].
- Buschinger A. and Verbeek B.** (1970), "Freiland studien an Ta-182 markierten Bergeidechsen (*Lacerta vivipara*)," *Salamandra*, 6(1/2), 26 – 31.
- Damm R. V., Bauwens D., and Verheyen R. F.** (1987), "Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*," *Herpetologica*, 43(4), 405 – 415.
- Darevsky I. S.** (1987), "Methods of study reptiles in national parks," in: Darevsky I. S. and Krever V. G. (eds.), *Amphibians and Reptiles of the Protected Territories*, Moscow, pp. 25 – 32 [in Russian].
- Dinesman L. G. and Kaletskaya M. L.** (1952), "Methods of quantitative accounts of amphibians and reptiles," in: *Methods of Quantitative Accounts and Geographic Dispensation of Vertebrates*, Vysshaya Shkola, Moscow, pp. 329 – 341 [in Russian].
- Gasso V. Y.** (2001), "Ecological and biochemical peculiarities of *Lacerta agilis* under the conditions of industrial transformation of ecosystems," in: *Problems of Herpetology*, Izd. MGU, Pushchino – Moscow, pp. 69 – 72 [in Russian].
- Giller P. S.** (1988), *Community Structure and the Niche*, Mir, Moscow [Russian translation].
- Glandt D.** (1987), "Substrate choice of the sand lizard," in: *Proc. of the 4th Ord. Gen. Meet. Soc. Eur. Herpetol.*, Nijmegen, pp. 143 – 146.
- Kuranova V. N.** (1983), "Some aspects of activity and behavior of *Lacerta vivipara* in Tomsk region," in: *Ecology of Vertebrates at Siberia*, Izd. TGU, Tomsk, pp. 140 – 148 [in Russian].
- Kuranova V. N. and Kolbintsev V. G.** (1983), "Methods of unblooding study nutrition of snakes," in: *Ecology of Vertebrates at Siberia*, Izd. TGU, Tomsk, pp. 161 – 169 [in Russian].
- Kuranova V. N., Moskvitina N. S., and Suchkova N. G.** (1981), *Methods of Organization Field Practice in Zoology of Vertebrates*, Izd. TGU, Tomsk [in Russian].
- Lakin G. F.** (1980), *Biometry*, Vysshaya Shkola, Moscow [in Russian].
- Nerouchev V. V. and Kopoustina S. F.** (1983), "Behavioral aspects of adaptation in *Phrynocephalus helioscopus* to natural and anthropogenic landscapes," in: *Application Ethology. Proc. of the 3rd All-Union Sci Conf. of Ethology. Vol. 3*, Nauka, Moscow, pp. 115 – 118 [in Russian].
- Nuland G. J. V. and Strijbosch H.** (1981), "Annual rhythmicity of *Lacerta vivipara* Jaquin and *Lacerta agilis agilis* L. (Sauria, Lacertidae) in the Netherlands," *Amphibia-Reptilia*, 8(2), 83 – 95.
- Pianka E. R.** (1981), *Evolutionary Ecology*, Mir, Moscow [Russian translation].
- Pilorge T. and Xavier F.** (1981), "Le lezard *vivipara* (*Lacerta vivipara* J.) dans la region du Puy-de-Dome: Ecologie et strategie de reproduction," *Ann. Stat. Boil. Besse-en-Chandresse*, 15, 32 – 59.
- Reymers N. F.** (1990), *Naturensering (Handbook)*, Mysl', Moscow [in Russian].
- Rokitsky P. F.** (1973), *Biological Statistics*, Minsk [in Russian].
- Shenbrot Yu. G.** (1986), "Niche: methods of study," in: *Methods of Study Ecology and Ethology*, Pushchino, pp. 76 – 93 [in Russian].
- Strijbosch H.** (1986), "Niche segregation in sympatric *Lacerta agilis* and *Lacerta vivipara*," in: *Studies in Herpetology*, pp. 449 – 454.
- Tuniyev B. S. and Beregovaya S. Yu.** (1986), "Sympatric amphibians of the yew-box groove," in: Ananjeva N. B. and Borkin L. J. (eds.), *Systematics and Ecology of Amphibians and Reptiles*, Izd ZIN AN SSSR, Leningrad, pp. 136 – 151 [in Russian].
- Yablokov A. V.** (ed.) (1976), *Sand Lizard*, Nauka, Moscow [in Russian].
- Zamolodchikov D. G. and Avilova K. V.** (1989), "Data in *Lacerta vivipara* at marsh habitats of Western Moscow region," in: *Amphibians and Reptiles of Moscow Region*, Moscow, pp. 140 – 148 [in Russian].

A COMPARATIVE SKELETOCHRONOLOGICAL ANALYSIS OF DEMOGRAPHY OF FOUR AMPHIBIAN SPECIES (ANURA, RANIDAE) FROM IVANOV OBLAST', EUROPEAN RUSSIA

O. Lazareva¹

Keywords: frog, longevity, age of maturity, age structure.

INTRODUCTION

Skeletochronological method of age determination permits obtaining information on growth rates, age at sexual maturity, duration of life, and ratio of different age groups in amphibian populations in the temperate zone. The study of widely distributed species is of particular interest, as the age structures of the populations in different localities can reflect characters of population dynamics. Some of the demographic characteristics (longevity, age of maturity) are effected by the latitude and the climate of the territory (Hemelaar, 1986).

The aims of the present work were as follows: 1) for the first time for species from Ivanovo Oblast' (European Russia) on vast material to establish the basic demographic characteristics of the frogs populations by skeletochronology; 2) to compare demographic data on four species from the same territory in light of their life strategies.

MATERIAL AND METHODS

The study was conducted in Ivanovo Oblast' (European Russia), in 1994 – 2000. Femurs from 1233 individuals of the frogs were sectioned and examined to estimate the age of the frog individuals by the methods of Kleinenberg and Smirina (Kleinenberg and Smirina, 1969; Smirina, 1972). Sections were examined at least three times using a light microscope with phase-contrasting appliance (Shaldybin, 1987). Endosteal resorption was established by the method of comparison of the LAGs (Lines of Arrested Growth) diameters (Hemelaar, 1986).

Frogs were captured in 15 populations, most of them — in 10 populations: 234 individuals of *Rana lessonae*, 384 individuals of *R. ridibunda*, 569 individuals of *R. temporaria*, and 46 individuals of *R. arvalis*.

RESULTS AND DISCUSSION

The skeletochronological method has been used for age determination in a great variety of different amphibian taxa, because it is based on the assumption that layers of

new bone are added annually during the life of the individual and the resting lines reflect hibernations (Smirina, 1972; Acker et al., 1986). Skeletochronological age determination has been conducted in Caudata (Hynobiidae, Salamandridae) and Anura (Discoglossidae, Bufonidae, Hylidae, Leptodactylidae, Ranidae): *Hinobius kimurae* (Mitsawa and Matsui, 1999), *Triturus alpestris* (Guyetant et al., 1995), *T. marmoratus* (Caetano et al., 1985), *Mertensiana luschani* (Leskovar et al., 1998), *Batrachoseps attenuatus* (Wake and Castanet, 1995), *Bombina variegata* (Seidel, 1992; Guarino et al., 1995), *Bufo raddei* (Kuzmin and Ischenko, 1997), *B. americanus* (Acker et al., 1986; Kalb and Zug, 1990), *B. bufo* (Hemelaar and Gelder, 1980; Smirina, 1983; Hemelaar, 1986, 1988), *B. gargarizans* (Lazareva, 1998), *Pseudoacris crucifer* (Lykens and Forester, 1987), *Physalaemus biligonigerus* (Martino et al., 1999), *Rana sylvatica* (Bastien and Leclair, 1992), *R. temporaria* (Smirina, 1972; Ryser, 1988; Esteban, 1990; Guyetant et al., 1995), *R. pipiens* (Leclair and Castanet, 1987), *R. dalmatina* and *R. italica* (Guarino et al., 1995), and other species. Our research is the attempt to apply this method for aging and demographic studies of *Rana lessonae*, *R. ridibunda*, *R. temporaria*, and *R. arvalis* from Ivanovo Oblast' (European Russia).

Endosteal resorption in femur completely destroys only LAG1 in 59 – 63% of adults of *R. temporaria*, 65 – 73% of adults of *R. lessonae*, 23 – 67% of adults of *R. ridibunda* in our study populations. In addition to it LAG2 is destroyed in 11 – 29% of adults of *R. temporaria*, 23 – 35% of adults of *R. lessonae*, 33 – 77% of adults of *R. ridibunda*.

The maximum age of the frogs from Ivanovo Oblast' was found to be 5, 6, 7, 7 years for *R. lessonae* from different localities, 7, 7, 7, 11, 11 (12) years for *R. ridibunda*, 7, 8, 9, 9 years for *R. temporaria*, and 9 years for *R. arvalis*.

Similar data are known for Central Russia. In Moscow Oblast' the females of moor frog (*R. arvalis*) usually are able to reproduce up to the age of 6 years, sometimes up to the age of 9 years (Cherdantsev et al., 1997). In the same region the common frogs (*R. temporaria*) live up to the age of 9 years (Kleinenberg and Smirina, 1969). In the Volga–Kama region the pool frogs (*R. lessonae*) live up to the age of 7 years, and only a few of them, up to 8 – 12 years, the lake frogs (*R. ridibunda*), also up to 7 years,

¹ Department of Zoology, Ivanovo State University, Leninsky Pr. 136, 153002 Ivanovo, Russia; E-mail: herpet.log@mail.ru.

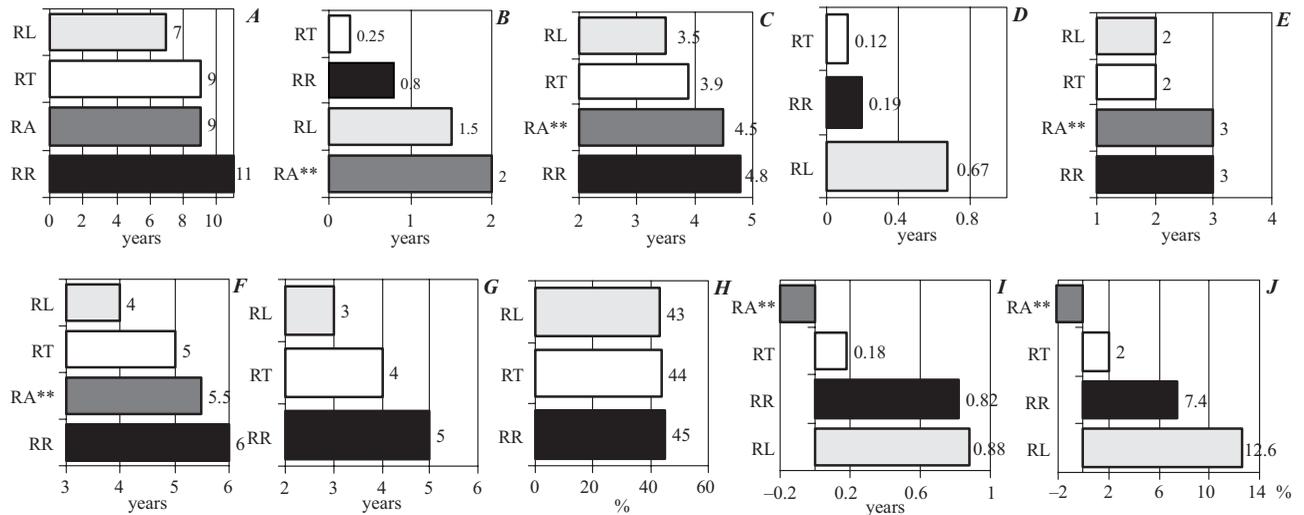


Fig. 1. Summary of the demographic characteristics of four amphibian species (Anura, Ranidae) from Ivanovo Oblast', European Russia. **A**, longevity; **B**, longevity difference between females and males*; **C**, basic age at maturity*; **D**, difference in basic age at maturity between females and males*; **E**, minimal age at maturity; **F**, basic age of reproductive gist of the populations*; **G**, basic section limits of reproductive gist of the populations (absolute)*; **H**, basic section limits of reproductive gist of the populations (in percentage of longevity)*; **I**, absolute difference in mean age of adult females and males*; **J**, difference in mean age of adult females and males (in percentage of longevity)*. *, on the average for all localities of every species; **, data are not enough for the species; RL, *Rana lessonae*; RR, *R. ridibunda*; RT, *R. temporaria*; RA, *R. arvalis*.

sometimes, up to 11 years (Shaldybin, 1976). On the territory with a milder climate (Leningradskaya Oblast') pool frog lives up to the age of 6 years (Borkin and Tikhenko, 1979). In northern and mountain populations frogs live longer: for example, *R. temporaria* from Northern Alps lives up to 15 years (Guyetant et al., 1995).

In the frog populations of Ivanovo Oblast' longevity of females is usually more than that of males: 1–2 years for *R. lessonae* and *R. arvalis*, 1 (0–2) years for *R. ridibunda*, 0–1 years for *R. temporaria*. In one *R. temporaria* population males are older than females.

Longevity of *R. temporaria* decreases in abundant populations or in recreation zones (the town of Ples), or in populations with very low density (Rusino Village, Yuzha Raion). Longevity of *R. lessonae* decreases in urbanized territories (Ivanovo City). Longevity of *R. ridibunda* decreases in urbanized territories (Ivanovo City), in populations with low density (Rusino Village, Yuzha Raion), or under similar conditions with numerous *R. lessonae* (Shuya Raion).

Mean age of adults in various populations is variable, especially in *R. ridibunda* (distinctions reach to 2.9 years for males and 3.3 years for females). In the same populations interannual variations of age structure are more pronounced in *R. ridibunda*. This is likely to be connected with the longer length of life of this species on the whole in comparison with that of the three other species, which results in larger varieties of the types of the population structures.

Because of delayed maturity, adult females are on average older than adult males: in *R. lessonae*, on 0.7–1.0 years, in *R. ridibunda*, on 0.6–1.4 years (except the population from Shuya Raion), in *R. temporaria*, on 0.3–1.1 years in different populations. Females are significantly older than males in 83% of *R. ridibunda* samples, in 60% of *R. lessonae* samples, and only in 33% of *R. temporaria* samples.

R. temporaria attains sexual maturity at the age of 2–5 years, mainly at 3–4 years. In *R. temporaria* populations with longevity of 7 years, the frogs reach sexual maturity earlier (at 3 years) than in the ones with 8–9-year longevity (at 4 years on the average). *R. lessonae* reach sexual maturity at 2–4 years, mainly at 3 or 3–4 years. *R. ridibunda* matures later — at 3–6 years, mainly at 4–5 years.

There were found very variable demography characteristics (especially in *R. ridibunda* populations): 5 (3–6) years for *R. temporaria*, 5–6 years for *R. arvalis*, 4 (3–5) years for *R. lessonae*, and 6 (4–8) years for *R. ridibunda*. It is determined by different size of the generations.

Summing up the results of our investigation we tried to compare the demographic characteristics obtained by the using of the skeletochronology method for four species of *Rana* genus of the same area (Fig. 1). The following characteristics of the life strategies of the species have been found:

1. Species of genus *Rana* with the least longevity (such as *Rana lessonae*) attain sexual maturity earlier and

have maximal sexual dimorphism in age demographic characteristics.

2. Species with the largest longevity (*Rana ridibunda*) is characterized by the largest interpopulation variability of age compositions of adult part of populations.

3. *Rana temporaria* has minimal sexual dimorphism in attainment of sexual maturity.

Acknowledgments. We are thankful to S. Sokolov, M. Gro-mova, S. Euseyeva, T. Shumarina, and N. Sudakova for their assistance in the field and laboratory research.

REFERENCES

- Acker Ph. M., Kruse K. C., and Krehbiel E. B. (1986), "Aging *Bufo americanus* by skeletochronology," *J. Herpetol.*, **20**(4), 570 – 574.
- Bastien H. and Leclair R., Jr. (1992), "Aging wood frogs (*Rana sylvatica*) by skeletochronology," *J. Herpetol.*, **26**(2), 222 – 225.
- Borkin L. J. and Tikhenko N. D. (1979), "Some aspects of morphological variability, color polymorphism, growth, population structure and activity of the pool frog, *Rana lessonae*, at the northern border of its range," in: Ananjeva N. B. and Borkin L. J. (eds.), *Ecology and Systematics of Amphibians and Reptiles*. Vol. 89, Izd. ZIN AN SSSR, Leningrad, pp. 18 – 54 [in Russian].
- Caetano M. H., Castanet J., and Francillon H. (1985), "Détermination de l'âge de *Triturus marmoratus marmoratus* (Latreille 1800) du Parc National de Peneda Gerês (Portugal) par squeletochronologie," *Amphibia-Reptilia*, **6**, 117 – 132.
- Castanet J. and Smirina E. (1990), "Introduction to the skeletochronological method in amphibians and reptiles," *Ann. Sci. Nat. Zool. Paris. Ser. 13^{eme}*, **11**, 191 – 196.
- Cherdantsev V. G., Lyapkov S. M., and Cherdantseva E. M. (1997), "Mechanisms of forming fecundity in *Rana arvalis*," *Zool. Zh.*, **76**(2): 187 – 198 [in Russian].
- Esteban M. (1990), "Environmental influences on the skeletochronological record among recent and fossil frogs," *Ann. Sci. Nat. Zool. Paris. Ser. 13^{eme}*, **11**, 201 – 204.
- Guarino F. M., Angelini F., and Cammarota M. (1995), "A skeletochronological analysis of three syntopic amphibian species from Southern Italy," *Amphibia-Reptilia*, **16**, 297 – 302.
- Guyetant R., Miaud C., and Humbert A. (1995), "Caractéristiques démographiques des populations d'amphibiens en altitude: exemple des structures d'âges *Tritons alpestris* (*Triturus alpestris* Laur.) et des Grenouilles rousses (*Rana temporaria* L.) dans le parc National de la Vanoise (Alpes du Nord)," *Bull. Soc. Ecophysiol.*, **XX**(1 – 2), 47 – 51.
- Hemelaar A. S. M. (1986), *Demographic Study on Bufo bufo L. (Anura, Amphibia) from Different Climates by Means of Skeletochronology. Thesis*, Univ. of Nijmegen.
- Hemelaar A. S. M. (1988), "Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes," *J. Herpetol.*, **22**(4), 369 – 388.
- Hemelaar A. S. M. and van Gelder J. J. (1980), "Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination," *Netherlands J. Zool.*, **30** (1), 129 – 135.
- Kalb H. J. and Zug G. R. (1990), "Age estimates for a population of American toads, *Bufo americanus* (Salientia: Bufonidae), in northern Virginia," *Brimleyana*, **16**, 79 – 86.
- Kleinenberg S. E. and Smirina E. M. (1969), "A contribution to the method of age determination in amphibians," *Zool. Zh.*, **48**, 1090 – 1094 [in Russian].
- Kuzmin S. L. and Ischenko V. G. (1997), "Skeletochronology of *Bufo raddei* from the Gobi Desert," *J. Herpetol.*, **31**(2), 306 – 309.
- Lazareva O. G. (1998), "Life strategy of *Bufo gargarizans* from the north-eastern part of its area (Khabarovsk Region, Russian Far East)," in: *Proc. of the Third Asian Herpetol. Meeting, Almaty, September 1 – 5, 1998*. Almaty, p. 26.
- Leclair R., Jr., and Castanet J. (1987), "A skeletochronological assessment of age and growth in the frog *Rana pipiens* Schreber (Amphibia, Anura) from south-western Quebec," *Copeia*, **36**, 361 – 369.
- Leskovar C., Veith M., and Sinsch U. (1998), "Age structure and growth of two Turkish salamander subspecies (*Mertensiana luschani atifi*, *M. l. billae*)," in: *Proc. of the 9th Ord. Gen. Meeting of Soc. Eur. Herpetol., Chambezy (France), August 25 – 29, 1998*, Univ. Savoie.
- Lykens D. V. and Forester D. C. (1987), "Age structure in the spring peeper: Do males advertise longevity?," *Herpetologica*, **43**(2), 216 – 223.
- Martino A. L., Sinsch U., and Di Tada I. E. (1999), "Age structure, sex distribution, mating call and food diversity of the frog *Physalaemus biligonigerus* (Cope, 1860) (Anura: Leptodactylidae), Cordoba — Argentina," in: *Proc. of the 10th Ord. Gen. Meeting of Soc. Eur. Herpetol., Irakleio (Greece), September 6 – 10, 1999*, pp. 102 – 103.
- Misawa J. and Matsui M. (1999), "Age determination by skeletochronology of the Japanese Salamander *Hinobius kimurae* (Amphibia, Urodela)," *Zool. Sci.*, **16**, 845 – 851.
- Ryser J. (1988), "Determination of growth and maturation in the common frog, *Rana temporaria*, by skeletochronology," *J. Zool. London*, **216**, 673 – 685.
- Seidel B. (1992), "Age structure in a yellow-bellied toad population," in: *Proc. of the 6th Ord. Gen. Meeting of Soc. Eur. Herpetol., Budapest, August 19 – 23, 1991*, pp. 403 – 408.
- Shaldybin S. L. (1976), "Age and sex structure of populations of anurans," *Privod. Zapoved. Volgo-Kamskogo Bass.*, **4**, 112 – 117 [in Russian].
- Shaldybin S. L. (1987), "Age structure of populations and methods of age determination of anurans," in: Darevsky I. S. and Krever V. G. (eds.), *Amphibians and Reptiles of Protected Territories*, Izd. TsNIL Glavokhota RSFSR, Moscow, pp. 33 – 35 [in Russian].
- Smirina E. M. (1972), "Annual layers in bones of *Rana temporaria*," *Zool. Zh.*, **51**, 1529 – 1534 [in Russian].
- Smirina E. M. (1983), "Age determination and retrospective body size evaluation in the live common toad, *Bufo bufo*," *Zool. Zh.*, **62**(3), 437 – 444 [in Russian].
- Wake D. B. and Castanet J. (1995), "A skeletochronological study of growth and age in relation to adult size in *Batrachoseps attenuatus*," *J. Herpetol.*, **29**(1): 60 – 65.

ENVIRONMENT AND BODY TEMPERATURES OF REPTILES IN VOLGA–URAL REGION

N. Litvinov¹ and S. Ganshchuk¹

Keywords: body temperature, thermopreferenda, cardiac electrical activity.

INTRODUCTION

A number of studies have been dedicated to the thermoregulatory mechanisms of ectotherms (Sergeyev, 1939; Chernomordikov, 1943; Khozatsky, 1959; Pianka, 1975; Hutchison, 1976; Hutchison and Maness, 1979; Bartholomew, 1982; Huey, 1982; Cherlin, 1983; Cherlin and Muzychenko, 1988; Cherlin and Chikin, 1991; Hutchison and Dupre, 1992; Du Wei-Guo et al., 2000; Blumberg et al., 2002; Sartorius et al., 2002; Franklin and Seebacher, 2003).

The majority of ectotherms, when taken in the same temperature ranges, warm up more rapidly than cool down. This so-called thermal hysteresis is usually connected with the changes in bodily thermal conductivity, resulting in blood vessel diameter change (Bartholomew et al., 1965; Myhre and Hammel, 1969). Some amphibians and reptiles thermoregulate by evaporation. These thermoregulatory reactions, as well as the behavioral ones, are affected both by environment temperatures and brain stem and core temperature (Crawford and Barber, 1974). The rate of heat exchange with the environment is under neural control in reptiles. The rate of the reptile-environment heat exchange is neurally controlled due to cardiac output changes and blood redistribution. Thermoregulatory behavior helps reptiles to avoid overheating, though under some circumstances they may be subject to subcritical temperatures. In such cases the thermal tolerance is limited by functional capabilities of the cardiovascular system and its regulatory mechanisms.

We studied the changes of the electric cardiac activity as reaction to extreme temperature influence.

MATERIAL AND METHODS

The environment, body temperatures and their ratios have been studied for 11 species of snakes and lizards (*Anguis fragilis*, *Eremias arguta*, *Lacerta agilis*, *Zootoca vivipara*, *Natrix natrix*, *N. tessellata*, *Elaphe dione*, *Coronella austriaca*, *Vipera berus*, *V. nikolskii*, *V. renardi*). The tem-

peratures were taken with sensor thermistors at the eight body spots for lizards: the top and the bottom of a head, back and belly, the top and the bottom of a tail in the mean (external temperatures), esophagus and anus (internal temperatures). For snakes the temperatures at the top and the bottom of a head, back and belly, esophagus and anus were taken. The topography of body temperatures at different environment temperatures was studied.

Sometimes we were measuring the heat flux density (irradiance) (W/m^2) of the solar incident radiation and of that emitted from the surface, rather than the temperature. The special spiral heat flux sensor was used in the measurements. This particular sensor was selected due to its favorable operating characteristics such as high sensitivity and tiny dimensions that made it possible to carry out localized measurements. Environment temperature measurements have been correlated with the duration of daily activity. First of all, we should remark that we were terming esophagus temperature as the “body temperature.” For the vast majority of cases this temperature is somewhat higher than the anal temperature (Table 1). In this paper we are using some widely accepted terms as the body and environment temperature optima, the maximum and minimum voluntary activity temperatures and the temperature range of activity. The correlation ratio (h) has been derived in order to reveal the degree of dependence between the body and the surface temperatures, as well as for the surface air (3 – 5 cm above the surface) temperatures. These data are summarized for the snakes in Table 1.

Cardiac activity was registered with needle electrode of a single channel portable cardiograph with heat recording. The grass-snakes were cooled down before recording: *Natrix tessellata* ($n = 12$) down to $6.0^\circ C$ in esophagus, *Natrix natrix* ($n = 14$) down to $-1.0^\circ C$; followed by gradual warm-up to $38.0^\circ C$ in esophagus.

RESULTS AND DISCUSSION

As long as reptiles actively pursue their optimal temperatures throughout the season, we found it possible to

¹ Perm State Pedagogical University, Sibirskaya str. 32-39, 614000 Perm, Russia; E-mail: Ganshchuk@mail.ru.

TABLE 1. Basic Temperature Indices of Snakes for the 1999 – 2002 Activity Season

Basic temperature indices	Species and their origin								
	<i>Natrix natrix</i> , Ural Foothills	<i>N. natrix</i> , Mid-Volga	<i>N. tessellata</i> , Mid-Volga	<i>Elaphe dione</i> , Mid- and Lower Volga	<i>Coronella austriaca</i> , Ural Foothills and Mid-Volga	<i>Vipera berus</i> (bright morph), Ural Foothills	<i>V. berus</i> (black morph), Ural Foothills	<i>V. nikolskii</i> , Mid-Volga	<i>V. renardi</i> , Mid- and Lower Volga
Mean body temperature and sample volume	25.2 ± 0.19 n = 322	25.3 ± 0.66 n = 45	25.9 ± 0.37 n = 94	29.3 ± 0.09 n = 16	27.9 ± 2.13 n = 4	27.7 ± 0.57 n = 88	23.9 ± 1.02 n = 23	28.9 ± 0.45 n = 40	29.7 ± 0.95 n = 18
Mean external body temperatures, back/belly	21.8 ± 0.23	22.0 ± 0.54	23.0 ± 0.34	24.9 ± 1.36	23.8 ± 2.23	23.2 ± 0.60	20.8 ± 1.04	23.6 ± 0.84	22.2 ± 2.49
Mean environment temperatures, land surface air 5 cm/surface	21.0 ± 0.23	21.9 ± 0.52	23.2 ± 0.32	25.2 ± 1.51	23.1 ± 1.30	23.2 ± 0.57	20.8 ± 1.09	23.3 ± 0.89	21.1 ± 2.63
	21.1 ± 0.31	20.9 ± 0.59	23.3 ± 0.46	32.6 ± 2.12	21.1 ± 2.70	22.9 ± 1.31	—	22.5 ± 0.67	26.9 ± 2.96
	24.4 ± 0.28	21.5 ± 0.61	25.6 ± 0.65	33.3 ± 2.45	20.7 ± 0.67	25.2 ± 0.79	19.6 ± 1.24	22.7 ± 0.63	27.7 ± 2.18
Max and min voluntary body temperatures	13.6 – 33.2	16.0 – 32.4	14.8 – 33.2	22.1 – 32.9	24.7 – 31.1	6.2 – 34.3	12.7 – 29.7	21.9 – 34.0	21.3 – 35.8
Max and min voluntary external temperatures, back/belly	8.2 – 32.2	16.0 – 33.4	14.8 – 33.0	19.8 – 30.3	20.5 – 27.1	5.2 – 31.2	11.6 – 27.5	15.5 – 30.2	11.0 – 30.7
	9.2 – 32.9	16.0 – 32.1	15.1 – 32.2	19.3 – 30.1	21.0 – 25.2	5.4 – 31.8	12.2 – 27.3	15.1 – 32.3	10.3 – 29.4
Max and min voluntary external temperatures, land surface air 5 cm/surface	13.5 – 35.3	13.9 – 31.6	14.0 – 38.6	24.2 – 43.2	21.1 – 29.0	14.1 – 32.3	—	14.1 – 27.6	14.2 – 43.3
	14.2 – 40.0	14.2 – 34.3	15.8 – 40.7	23.6 – 45.6	20.4 – 21.0	3.7 – 39.9	6.6 – 28.7	15.1 – 31.4	14.5 – 44.8
Body temperature optimum	21.7 – 29.5	24.3 – 29.7	24.7 – 30.1	27.9 – 31.4	—	26.7 – 32.0	22.4 – 29.4	26.3 – 31.0	28.6 – 34.5
External temperatures optimum, land surface air 5 cm/surface	18.2 – 22.3	18.3 – 24.5	20.7 – 27.5	—	—	20.6 – 26.3	—	20.3 – 26.0	—
	20.0 – 28.9	17.3 – 26.0	20.8 – 28.5	30.7 – 32.9	—	22.5 – 33.2	12.9 – 26.3	20.8 – 28.9	23.9 – 39.3
Temperature range of activity by body temperature	19.6	16.4	18.4	10.8	—	28.1	17.0	12.1	14.5
Temperature range of activity by external temperatures, land surface air 5 cm/surface	21.8	17.7	24.6	19.0	—	18.2	—	13.5	29.1
	25.8	20.1	24.9	22.0	—	36.2	22.1	16.3	30.3
Degree of dependence between body and surface temperatures, η	0.48 ± 0.04	0.72 ± 0.07	0.82 ± 0.03	0.75 ± 0.13	—	0.84 ± 0.03	0.99 ± 0.004	0.62 ± 0.09	0.91 ± 0.04
Degree of dependence between body and land surface air (5 cm) temperatures, η	0.46 ± 0.08	0.93 ± 0.02	0.78 ± 0.04	0.86 ± 0.08	—	0.76 ± 0.12	—	0.71 ± 0.11	1.00

introduce the “mean body temperature.” For both species of grass snakes, originating from different locations these temperatures are surprisingly close. *Vipera renardi* and *Elaphe dione* were found to have the highest mean body temperature, the fact that can be rationalized by taking into account their adaptation to relatively high surface temperatures, 33.7 and 27.7°C, respectively. *V. renardi* has the highest maximum voluntary body temperature. All the studied snakes have this maximum within 29.7 – 35.8°C. The minimum voluntary range is almost three times as wide: from 6.2°C for *Vipera berus* (bright morph) to 22.1°C for *Elaphe dione*. The bright morph of *Vipera berus* has the widest temperature range of activity, 28.1°C. The other vipers have substantially narrower values: 17.0°C for *V. berus* (black morph), 12.1° for *V. nikolskii*, and 14.5° for *V. renardi*. The dependence of body temperatures on environment temperatures (surface and surface air) is high for all the snakes, but *N. natrix* of Ural Foothills. Its relatively low correlation is not quite clear. *Eremias arguta* is the most thermophilic among the studied lizards, having its surface temperature optimum within 41.5 – 46.0°C and body temperature optimum within 31.3 – 33.0°C. *Lacerta agilis* is less thermophilic with surface temperature optimum is 28.4 – 45.3°C and body temperature optimum is 29.6 – 33.9°C. The least thermophilic are *Anguis fragilis* and *Zootoca vivipara*. The surface temperature optima are 18.1 – 22.5°C for the former and 20.6 – 28.7°C for the latter, while the body optima are 20.6 – 26.7 and 26.4 – 32.5°C, respectively. It is apparent that body temperature exceeds surface temperature until a certain point where they become equal, henceforth this ratio becomes reversed. Why? Here the body temperature regulatory mechanisms come into effect. The body temperature overcoming the optimum, the animal lowers its temperature escaping to a shady place or a shelter. It is behavioral mechanism. If there is a situation, rare (if possible) in nature, but quite possible in the experiment, when the animal cannot escape, there comes into effect physiological mechanism, evaporating moisture from the mouth mucosa. All the animals in our experiment were caught being out of their shelters. If these described regulatory mechanisms are not effective enough, then escape into a shelter follows. Thus for *Natrix tessellata* ($n = 45$) of Middle Volga this point of overheating protection lies about 27.5°C (ground temperature), for *Lacerta agilis* ($n = 67$), about 32.0°C. Diurnal activity for the lizards in summer is definitely two-peaked.

As for the aforementioned topography of temperature, the esophagus temperatures of both snakes and lizards exceed the anal temperatures. For *Natrix natrix* ($n = 227$) by 3.7°C, for *N. tessellata* by 2.0°C, for *Vipera berus*

(bright morph) ($n = 86$) and *V. nikolskii* ($n = 40$) equally by 2.7°C; for *V. berus* black morph ($n = 23$) by 3.0°C; *V. renardi* ($n = 18$) by 2.8°C; *Elaphe dione* ($n = 16$) by 1.0°C; *Zootoca vivipara* ($n = 71$) by 1.6°C; and for *Lacerta agilis* ($n = 89$) by 0.6°C. The topography of body temperature has been most consistently studied for *L. agilis* of Middle Volga ($n = 82$): back, belly and throat region temperatures are equal, 26.4 – 26.5°C. Top-head temperature is lower by approximately 1.0°C. These temperatures have been obtained at 26.5 – 31.8°C of environment temperature, corresponding to the pinnacle of activity. An attempt to determine the difference in the mean temperatures between males ($n = 19$) and females ($n = 39$) of Ural foothills origin has shown male’s back temperature (30.2°C) being significantly higher ($P < 0.01\%$), than that for the females (27.6°C) by 2.6°C during spring-summer season. Belly temperature for the males (29.8°C) is significantly higher ($P < 0.05\%$) by 2.1°C than that for the females (27.7°C). The difference between the temperatures of the other body spots is statistically insignificant for such a small sample.

At low body temperature -1.0°C the heartbeat rate reduces to 3 bpm, all cardiogram indices being incredibly extended and elongating. This points to the slow ventricular impulse conduction and its repolarizatory elongation. It may be assumed that there is an atrioventricular node impulse delay. As the internal body temperature approaches its optimum (24.7 – 30.1°C), all cardiogram indices normalize. Heartbeat rate is from 48 – 68 bpm PR interval 0.20 sec, average QRS duration 0.06 sec, QT interval 0.43 sec. At high body temperatures (38.0°C), heartbeat rate increases up to 167 bpm All intervals are shortened, indicating premature ventricular excitation.

REFERENCES

- Bartholomew G. A., Tucker V. A., and Lee A. K. (1965), “Oxygen consumption, thermal conductance and heart rate in the Australian scink *Tiliqua scincoides*,” *Copeia*, **1965**, 169 – 173.
- Bartholomew G. A. (1982), “Physiological control of body temperature,” *Biol. Reptilia. Physiol. C. London*, **12**, 167 – 211.
- Blumberg M. S., Lewis S. J., and Sokoloff G. (2002), “Incubation temperature modulates post-hatching thermoregulatory behavior in the Madagascar ground gecko, *Peroedura pictus*,” *J. Exp. Biol.*, **205**(18), 2777 – 2784.
- Cherlin V. A. (1983), “The mechanisms of reptile adaptations to the thermal conditions of environment,” *J. Gen. Biol.*, **6**, 753 – 764.
- Cherlin V. A. and Muzychenko I. V. (1988), “The seasonal variability of thermobiological indices of some arid lizards,” *Zool. J.*, **67**, 406 – 416.

- Cherlin V. A. and Chikin Yu. A.** (1991), "The thermal biology of lizards of the mountain regions of Uzbekistan," *Gerpetol. Issled.*, No. 1, 119 – 129 [in Russian].
- Chernomordikov V. V.** (1943), "On temperature reactions of reptiles," *Zool. Zh.*, **22**, 274 – 279 [in Russian].
- Crowford E. C. and Barber B. J.** (1974), "Effects of core, skin and brain temperature on panting in the lizard *Sauromalus obesus*," *Am. J. Physiol.*, **226**, 569 – 573.
- Du Wei-Guo, Yan Shui-Juan, and Ji Xiang** (2000), "Selected body temperature, thermal tolerance and thermal dependence of food assimilation and locomotor performance in adult blue-tailed skinks, *Eumeces elegans*," *J. Therm. Biol.*, **25**(3), 197 – 202.
- Franklin C. E. and Seebacher F.** (2003), "The effect of heat transfer mode on heart rate responses and hysteresis during heating and cooling in the estuarine crocodile *Crocodylus porosus*," *J. Exp. Biol.*, **206**(7), 1143 – 1151.
- Huey R. B.** (1982), "Temperature, physiology, and the ecology of reptiles," *Biol. Reptilia. Physiol. C. London*, **12**, 25 – 91.
- Hutchison V. H.** (1976), "Factors influencing thermal tolerances of individual organisms," in: Esch G. W. and McFarlane R. W. (eds.), *Thermal Ecology*, U.S. Natl. Tech. Inf. Serv., Oak Ridge (TN), pp. 10 – 26.
- Hutchison V. H. and Maness J. D.** (1979), "The role of behavior in temperature acclimation and tolerance in ectotherms," *Am. Zool.*, **19**, 367 – 384.
- Hutchison V. H. and Dupre R. K.** (1992), "Thermoregulation," in: Feder M. E. and Burggren W. W. (eds.), *Environmental Physiology of the Amphibians*, Univ. of Chicago Press, Chicago, pp. 206 – 249.
- Khozatsky L. I.** (1959), "The body surface temperature of some amphibians and reptiles," *Byull. LGU*, **21**, 92 – 105 [in Russian].
- Morgareidge K. R. and Hammel H. T.** (1975), "Evaporative water loss in box turtles. Effects of rostral brainstem and other temperatures," *Science*, **187**, 366 – 368.
- Myhre K. and Hemmel H. T.** (1969), "Behavioral regulation of internal temperature in the lizard *Tiliqua scincoides*," *Am. J. Physiol.*, **217**, 1490 – 1495.
- Pianka E. R.** (1975), "Niche relations of desert lizards," in: Cody M. and Diamond J. (eds.), *Ecology and Evolution of Communities*, Harvard Univ. Press, Cambridge, pp. 292 – 314.
- Sartorius S. S., do Amaral J. P. S., Durtsche R. D., Deen C. M., and Lutterschmidt W. I.** (2002), "Thermoregulatory accuracy, precision and effectiveness in two sand-dwelling lizards under mild environmental conditions," *Can. J. Zool.*, **80**, 1966 – 1976.
- Sergeyev A.** (1939), "The temperature of reptiles in nature," *Dokl. AN SSSR*, **22**, 49 – 52 [in Russian].

CONSERVATION AND RECOVERY OF RARE AMPHIBIAN SPECIES OF EUROPEAN RUSSIA: DEVELOPMENT OF BASIC PRINCIPLES AND EFFECTIVE PRACTICAL MEASURES

S. M. Lyapkov¹

Keywords: population recovery, life-history characteristics, egg and tadpole translocation, *Bufo viridis*.

INTRODUCTION

A new approach is proposed to increase population stability and to recover of populations of rare amphibian species in the central European Russia. The development of general principles and effective practical measures should be based on the data of intraspecific (geographical, inter- and intrapopulation) variation of life-history and demographic characteristics. Protected areas should be selected for testing the effectiveness of these measures, since the negative anthropogenic impact is minimal there and hence the natural factors (primarily the density) play an important role in regulation or limitation of population size.

The selected ponds will be populated with eggs or larvae of rare species. The effectiveness of the proposed practical measures is dependent of the improvement of premetamorphic conditions and thereby — “the quality” of metamorphs leaving the ponds, i.e., an increase in their body size and a decrease in time of premetamorphic development. It is generally accepted that the metamorphs, characterized by a larger body size and a lower time of premetamorphic development, can mature at an earlier age and will therefore substantially increase the net rate of reproduction of a given generation and hence of a whole population. It is important to consider the geographical variation: individuals that mature earlier in southern populations possess the higher reproductive potential that is also important for population recovery. The use of local translocations for enhancing of metamorphs’ quality and net rate of reproduction seems to be quite original (for a review see Seigel and Dodd, 2002; Marsh and Trenham, 2001). My first two years with field experiments on the translocation of *Bufo bufo* clutches to create a new local population was successful (Lyapkov, 2003; unpublished data). The aim of this study was to confirm the effectiveness of translocations of rare species larvae to natural ponds.

MATERIAL AND METHODS

1. Translocation of *Bufo viridis* and *Pelobates fuscus* Tadpoles to a New Pond in the Moscow Oblast’.

The habitat conditions of sympatric and partially syntopic populations of *B. viridis* and *P. fuscus* in the Moscow Oblast’ (near Zvenigorod Biological Station, about 55 km westward of Moscow) were substantially deteriorated in early spring of 2003. The cause was an extensive exploitation of sandpit started on the bank-side of the pond represented the main breeding site of *B. viridis*. The pond itself was not destroyed or polluted, but the access roads to the sandpit crossed the routes of dispersing metamorphs and the traffic by trucks was very heavy. For this reason it was decided to translocate a part of the tadpole population. An artificial well-warmed (not-shaded) pond was chosen as a new (experimental) pond. The size of the pond was about 200 × 60 m, i.e., its area was two times smaller than this of the native pond. The distance between these two ponds was about 1 km. The anthropogenic impact (non-intensive grazing only) on the banks of the experimental pond was substantially weaker. The preliminary survey in May 2003 revealed the presence of only two species, *B. bufo* and *Rana arvalis*, and their total density was about by a factor of 10² as low as in the native pond. In the native pond, besides predominating tadpoles of *B. viridis*, the larvae of *B. bufo*, *R. temporaria*, and *R. arvalis* were recorded. From 13 to 23 June, 23,430 *B. viridis* tadpoles were translocated to experimental pond. In addition, 13,400 *P. fuscus* tadpoles were translocated to that pond from May 18 to June 24. These *P. fuscus* tadpoles populated another pond (about 60 × 50 m) at the same site. Three samples of *B. viridis* tadpoles, three samples of *B. viridis* metamorphs, and six samples of *P. fuscus* tadpoles from the experimental and native ponds were measured (minimal sample size 50). The stages of larval development were determined according to the tables (Dabagyan and Sleptsova, 1975).

2. Study of larval growth and development in *B. viridis* and *P. fuscus* in the Bryansk Oblast’.

B. viridis metamorphs were also collected June 12 and 26, 2003, in the

¹ Biological Evolution Department, Biological Faculty, Moscow State University, Leninskie Gory 1/12, 119899 Moscow, Russia;
E-mail: lyapkov@mail.ru.

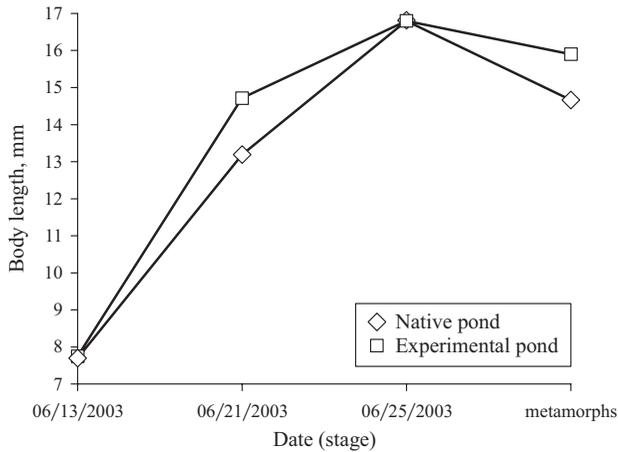


Fig. 1. Tadpole growth and development in *Bufo viridis*, Moscow Oblast'. Tadpoles collected on June 21 and metamorphs from the experimental pond were significantly ($p < 0.01$) larger than those from the native pond.

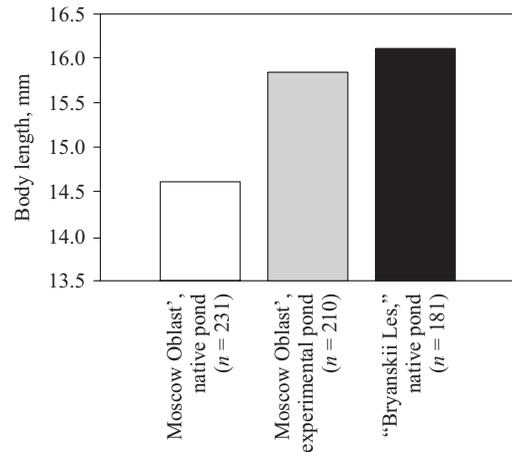


Fig. 3. Average body length of *Bufo viridis* metamorphs.

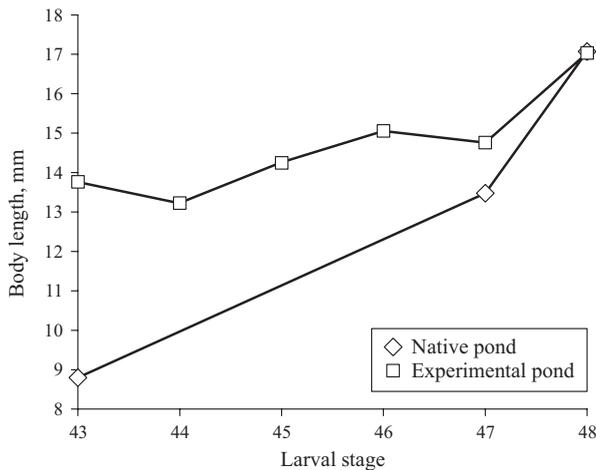


Fig. 2. Tadpole growth rate in *Bufo viridis*, Moscow Oblast'.

vicinity of the "Bryanskii Les" State Nature Reserve (southeast Bryansk Oblast'), on the banks of a relatively large (about 150×350 m) artificial village pond. Data on *P. fuscus* tadpole growth and development were obtained from samples collected from June 7 to July 1, 2003, in five small and partially drying ponds of the "Bryanskii Les" Reserve.

For the estimation the variance components of body length of *Pelobates fuscus* tadpoles the hierarchical design of three-way ANOVA was used ("stage" was nested in "pond" and "pond" was nested in "locality").

RESULTS AND DISCUSSION

1. Translocation of *B. viridis* tadpoles. Tadpoles collected in the experimental pond were larger than those in the native pond (Fig. 1) in 2 samples (including metamorphs) and at most larval stages (Fig. 2). The main cause of these differences is the substantially lower density in the experimental pond: according to visual surveys, the proportion of translocated *B. viridis* tadpoles was at maximum 5% of their numbers in the native pond, hence tadpole density in the native pond was 10 times higher than in the experimental one (because its area was twice smaller than this of the native pond). In both ponds the metamorphosis began at the same time; the modal time of premetamorphic development was 60 days. But metamorphs leaving the experimental pond were significantly larger than those from the native pond (Fig. 3). From July 13 to July 23, 10,000 *B. viridis* metamorphs were collected at the banks of the experimental pond, marked by group-mark and released. In addition, 5215 metamorphs were collected at the bank of the native pond, also group-marked and released near the experimental pond. These marks will enable to determine the origin of these toads in the future, when breeding adults are found. The tadpole survivorship until metamorphosis in the experimental pond was at least 43% ($10,000/23,430$), which represents the maximal value in comparison with literature data on European *Bufo* species (for a review, see Reading and Clarke, 1999).

2. Translocation of *P. fuscus* tadpoles. According to a census on May 31, the number of *P. fuscus* tadpoles of earlier stages in the native pond was at least 90000 individuals with a density of 30 tadpoles per 1 m^2 . In the na-

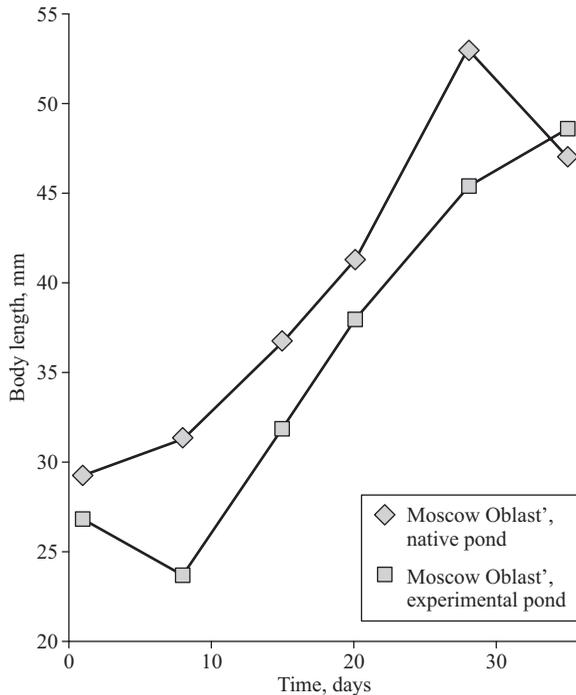


Fig. 4. Tadpole growth rate in *Pelobates fuscus*, Moscow Oblast'.

tive pond, *P. fuscus* tadpoles grew somewhat faster than in the experimental pond but at the end of the larval period tadpole the body lengths of these two groups were equal (Fig. 4).

3. Geographical variation of metamorph size in *B. viridis*. In the Bryansk Oblast', the premetamorphic development completed earlier (modal developmental time 45 days) than in both ponds of the Moscow Oblast'. This difference was due to warmer climatic conditions in the Bryansk Oblast'. In addition, the mean metamorph size was significantly larger (Fig. 3), apparently corresponding with the lower larval density.

4. Geographical variation of larval growth and development in *P. fuscus*. The data on tadpole growth and development in *P. fuscus* in several ponds of "Bryanskii Les" Nature Reserve were compared with corresponding data from the Moscow Oblast' (Fig. 5). The significantly larger mean values of body length for stages 43 – 48 in the population from the Moscow Oblast' (the influence of "locality" was highly significant according to two-way ANOVA results: $F = 68.0$; $d.f. = 1$; $p < 0.00001$), despite of substantially higher density revealed in this population, were unexpected results. The so-called "capacity factor" can be a possible explanation: in Moscow Oblast', both ponds were substantially larger than small and partially drying ponds in "Bryanskii Les."

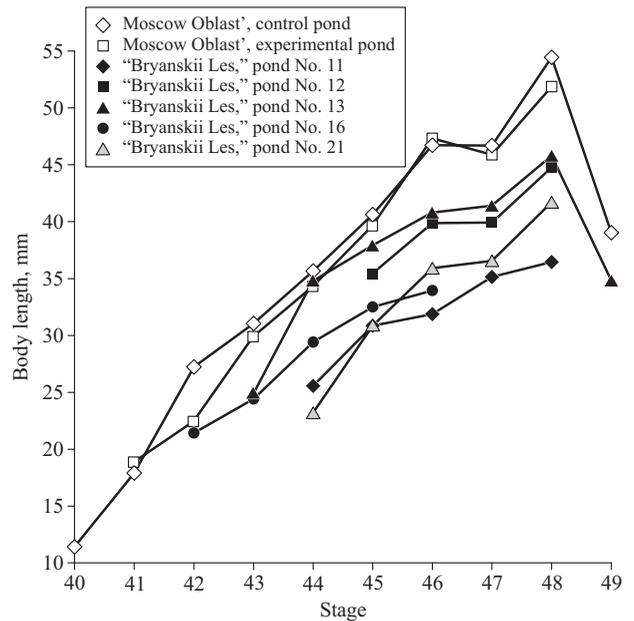
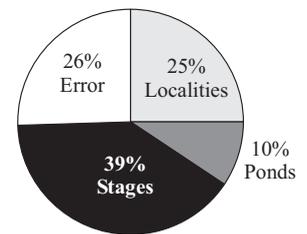


Fig. 5. Geographical and local variation of tadpole growth and development in *Pelobates fuscus*.

Fig. 6. Variance components of body length of *Pelobates fuscus* tadpoles on stages 44 – 48. Only the variance components of factors with significant effects ($p < 0.05$) are presented.



Our data enabled to estimate the effects of geographical and within-locality differences on tadpole body length (Fig. 6). The maximal variance component was due to larval stages; the influence of differences between localities was higher than the differences between ponds at each locality. Obviously, the higher inter-locality variation corresponds to the above mentioned differences between pond conditions.

CONCLUSIONS

1. The application of life history theory enables to develop the basic principles and effective practical measures of management, conservation and recovery of rare amphibian species.
2. The tolerance to high tadpole density was revealed in the two studied species with different larval characteristics. In *B. viridis*, the increase of initial density lead to a

decrease of body size of metamorphs but not of the time of premetamorphic development (at least in the Moscow Oblast'). In *P. fuscus*, the influence of high density on body length of tadpoles and metamorphs was not so predictable and could be compensated by a relatively large size of the pond where tadpoles grew and developed.

3. These characteristics of the species should be considered in programs including the translocation and recovery of populations in a given amphibian species.

Acknowledgments. The project was supported by the John D. and Catherine T. MacArthur Foundation (Research and Writing Grants for Individuals). In addition, I am grateful to students of the Departments of Vertebrate Zoology and Biological Evolution of the Moscow State University and to school children — members of the natural history study group of the Zvenigorod biological station for their assistance with my field work.

REFERENCES

- Dabagyan N. V. and Sleptsova L. A.** (1975), "Common frog *Rana temporaria*," in: *Ob'ekty Biologii Razvitiya [The Objects of Developmental Biology]*, Nauka, Moscow, pp. 442 – 462 [in Russian].
- Lyapkov S. M.** (2003), "Recovery and maintenance of amphibian populations: a model field experiment on translocation of *Bufo bufo* eggs," in: *Proc. of the Third Conf. of Volga Region Herpetologists*, Tol'yatti, pp. 47 – 49 [in Russian].
- Marsh D. M. and Trenham P. C.** (2001), "Metapopulation dynamics and amphibian conservation," *Conserv. Biol.*, **15**(1), 40 – 49.
- Reading C. J. and Clarke R. T.** (1999), "Impacts of climate and density on the duration of the tadpole stage of the common toad *Bufo bufo*," *Oecologia*, **121**(3), 310 – 315.
- Seigel R. A. and Dodd C. K.** (2002), "Translocations of amphibians: proven management method or experimental technique?" *Conserv. Biol.*, **16**(2), 552 – 554.

GEOGRAPHICAL AND LOCAL VARIATION OF REPRODUCTIVE AND DEMOGRAPHIC CHARACTERISTICS IN BROWN FROGS

S. M. Lyapkov¹

Keywords: geographical and local variation, growth rate, age, reproductive characteristics, survivorship.

INTRODUCTION

The comparative studies of life-history traits in *Rana temporaria* revealed the large scale of variation between geographically removed localities (Ishchenko, 1999; Miaud et al., 1999). The similar studies of reproductive and demographic characteristics in other brown frog species, are scarce (for review see Ishchenko, 1999; Lyapkov et al., 2002b). In addition, the relative value of intrapopulation variation is mostly underestimated. The intrapopulation variation includes the age-specific changes and the differences between generations. So, the long-term study of *R. arvalis* population allowed to reveal differences in survival and reproductive characteristics of females of distinct ages and generations (Lyapkov et al., 2001a, 2001b). The aim of the present study was to compare the reproductive and demographic characteristics of the populations of *R. temporaria* and *R. arvalis*, inhabiting two different localities. For each species it was necessary to compare the scale of intrapopulation variation with the differences between two localities of the range, basing on long-term and simultaneous studies of populations in both localities. For this purpose the reproducing individuals of these two species have been studied in relatively favorable conditions of their ranges (in Moscow Oblast') as well as in northern part of ranges (in Kirov Oblast').

MATERIAL AND METHODS

For 5 years (1998 – 2002), the amplexant pairs of both species were collected in breeding ponds during reproduction period in two localities: in Moscow Oblast' — near Zvenigorod Biological Station of Moscow State University, 55 km westward of Moscow (55°44' N 36°51' E, 50 m above sea level (a.s.l.); the name of this locality is abbreviated to "ZBS"), and in Kirov Oblast' — near Kipenevshchina village, Orlovskii raion, 40 km westward of Kirov (58°40' N, 49°5' E, 100 m a.s.l., "Kirov"). The body

length and the age were determined in each individual, fecundity and egg diameter (with eyepiece micrometer, accurate to 0.1 mm) — in each female. The fecundity was estimated by mass of whole clutch taken out of dissected females (previously weighted accurate to 0.1 g) and by mass of clutch portion (weighted accurate to 1 mg) in which all eggs were counted. The relative clutch mass, i.e., clutch mass relative to gravid female mass was estimated also. Age was determined by standard skeletochronological method (preparation of cross-sections of tibio-fibula stained by Ehrlich hematoxiline — Smirina, 1994). The survivorship of both sexes was estimated as calculated number of frogs of a given age (based on the percentage of individuals of this age and the total number of frogs reproducing in a given year) relative to initial number of generation (i.e., total egg number laid in a given year divided by 2). To obtain required data the censuses of clutches of both species in all ponds used by each studied population were conducted since 1988.

RESULTS AND DISCUSSION

The age distributions of matured *R. temporaria* differ substantially between two localities (Fig. 1): the proportion of two-year-olds (both females and males) was higher in ZBS population whereas the proportion of older ages was lower than in Kirov population. Contrarily (and surprisingly), ZBS population of *R. arvalis* (Fig. 2) is characterized by lack of two-year-old females and by very low proportion of two-year-old males. Thus, in Kirov Oblast' the females can mature at age of two years, unlike to those of Moscow Oblast'. The proportion of 3-year-old females and males was also higher in Kirov Oblast'. The only explanation is that in Kirov Oblast' both species are largely syntopic and reproduce in the same ponds. Correspondingly, the proportion of older ages was higher in ZBS population of *R. arvalis*.

In ZBS population of *R. temporaria*, the body length of each age was significantly larger than in Kirov population (Fig. 3), both in males and females (for each age from

¹ Biological Evolution Department, Biological Faculty, Moscow State University, Leninskie Gory 1/12, 119899 Moscow, Russia;
E-mail: lyapkov@mail.ru.

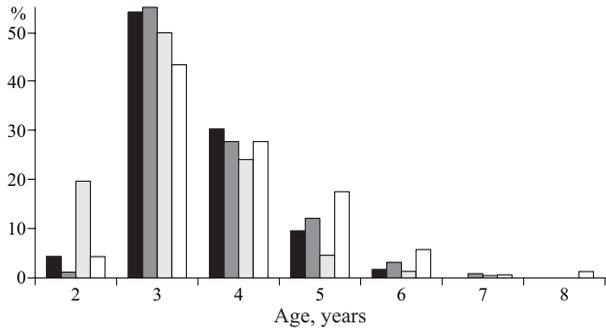


Fig. 1. Age distributions of adult *R. temporaria*, collected in breeding ponds. **Moscow Oblast:** ■, *Rana temporaria* females (n = 284); ▒, *R. temporaria* males (n = 326). **Kirov Oblast:** ▒, *R. temporaria* females (n = 264); □, *R. temporaria* males (n = 263).

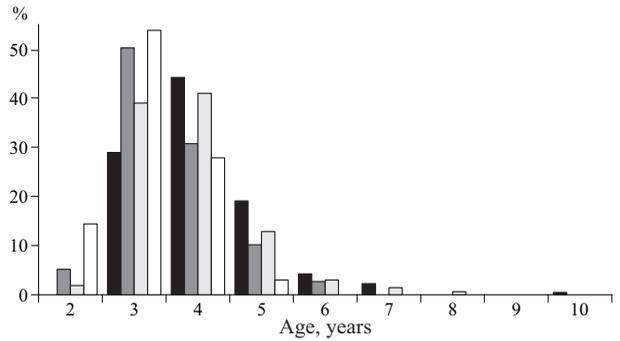


Fig. 2. Age distributions of adult *R. arvalis*, collected in breeding ponds. **Moscow Oblast:** ■, *Rana arvalis* females (n = 280); ▒, *R. arvalis* males (n = 280). **Kirov Oblast:** ▒, *R. arvalis* females (n = 77); □, *R. arvalis* males (n = 103).

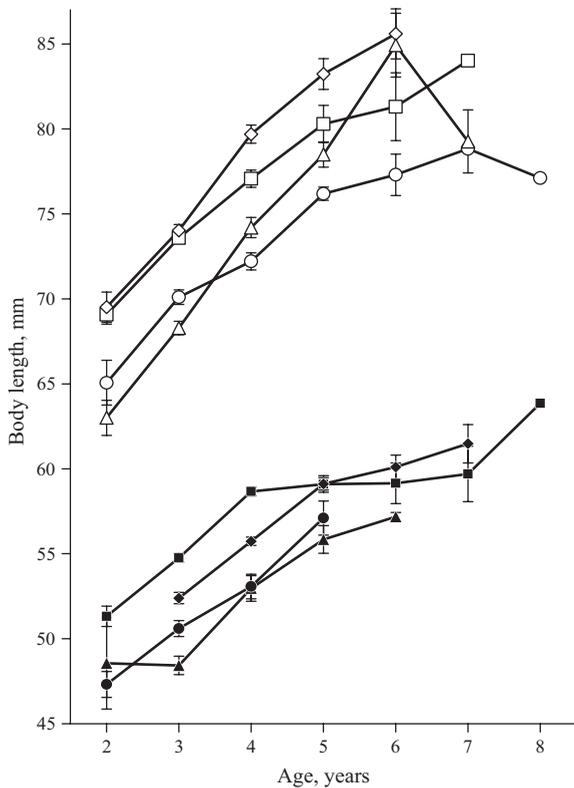


Fig. 3. Dependence of body length on age in *R. temporaria* and *R. arvalis*. The average values and standard errors are given. **Moscow Oblast:** ◇, *Rana temporaria* females; □, *R. temporaria* males; ◆, *R. arvalis* females; ■, *R. arvalis* males. **Kirov Oblast:** △, *R. temporaria* females; ○, *R. temporaria* males; ▲, *R. arvalis* females; ●, *R. arvalis* males.

2 to 5, $p < 0.01$, accordingly to results of 1-way ANOVA). It indicates on lower annual growth rate in Kirov population, though males matured at the same age as in Moscow Oblast (after second or third wintering). In ZBS population of *R. arvalis*, the body length at each given age was

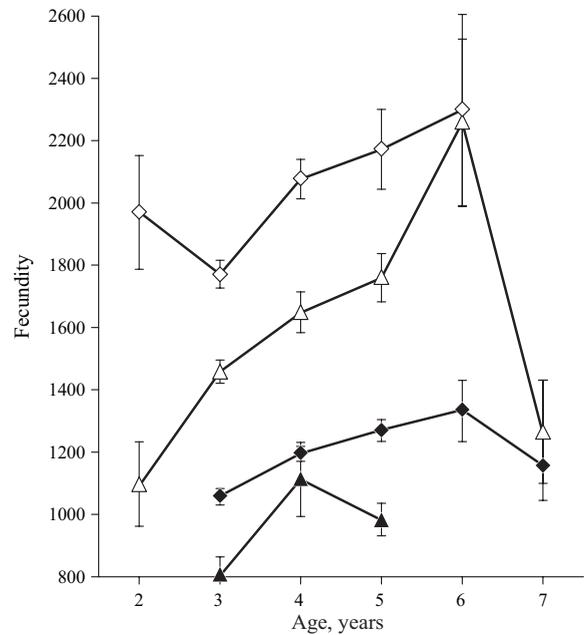


Fig. 4. Dependence of fecundity on age in *R. temporaria* and *R. arvalis*. For designations see Fig. 3.

also considerably larger than in Kirov population, both in males (at the ages from 2 to 5) and females (at the ages from 3 to 5; $p < 0.01$, accordingly to results of one-way ANOVA).

The general trend in populations of both species studied in both localities was the significant increase of fecundity, egg diameter and relative clutch mass with the age of females. At the same time, in Kirov females of both species the age-specific changes of fecundity and egg diameter occur at lower level that corresponds to smaller body length at each age (Figs. 4 and 5). It in turn is determined by stronger restriction of activity season. The number of

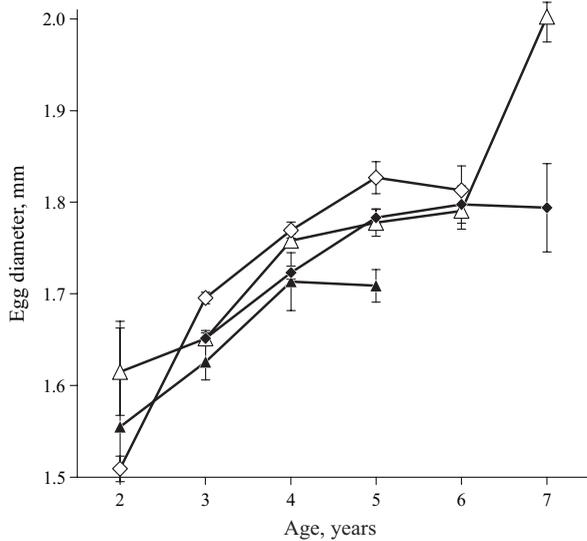


Fig. 5. Dependence of egg diameter on age in *R. temporaria* and *R. arvalis*. For designations see Fig. 3.

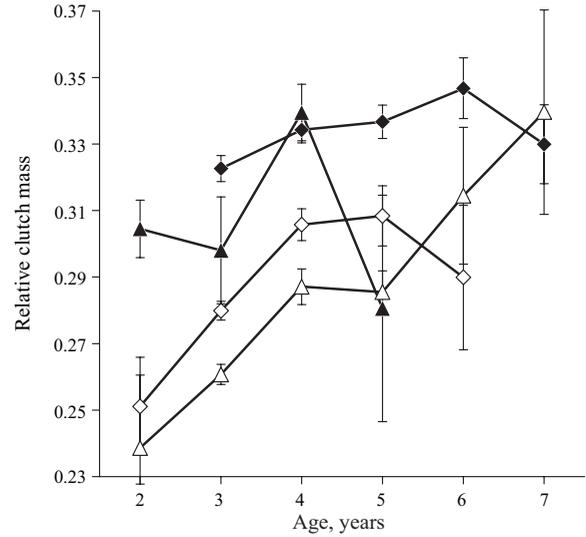


Fig. 6. Dependence of relative clutch mass on age in *R. temporaria* and *R. arvalis*. For designations see Fig. 3.

TABLE 1. Mean Annual Survivorship (%) in *Rana temporaria* and *R. arvalis* in Two Localities

Year	<i>Rana temporaria</i>				<i>Rana arvalis</i>			
	Moscow Oblast'		Kirov Oblast'		Moscow Oblast'		Kirov Oblast'	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1998	0.1704	0.0752	0.0181	0.0071	0.1149	0.1042	0.0376	0.0407
1999	0.0893	0.0576	0.0342	0.0071	0.1165	0.0751	0.0418	0.0403
2000	0.1708	0.0776	0.0690	0.0021	0.0609	0.0620	0.1165	0.0123
2001	0.0546	0.0354	0.0261	0.0279	0.0533	0.0334	0.1944	0.1590
2002	0.0155	0.0094	0.0255	0.0000	0.0146	0.0200	0.0695	0.0000

days with air temperature above +5°C in Moscow Oblast' is on an average on 15 higher than in Kirov Oblast'. In *R. arvalis*, the differences in relative clutch mass between two localities within each age were non-significant (with the exception of 3- and 5-year-old females). In addition, the mean values of relative clutch mass were significantly higher in *R. arvalis* than in *R. temporaria* in each locality (Fig. 6) despite of smaller body size in this species.

The survivorship of separate generations differed between species, localities and sexes. So, in ZBS populations the survivorship of *R. temporaria* females was higher than in the same generations of *R. arvalis*. Survivorship of many ZBS generations in *R. arvalis* females was higher than in Kirov generations. Survivorship of many ZBS generations in *R. temporaria* females was higher than in males. In *R. temporaria*, mean annual survivorship (i.e., average over all generation in a given year, Table 1) of females was higher than in males; mean survivorship in ZBS population was higher than in Kirov population, both in

females and males. In *R. arvalis* (Table 1), mean survivorship of females was usually higher than in males, but the differences between two localities were not as distinct as in *R. temporaria*.

The rate of population turnover of each species differs between two localities. As the measures of these differences we can use the values of R_0 (net rate of reproduction) and T (generation time) calculated according to formulas (Pianka, 1978):

$$T = \frac{\sum_{x=0}^{T_{max}} x l_x m_x}{\sum_{x=0}^{T_{max}} l_x m_x}; R_0 = \sum_{x=0}^{T_{max}} l_x m_x,$$

where T_{max} is the maximal age, m_x is the average female fecundity of age class x , l_x is the proportion of individuals that have survived till age x .

TABLE 2. Net Rate of Reproduction (R_0) and Generation Time (T) in *Rana temporaria* and *R. arvalis*

Year of birth	R_0				T			
	<i>Rana temporaria</i>		<i>Rana arvalis</i>		<i>Rana temporaria</i>		<i>Rana arvalis</i>	
	Moscow Oblast'	Kirov Oblast'	Moscow Oblast'	Kirov Oblast'	Moscow Oblast'	Kirov Oblast'	Moscow Oblast'	Kirov Oblast'
1994	0.860	2.460	2.978	2.013	4.222	3.687	4.279	3.725
1995	4.401	0.943	5.046	1.406	3.610	4.437	4.048	4.074
1996	2.732	2.797	3.657	1.475	3.616	3.712	4.389	3.811
1997	4.688	3.891	2.460	4.181	3.608	3.836	4.228	3.798

In *R. temporaria*, relatively high proportion of younger ages among breeding adults (Fig. 1) corresponds to lower value of T in most generations of ZBS population (in comparison with Kirov one); in *R. arvalis*, the converse relationship was revealed (Table 2). The variation of R_0 depends not only on age structure and survivorship but on the fecundity. Therefore, the values of R_0 in both localities did not differ distinctly between two species. The values of R_0 in both species are usually higher in Moscow Oblast' than in Kirov Oblast'.

CONCLUSIONS

1. In both species, the variation between localities was comparable with intrapopulation variation that is due to age and belonging to given generation. Therefore, long-term studies are necessary for evaluation of interrelation between geographical and local variation.

2. The constraints, associated with unfavorable climatic conditions in northern locality, influence on both species in the same way.

3. Within each locality, the differences between species in fecundity and egg diameter correspond to larger body length of *R. temporaria* females. The higher values of relative clutch mass in *R. arvalis* females are due to other reasons.

4. The values of R_0 in most generations of both species are usually higher in Moscow Oblast' than in Kirov Oblast'.

Acknowledgments. This study was financially supported by the Russian Foundation for Fundamental Research (grant No. 02-04-49230). I am grateful to J. A. Kabardina for her help in field work and age determination.

REFERENCES

- Ishchenko V. G.** (1999), *The Population Ecology of Brown Frogs of Russia and Adjacent Areas. Author's Abstract of Doctoral Thesis*, Zoological Institute, Russian Academy of Sciences, St. Petersburg.
- Lyapkov S. M., Cherdantsev V. G., and Cherdantseva E. M.** (2001a), "Structure of relationship between fitness components in life history of *Rana arvalis*: 1. Dynamics of reproductive effort and its components," *Entomol. Rev.*, **81**(Suppl. 1), S161 – S169 {Translated from *Zool. Zh.*, 2001, **80**(4), 438 – 446 [in Russian]}.
- Lyapkov S. M., Cherdantsev V. G., and Cherdantseva E. M.** (2001b), "Structure of relationship between fitness components in life history of *Rana arvalis*: 2. Dynamics of fitness components," *Entomol. Rev.*, **81**(Suppl. 1), S170 – S179. {Translated from *Zool. Zh.*, 2001, **80**(5), 567 – 575 [in Russian]}.
- Lyapkov S. M., Kornilova M. B., and Severtsov A. S.** (2002a), "Variation structure of reproductive characteristics in *Rana temporaria* and its relationship with size and age of the frogs," *Zool. Zh.*, **81**(6), 719 – 733 [in Russian].
- Lyapkov S. M., Kornilova M. B., and Severtsov A. S.** (2002a), "Demographic characteristics and number dynamics of *Rana temporaria* population," *Zool. Zh.*, **81**(10), 1251 – 1259 [in Russian].
- Miaud C., Guyétant R., and Elmberg J.** (1999), "Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps," *J. Zool. Lond.*, **249**, 61 – 73.
- Pianka E.** (1978), *Evolutionary Ecology*, Harper and Row, New York.
- Smirina E. M.** (1994), "Age determination and longevity in amphibians," *Gerontology*, **40**(4), 133 – 146.

ILLEGAL EXPORT OF AMPHIBIANS AND REPTILES FROM THE RUSSIAN FAR EAST TO COUNTRIES OF THE ASIAN REGION: THE SITUATION IN 2003

I. V. Maslova¹ and S. N. Lyapustin²

Keywords: Russian Far East, China, illegal export, *Rana dybowskii*, *Pelodiscus sinensis*.

INTRODUCTION

Russia's Far East is facing a critical situation connected with mass illegal export of its natural resources, such as ginseng, paws of Asiatic black bear, skins of tigers and leopards and many other things, to the countries of South East Asia. This leads to inconvertible changes in the ecosystems of the Far East region. The situation is especially critical in the Primorskii Krai area. Many representatives of local herpetofauna are among the most exported bioresources. Chinese and sometimes Koreans catch amphibians and reptiles for traditional oriental medicine and culinary. Over the past 10 years, a comprehensive network of poachers, co-dealers and Chinese traders was established in Primorskii Krai. This illegal network also includes many Russian people whose present-day living standard has become particularly low. In this report we give a general overview of the situation connected with poaching.

MATERIAL AND METHODS

We have been collecting data over 11 years (from 1993 to 2003). These are the archives' data of several local Nature Conservancy committees, the data of the Primorskii Krai ecological group "Tiger," the information from the border guards and regional customs officials, and also the materials of the local mass media and police reports. We also included the information received during the interviews of the residents of 18 districts of Primorskii Krai.

RESULTS AND DISCUSSION

Two species are in greatest danger: *Rana dybowskii* and *Pelodiscus sinensis*. However, according to the reports of the Far East customs officials, live specimens and parts of *Bombina orientalis*, *Bufo gargarizans*, *Hyla japonica*, and *Rana nigromaculata* are also exported.

¹ Khankaiskii State Nature Reserve, Ershov str. 10, Spassk-Dalnii, 692245 Primorskii Krai, Russia; E-mail: rana@mail.primorye.ru.

² Far East Task Force Customs, Krasnogo Znameni str. 66a, Vladivostok, Russia.

Rana dybowskii is a common species in the southern Far East, and is not listed in the Red Data Book. However, its population has notably decreased in this region because of poaching.

Before 1917 the Chinese harvested these species of frogs in the Suifun River valley (which is now called Razdol'naya River valley). *Rana dybowskii* is valuable because of its fat, which is used in oriental medicine. Later, the main catches of frogs were conducted in the Manchuria area. Beginning from the 1990s the borderline between China and Russia has become more open. Many Chinese began to travel to the Primorskii Region, and some of them purchased *Rana dybowskii*.

Chinese poachers harvest *Rana dybowskii* in autumn and early winter for pharmacological needs, and in spring for culinary purposes. Poachers use different types of traps to catch amphibians. These traps may include polyethylene fences put along the rivers in the mountains, in order to collect frogs on their way during the autumn migration.

Thus, for example, in October 1994, members of the Ussuriisk Regional Environmental Committee found a 3-km polyethylene fence near the Komarovka River, which was installed by Chinese farmers.

Another instrument taking frogs are fishing nets. For example, (Protocol 13 of 03/24/1999, Ussuriisk Regional Environmental Committee), near Kondratenovka village two citizens from China had illegally taken wintering frogs from the left tributary of the Kamenyushka River. For their purposes they used nets (length 1.5 m, height 0.8 m). When these Chinese were detained, they had 500 live animal specimens in their bags.

Poachers also used electric fishing rods and traps. This was recorded from the Pogranichnyi raion.

Poaching has drastically increased since 2002. Mass poisoning of rivers has started. There was a newspaper report, presenting materials and photographs, which were handed over by the border guards. The analysis of videos made by border guards on the Ryazanovka River showed that the Chinese took motionless frogs and did this for a long time. They had managed to take 400 specimens of *Rana dybowskii*.

Rangers who took the water from the river, 20 km downstream from this place, got seriously poisoned. Members of the Far East branch of WWF inspected the Ryazanovka River and showed that a lot of water life (fish, amphibians, water invertebrates) of the river was destroyed. Also a large part of the rivers in the Khasan raion had been poisoned recently.

Besides the border guards, the Russian Far East customs officials play an important role in minimizing illegal trade. Since 1996 they are making attempts to prevent illegal export of amphibians and their parts from Russia. Over the period 1996 – 2003 the customs officials have prevented attempts of the citizens of China to take out 3720 live frogs, 90 kg of dried frogs, 39 kg of dried frog meat, 55 kg of fatlike frog substance, 74 g of frog brown fat, 3.3 kg of frog eggs.

They also prevented the export of 2.4 kg of fatlike substance of *Hyla japonica* and 3 kg of secretor substance of *Bombina orientalis*.

Mass illegal collection of soft-shelled turtles has also begun. In 2001 we conducted expeditions in Primorskii Krai and studied places with *Pelodiscus sinensis* habitat. We found out that at present, out of 13 districts inhabited by the turtles, there are only two regions with few cases of poaching. Reptilian sales have existed a long time and have a mass character. It is perfectly clear that in some districts turtles are collected by the local fishery inspection. Lesozavodsk and Dal'nerechensk are the main illegal bases for transporting these species to the Chinese territory.

The population of one of the villages on the Malinovka River lives on the money from the sales of soft-shelled turtles. According to reliable sources, one of the poachers bought his car with the money from the turtle trade. It was estimated that he collected and sold no less than 500 specimens.

Villagers of all the regions report that large turtles have not been found in these areas. At present the length of carapax does not exceed 20 – 25 cm. It has been pointed out that the number of these animals has decreased. There are lakes and river areas, where these species were met earlier, but are no longer found at present.

There is the following information about the Far East customs service. Beginning in the middle 1990s, the Far East customs inspection reported about numerous attempts of illegal export of *Pelodiscus sinensis* to China. The export is conducted in different ways. For example, in 1999 the Ussuriisk Customs officials detected several turtles

bound by metal wires and attached to the bottom of a railway cargo carriage.

During the last 8 years, the Far East customs inspectors have withdrawn 215 specimens and 40 eggs of *Pelodiscus sinensis* from illegal export. However, the amount of illegal export to China have been significantly increased. There is testimony that the Chinese market is overflowed by *Pelodiscus sinensis* taken from Russia.

Very recently, police officers from the October District detained a car, in which they discovered big amounts of soft-shelled turtles, whose weight totaled 184 kg. Co-dealers purchased these turtles for 70 – 100 rubles from the local poachers in order to sell them to China.

Thus, the situation is as follows. The numbers of the *Rana dybowskii* in the south of Primorskii Krai (Khasan, Ussuriisk, and Nadezhdinskii raions) have decreased to a disastrous extent (V. A. Kostenko, 2003, personal communication). In the rest of the districts the number of frogs has drastically decreased. After the winter of 2002 a big part of the rivers in the Khasan raion have been poisoned. *Pelodiscus sinensis* has disappeared in some places, while elsewhere the numbers have declined.

The problem of illegal collection of amphibians and reptiles in Primorskii Krai for the purpose of illegal export needs to be tackled urgently. Mass collection of different species from the wild has already brought ecosystems to imbalance. It is necessary to pass effective laws for conservation of herpetofauna in the Far East of Russia.

REFERENCES

- Maslova I. V. (2002), "New Data on the Distribution of *Pelodiscus sinensis* of the Primorskii Krai," in: Artamonov S. D. and Kolyada A. S. (eds.), *Animal and Plant World of the Far East*, Izd. UGPU, Issue 6 (Contribution to the Animal Ecology and Systematics), pp. 65 – 73 [in Russian with English summary].
- Maslova I. V. (2002), "Dybowsky's frog (*Rana dybowskii* Günther, 1876) — next object of illegal sale in China," in: Artamonov S. D. and Kolyada A. S. (eds.), *Animal and Plant World of the Far East*, Izd. UGPU, Issue 6 (Contribution to the Animal Ecology and Systematics), pp. 60–64 [in Russian with English summary].
- Protocols of Ussuriisk Regional Environmental Committee* (1993 – 2002).
- Protocols of Khasan Regional Environmental Committee* (1993 – 2002).
- Data of Far East Task Force Customs* (1994 – 2003).
- Data of Far East Border Guard* (1997 – 2002).

PRELIMINARY RESULTS ON THE GENETIC CONTROL OF DISPERSAL IN COMMON FROG *Rana temporaria* FROGLETS

C. Miaud,¹ J. Sérandour,¹ R. Martin,¹ and N. Pidancier²

Post-metamorphic dispersal in the common frog *Rana temporaria* (Amphibia, Anura) was studied with a combination of field (pit-fall traps) and laboratory (arena, artificial crossing) experiments. In the first studied population, the breeding place was surrounded by lines of fence-pitfall traps allowing capture of dispersing froglets. Dispersal was at random on the edge of the pond, but oriented in the most favorable terrestrial habitat at 10 m from the edge. Froglets of this population were then tested in orientation arena built on the University campus, where they also dispersed at random. The two other studied populations reproduced at each side (north and south) of a lake. Froglets from each population were tested in similar orientation arena, where they did not disperse at random but to the north and south direction respectively. In the laboratory we crossed males and females originated from these two populations. Resulting crossed froglets exhibited variable dispersal patterns, which significantly differed from those observed with their respective parents. These results argued for an at least partly genetic control of emigration direction in these two frog populations, that we interpreted as the result of directional selection due to landscape change during the XXth century.

Keywords: dispersal, juveniles, artificial crossing, orientation, local adaptation, amphibian.

INTRODUCTION

Dispersal is a major behavioral trait of many organisms (Clobert et al., 2001). A wide literature has been devoted to orientation in Amphibians, especially during breeding migration, e.g., between terrestrial and aquatic sites (Sinsch, 1992). Both Anura and Urodela can use numerous environmental cues, e.g., water odor to migrate from one place to another (e.g., Sinsch, 1990; Joly and Miaud, 1992 and references inside). These studies concern mainly adult stage and information on juvenile dispersal is particularly scarce.

The aim of this work was to use a combination of field and laboratory experiments to assess the post-metamorphic dispersal in the common frog *Rana temporaria* (Amphibia, Anura).

The first experiment was to study the direction of dispersal in post-metamorphic individuals (called froglets in this paper) from a breeding pond in the field. The second experiment was to test dispersal direction of the same froglets in an arena outside the pond environment. We also tested froglets from two other breeding populations: adults breed at opposite sides of a small lake and landscape structure (road, crops) imposed two obligatory migratory routes for frogs (north and south) in the terrestrial environment. Our first hypothesis was to test if froglet dispersal

followed direction used by adults. In the case of population-specific dispersal, the second hypothesis was to test the existence of a genetic basis of migratory behavior in froglets. We thus experimentally produced hybrids of “north” and “south” frog populations and compared froglets dispersal direction with those of the two parental populations.

MATERIAL AND METHODS

Dispersal in the Field

A peat bog pond (30 × 10 m), situated near “St. François de Sales” (Savoie Department, Southeast of France) at 1350 m a.s.l., is occupied by a large population of common frog. We placed 3 lines of fence-pitfall traps around the pond in 2001. Each line was composed of a fence made of fine plastic netting (1 mm mesh) 3 m long and 50 cm high. Three pitfall traps (diameter of the hole: 12 cm) equipped each system (one at each extremity and one in the middle of the fence). We installed 8 fence-pitfall traps in a circle around the pond (Fig. 1) at 1, 5, and 50 m from the edge of the pond. The traps were protected against the sun by an opaque wooden screen. The traps were visited from 1 to 3 times a week from the start of metamorphosis (June 23) until August 5.

Dispersal in Arena

Arenas were circular enclosures (4 m in diameter), similar to previously described fence-pit fall traps system

¹ UMR CNRS 5553 Laboratory Alpine Ecology, Population genomics and biodiversity, F-73376 Le Bourget du Lac Cedex, France; E-mail: claude.miaud@univ-savoie.fr.

² UMR CNRS 5553 Laboratory Alpine Ecology, Population genomics and biodiversity, F-38041 Grenoble, France.

(Fig. 2). The bottom was the natural ground with herbaceous coverage. Orientation of the froglets was estimated from their capture in 12 pitfall traps (diameter of the hole 20 cm) regularly placed along the net. The release site at the center of each arena consisted of a $30 \times 20 \times 10$ -cm plastic box with 2 cm depth of water. Tadpoles (about 30 at each experiment) were placed in this release site when the tail started to regress and froglets can leave it and disperse in the arena over the following days. Traps were controlled twice every 4 days from the beginning of each experiment. Five arenas were built on the campus of the University of Savoie, in an isolated piece of fallow land.

Tested froglets came from three populations: the peat bog pond of “St. François de Sales” where field dispersal



Fig. 1. Fence and pitfall traps used to study froglet dispersal around a natural breeding site.

was tested in 2001 and 2 populations spawning on each edge of Lake Aiguebelette (near the town of Chambéry, Savoie Department). This lake is about 4 km long and 1 km wide and common frog only breed in two breeding place on the north and south edges of the lake. Parts of several spawns were collected in 2002 in each of these populations and eggs and tadpoles were reared in similar conditions until orientation experiments started. Two other pools of froglets were obtained: Adults were caught on land during the breeding migration in the two lake populations. Four males and 4 females of each population were taken to the laboratory and anaesthetized. Ova were obtained by pressing lightly female abdomen, and were immediately fertilized with sperm obtained by male dissection. Each female of the north population was crossed with a male of the south population and reciprocally, to obtain two pools of crossed froglets. Eggs and larvae were reared in similar conditions as those from each parental population.

Froglet distribution around the arenas was summarized by a mean vector (data were grouped because animals were caught by trapping: Batschelet, 1981). Randomly distributed captures were tested by Raleigh test. Orientation towards an expected target was accepted if the target is included in the limits of the confidence interval of the mean vector. Circular statistics were from Batschelet (1981).

RESULTS

Dispersal in the Field

The common frog spawn in April in the studied peat bog pond of “St. François de Sales” (Savoie Department,



Fig. 2. Arena used to study froglet orientation on the University campus.

Southeast of France) at 1350 m a.s.l. The froglets started to metamorphose at the end of June. Dispersal from the pond was recorded with fence-pitfall traps. At the edge of the pond, froglets left the breeding site without a preferred direction (mean vector length $r = 0.082$, Raleigh test $P > 0.05$, $N = 408$, Fig. 3a).

At 10 m from the pond edge, froglets were significantly oriented toward a preferred direction (mean vector length $r = 0.652$, Raleigh test $P < 0.001$, $N = 93$, Fig. 3b).

Dispersal in Arena

Froglets of the bog pond were tested in the orientation arena on the University campus. Dispersal direction did not differ from random (mean vector length $r = 0,061$, Raleigh test $P > 0.05$, $N = 285$, Fig. 4a).

Froglets from populations at both north and south sides of the lake were tested in the arena. Dispersal directions of froglets originating from the “north” population significantly differed from random (mean vector length $r = 0.322$, Raleigh test $P < 0.001$, $N = 111$, Fig. 4b). The

mean vector angle $\Phi_m = -11.5 \pm 32^\circ$ (mean \pm confidence interval at $\alpha = 0.05$) did not differ significantly from the north ($\Phi = 0^\circ$) direction.

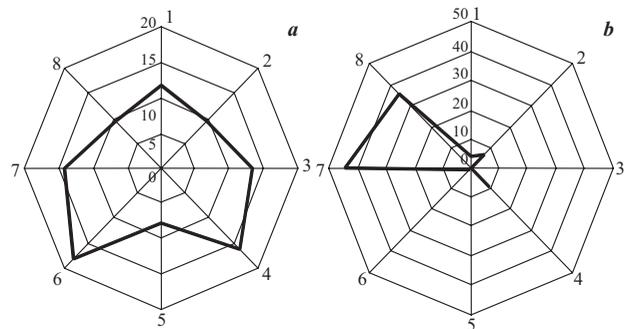


Fig. 3. Froglet dispersal from the pond recorded with fence-pitfall traps: (a) at the edge of the pond ($r = 0.082$, $N = 408$, no preferred direction); (b) at 10 m from the pond edge ($r = 0.652$, $N = 93$, froglets were significantly oriented toward one direction); 1 to 8, number of pit-fall traps with trap No. 1 orientated to the north, sample sizes in each direction expressed in %, scale on the vertical axis.

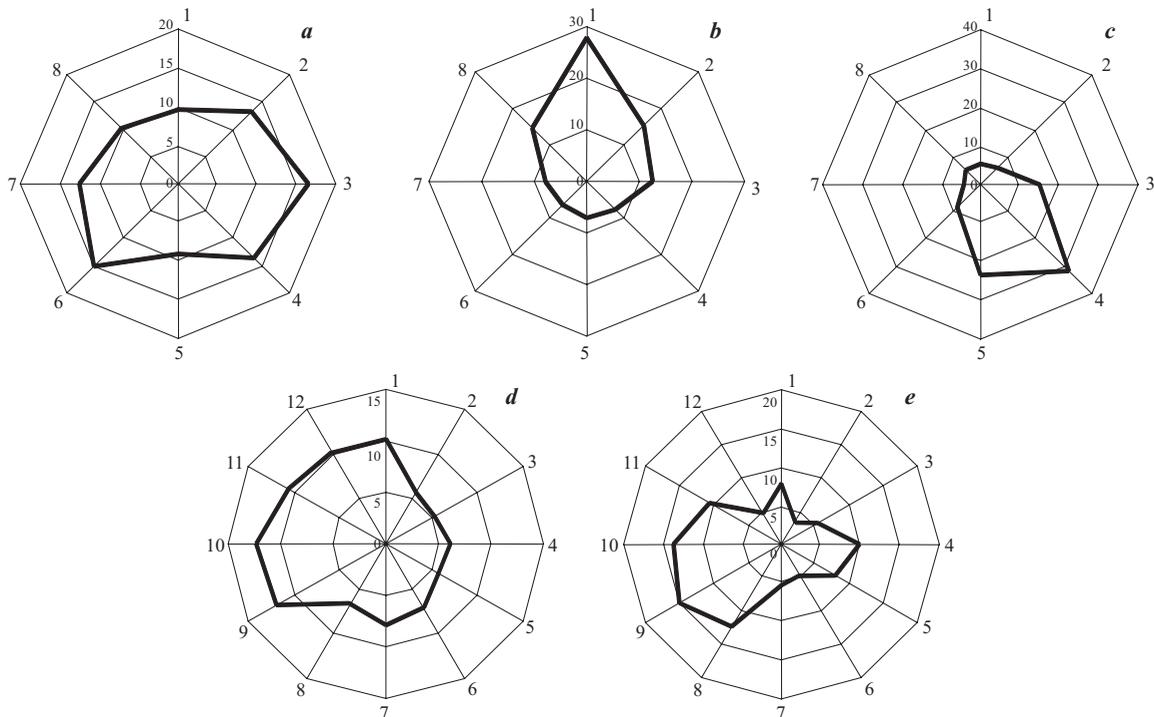


Fig. 4. Froglet dispersal in the orientation arenas: (a) Froglets originating from the bog pond of “St. François de Sales.” Mean vector length $r = 0.061$ ($N = 285$). There is no preferred direction; (b) froglets originating from the north population of the lake. Mean vector length $r = 0.322$, $N = 111$. The mean vector angle ($\Phi_m = -11.5 \pm 32^\circ$) did not differ significantly from the north ($\Phi = 0^\circ$) direction; (c) froglets originated from the south population of the lake. Mean vector length $r = 0.475$, $N = 112$). The mean vector angle ($\Phi_m = 126.7 \pm 24^\circ$) significantly differed from the south ($\Phi = 180^\circ$) direction; (d) froglets originated from artificial crosses [males (north) \times females (south)]. Mean vector length $r = 0.184$, $N = 226$). The mean vector angle ($\Phi_m = 280.1 \pm 35^\circ$) significantly differed from dispersal directions of froglets from parent populations (both north and south); (e) froglets originated from artificial crosses [males (south) \times females (north)]. Mean vector length $r = 0.212$, $N = 153$). The mean vector angle ($\Phi_m = 294.8 \pm 35^\circ$) significantly differed from dispersal directions of froglets from parent populations (both north and south).

Dispersal directions of froglets from the “south” population significantly differed from random (mean vector length $r = 0.475$, Raleigh test $P < 0.001$, $N = 112$, Fig. 4c). The mean vector angle ($\Phi_m = 126.7 \pm 24^\circ$) significantly differed from the south ($\Phi = 180^\circ$) direction.

Adults from the north and south populations were artificially crossed in the laboratory and the obtained froglets were tested in the arena. Froglets [males (north) \times females (south)] did not disperse at random (mean vector length $r = 0.184$, Raleigh test $P < 0.001$, $N = 226$, Fig. 4d). The mean vector angle ($\Phi_m = 280.1 \pm 35^\circ$) significantly differed from dispersal directions of froglets originating from parent populations (both north and south).

Froglets [males (south) \times females (north)] did not disperse at random (mean vector length $r = 0.212$, Raleigh test $P < 0.001$, $N = 153$, Fig. 4e). The mean vector angle ($\Phi_m = 294.8 \pm 35^\circ$) significantly differed from dispersal directions of froglets originating from parent populations (both north and south).

DISCUSSION

Froglet Dispersal

Following metamorphosis, froglets have to disperse in the terrestrial habitat surrounding the breeding and larval development aquatic area. Eggs and tadpoles are exposed to numerous predators and survival is low (e.g., Biek et al., 2002) give 0.06 and 0.34 as mean values of tadpole and metamorphosis survival in *Rana temporaria*). The post-metamorphic life is also risky for such small vertebrates (about 15 mm body length). Froglets left the peat bog pond of this study without preferred directions. However, at 10 m from the edge of the pond, they were caught in only one direction. The pond surroundings are composed of a small mixed forest and grassland. The preferred direction is towards the mixed forest habitat (this result is also obtained with pit-fall traps at 50 and 100 m from the pond, unpublished data). Froglets tested in the arena far from environmental cues of the pond surroundings also dispersed at random. We make the assumption that, at this breeding site, froglets dispersed at random, and those which survived were by chance in a favorable habitat. Adults, equipped over two successive years with transmitters, exhibited strong fidelity to routes in this habitat (Miaud and Martin, unpublished data). Therefore, it seems that adults migrated where they were successful as froglets, after a random emigration at the first breeding place.

Landscape structure is often shaped by human activities and amphibians are well known to be highly sensitive to landscape alteration. Agriculture, urbanism and road

network permitted only two obligatory migratory routes for the common frogs around the small lake of this study. Froglets from the “north” and “south” populations (tested in arena) dispersed in two opposite directions that corresponded to adult migratory routes. Distinction between genetic variation and environmentally induced phenotypic variation can be made using reciprocal transplant and common garden experiments (Mousseau et al., 2000). Our experiments in arena corresponded to this common garden design. Another approach is to obtain hybrids with individuals from populations where the studied traits vary. Our results argue for a genetic basis to froglet dispersal direction: “north” population froglets dispersed to the north while those from the “south” population dispersed to the south in the arena and hybrids exhibited variable dispersal patterns, mostly different from those observed with their respective parents. These results — which have to be considered as preliminary — lead to question which selective pressures act to generate evolutionary change in migration direction. Genetic determination in migratory direction is documented in arthropods (sandhoppers: Scapini and Fasinella, 1990), fishes (Salmon and trout: Raleigh, 1971), and birds (European blackcap: Helbig, 1991). In this last example, evolutionary changes in migratory direction occur relatively rapidly: 7 – 11% of the breeding population migrated to NW direction in 1990 whereas no birds were observed in this direction before 1960. The new NW migratory direction has a genetic basis and must have evolved through rapid microevolution (Helbig et al., 1994). We interpreted our results in the common frog as follow: 1) post-metamorphic dispersal is random and froglets survive in favorable environments. This strategy is successful because recruitment varies greatly from one year to another and favorable habitat move spatially in the landscape matrix. Specific situations can lead to directional selection which, under extreme conditions tends to favor local adaptation over plasticity (Piliucci, 2001); 2) Froglets dispersed on each side of the lake and survived if they reach the favorable habitat (relict forest patch). Old maps (beginning of the XXth century) show that the lake was almost completely surrounded by forest and one can imagine that frogs bred in numerous places around the lake. In less than 100 frog generations, landscape changes greatly and frogs can now reproduce in only two breeding places. Exchange of migrants between them was interrupted, favoring local adaptation in migratory direction.

Acknowledgments. We are grateful to P. Attenbourough for assistance with the pit-fall traps setting up, and field and laboratory work.

REFERENCES

- Batschelet E.** (1981), *Circular Statistics in Biology*, Acad. Press, London.
- Biek R., Funk W. C., Maxell B. A., and Mills L. S.** (2002), "What is missing in Amphibian decline research: insights from ecological sensitivity analysis," *Conserv. Biol.*, **16**, 728 – 734.
- Clobert J., Danchin E., Dhondt A. A., and Nichols J. D.** (eds.) (2001), *Dispersal*, Oxford Univ. Press, New York.
- Helbig A. J.** (1991), "Inheritance of migratory direction in a bird species: a cross-breeding experiment with SE- and SW-migrating blackcaps (*Sylvia atricapilla*)," *Behav. Ecol. Sociobiol.*, **28**, 9 – 12.
- Helbig A. J., Berthold P., Mohr G., and Querner** (1994), "Inheritance of a novel migratory direction in central European blackcaps," *Naturwissenschaften*, **81**, 184 – 186.
- Joly P. and Miaud C.** (1993), "How does a newt find its pond? The role of chemical cues in migrating newts (*Triturus alpestris*)," *Ethol. Ecol. Evol.*, **5**, 447 – 455.
- Mousseau T. A., Sinervo B., and Endler J.** (eds) (2000), *Adaptive Genetic Variation in the Wild*, Oxford Univ. Press, Oxford, USA.
- Piliucci M.** (2001), *Phenotypic Plasticity. Beyond Nature and Nurture*, The John Hopkins Univ. Press, Baltimore, MD.
- Raleigh R. F.** (1971), "Innate control of migrations of salmon and trout fry from natal gravels to rearing areas," *Ecology*, **52**, 291 – 297.
- Scapini F. and Fasinella D.** (1990), "Genetic determination and plasticity in the sun orientation of natural populations of *Taeniostichus saltator*," *Marine Biol.*, **107**, 141 – 145.
- Sinsch U.** (1990), "Migration and orientation in anuran amphibians," *Ethol. Ecol. Evol.*, **2**, 65 – 79.
- Sinsch U.** (1992), "Amphibians," in: Papi F. (ed.), *Animal Homing*, Chapman and Hall, London.

BASIS OF NATIVE POND FIDELITY IN ANURAN AMPHIBIANS: THE CASE OF CHEMICAL LEARNING

S. V. Ogurtsov¹

Keywords: native pond, Anura, chemical learning, sensitive period.

INTRODUCTION

Anuran amphibians come to breed to the native pond (Blair, 1953; Breden, 1987). Chemical stimuli from the pond could be among cues that guide them. That these cues are learnt early in ontogeny is often discussed but hasn't been tested yet (Grubb, 1973). *Rana lessonae* seems to learn the native pond odor during larval stages and is attracted by it after metamorphosis while keeping near the pond (Bastakov, 1986). We tried to find out whether other species possess the same type of reaction and whether sensitive periods to memorize chemical stimuli exist in larval development.

MATERIAL AND METHODS

Juveniles of 5 species (*Rana lessonae*, *R. ridibunda*, *R. temporaria*, *Bufo viridis*, *B. bufo*) were collected near the ponds of Moscow Oblast' (Russia) within 3 days after metamorphosis. They were tested in a plastic chamber $76 \times 12 \times 15$ cm divided into 5 sections by walls of 5 mm height (Fig. 1). A transparent glass cover had ventilatory holes at both ends. A 40-W incandescent lamp put 40 cm from the middle of one of the longest walls provided low illumination. A pair of odorants was positioned in Petri dishes at both ends. After each test the chamber was washed with tap water. Each experimental group was divided into 2 or 4 subgroups of 6–10 individuals. Each subgroup was tested separately with an altered position of stimuli. A subgroup was placed in the center of the chamber and left walking freely for 40 min while each 5 min we counted the number of individuals in sections. Results of subgroup tests were combined in accordance with the position of stimuli thus obtaining 8 sequential observations on the distribution of a group.

To describe frogs distribution in sections with stimuli we compared sequential observations with random-effect model using a classical procedure (Gotelli, 2000). We in-

roduced a distribution stability coefficient (S). For each test we calculated a sequence of 8 differences between the number of frogs in utmost sections. The differences were ranked (including "0" differences), and sums of ranks of "+", "-", and "0" differences were computed separately. The part that sum of ranks of "+" differences constitutes from the total sum of ranks is the coefficient S for the section with the stimulus of interest ("- differences describe S for the opposite section). S varies from 0 to 1. The more often individuals are observed in the section of interest and the more is the difference in the number of frogs in that section than in the opposite one the larger is the S value for it.

We used program Microsoft Excel 2000 (Microsoft Corp., 1985 – 1999) to generate a sequence of 8 random numbers taken with equal probability from $-n$ to $+n$ (n , group size). It models 8 differences between the number of frogs in the utmost sections. S value for "+" differences was then calculated. We chose one-tailed null hypothesis: experimental S does not exceed critical value. If the value is too low than S for the opposite section as well as S for "0" differences could exceed critical level. We used 95% and 99% percentiles to calculate confidence interval. For groups of 3 and more individuals and 225,000 iterations critical values of S come to 0.83 ($p = 0.05$) and 0.94 ($p = 0.01$). If experimental S is larger than the critical value (distribution is stable in time and nonrandom), we speak about preference of one of the stimuli (Fig. 2A). If it is lower than the critical value (frogs move at random), we speak about indifference (Fig. 2B).

To reveal a sensitive period we reared *R. lessonae* tadpoles with a natural marker (pond water) during one of the following stages of larval development (Gosner, 1960): till

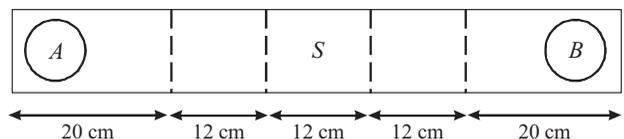


Fig. 1. Scheme of test-chamber (view from above): A, B, Petri dishes with chemical stimuli; S, starting position of froglets.

¹ Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Leninskie Gory 1/12, 119992 Moscow, Russia; E-mail: sk-ogurtsov@mtu-net.ru.

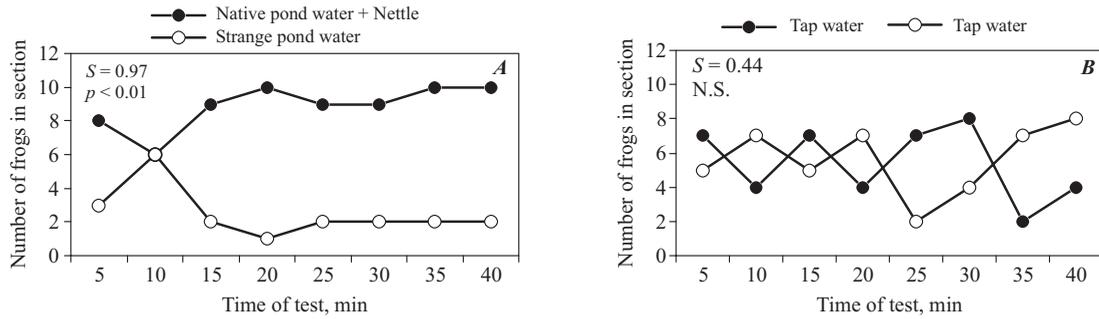


Fig. 2. Types of reaction: *A*, preference of *R. lessonae* froglets ($n = 12$) to familiar marker (native pond water with boiled nettle) compared to unfamiliar stimulus; *B*, indifference to two identical stimuli (dechlorinated tap water) in the same group.

hatching on stages 1 – 18 (Group 1), till the beginning of active feeding on 1 – 21 (Group 2), from the disappearance of external gills till the spade-shape hind limbs on 25 – 31 (Group 3), from the later one till the complete formation of hind limbs on 31 – 41 (Group 4). Group 4 received boiled nettle as a foodstuff during exposure. The water was changed once in 1 – 2 days. For the tests of Group 1 and 2 we used one pair of ponds (“native” vs. strange), and Group 5 that had passed the whole larval development in the native pond served as a control. For Group 3 and 4 we used another pair of ponds. Their “Control” group had no contact with pond water. Ponds treated in tests as “native” and strange were located within 0.7 – 1 km from each other.

RESULTS AND DISCUSSION

A long-lasting group test models the situation when juveniles keep near the pond before dispersal: they move back and forth from the native pond and keep their neighbors in view. According to our observations this period occupies from 3 – 7 days in terrestrial species (*R. tempora-*

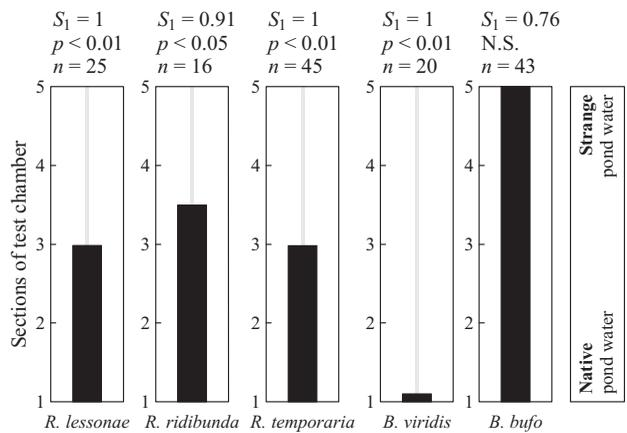


Fig. 3. Discrimination between water from the native pond and water from a strange one in juveniles of 5 anuran species soon after metamorphoses. Black rectangle, 25 and 75% percentiles (median 50% of individuals) of the median distribution of frogs in a chamber during a test (view from above); $S_1 - S$; value for section 1; p , difference from random distribution; n , number of frogs in a group.

ria, *B. viridis*) to 1 – 1.5 months in semiaquatic (*R. lessonae*). Juveniles of 4 species (*R. lessonae*, *R. ridibunda*, *R. temporaria*, *B. viridis*) caught near the native pond soon

TABLE 1. Influence of the Period of Exposure at Larval Stages on the Reaction of *R. lessonae* Froglets to the Native Pond Water

Group	Stages of exposure	Stimulus treated as “native”	Distribution between sections with stimulus, median (min – max)		Binomial p	S	Random model p	n
			native	strange				
Control	not exposed	pond water	6 (3 – 9)	5 (2 – 6)	N.S.	0.70	N.S.	12
Group 1	1 – 18	pond water	6 (1 – 8)	6 (3 – 9)	N.S.	0.39	N.S.	17
Group 2	1 – 21	pond water	17 (5 – 24)	9 (6 – 14)	<0.05	0.94	<0.05	37
Group 3	25 – 31	pond water	8 (6 – 10)	8 (5 – 9)	N.S.	0.68	N.S.	17
Group 4	31 – 41	pond water	6 (3 – 7)	6 (3 – 8)	N.S.	0.41	N.S.	12
		boiled nettle	5 (1 – 7)	4 (4 – 7)	N.S.	0.41	N.S.	12
		pond water + boiled nettle	9 (6 – 10)	2 (1 – 6)	<0.05	0.97	<0.01	12
Group 5	1 – 46	pond water	14 (11 – 18)	6 (4 – 7)	<0.05	1.00	<0.01	25

Note. n , Group size. S is given for section with “native” stimulus. Water from an unfamiliar pond was used as a strange stimulus, except for the test with boiled nettle where we used dechlorinated tap water.

after metamorphosis demonstrated preference to the native pond water. But *B. bufo* showed indifference to the same stimulus (Fig. 3). For *R. lessonae* our group tests gave the same results as individual tests (Bastakov, 1986).

R. lessonae tadpoles exposed to pond water on stages 1 – 21 (8 days) revealed preference to the “native” pond water as did Group 5 of natural froglets. As Group 1 did not form preference on stages 1 – 18, we consider learning to occur on stages 18 – 21 (4 days). Group 3 (exposed for 20 days) and Group 4 (48 days) showed no preference as did the Control group. But in Group 4 it appeared that only a complex stimulus actually used for exposure – a mixture of “native” pond water and boiled nettle (1 leaf stayed for 30 min in 200 ml of water), but not single components alone caused preference (Table 1, Fig. 2A). Video analysis of tests showed that movements of *R. lessonae* froglets were independent (they did not move in groups or follow each other). Thus we tried binomial test for time averaged (median) distribution, that gave us the same results (Table 1). They speak for the existence of 2 sensitive periods of chemical learning during larval development. We obtained the same results with artificial chemical markers: morpholine and β -phenylethanol (Ogurtsov and Bastakov, 2001).

REFERENCES

- Bastakov V. A.** (1986), “Preference by young-of-the-year of the edible frog (*Rana esculenta* complex) for their own reservoir ground smell,” *Zool. Zh.*, **65**(12), 1864 – 1868 [In Russian with an English abstract].
- Blair W. F.** (1953), “Growth, dispersal and age at sexual maturity of the Mexican toad (*Bufo valliceps* Wiegmann),” *Copeia*, **1953**(4), 208 – 212.
- Breden F.** (1987), “The effect of post-metamorphic dispersal on the population genetic structure of Fowler’s toad, *Bufo woodhousei fowleri*,” *Copeia*, **1987**, 386 – 394.
- Gosner K. L.** (1960), “A simplified table for staging anuran embryos and larvae with notes on identification,” *Herpetologica*, **16**(2), 183 – 190.
- Gotelli N. J.** (2000), “Null model analysis of species co-occurrence patterns,” *Ecology*, **81**(9), 2606 – 2621.
- Grubb J. C.** (1973), “Olfactory orientation in the breeding Mexican toad, *Bufo valliceps*,” *Copeia*, **3**, 490 – 497.
- Ogurtsov S. V. and Bastakov V. A.** (2001). “Imprinting on native pond odor in the pool frog, *Rana lessonae* Cam.,” in: Marchlewska-Koj A., Lepri J. J., and Müller-Schwarze D. (eds.), *Chemical Signals in Vertebrates. 9*, Kluwer Academic/Plenum Press, New York, pp. 433 – 441.

SOME ASPECTS OF REPRODUCTIVE BIOLOGY OF *Zootoca vivipara* (JACQUIN, 1787) IN THE ASIAN PART OF ITS AREA

V. F. Orlova,¹ V. N. Kuranova,² and N. A. Bulakhova²

Keywords: *Zootoca vivipara*, fecundity, body size, variability.

INTRODUCTION

Zootoca vivipara is the lacertid lizard with a large area in the northern part of Eurasia. This species is the only lacertid that has both oviparous and viviparous populations. Oviparous allopatric populations were found in the extreme south-western part of the species area, in the Pyrenean mountains, in Aquitaine in southwest France, and in northwest Spain (Lantz, 1927; Brana and Bea, 1987; Heulin, 1988). Recently the same populations were described from Slovenia, Lower Austria (Carinthia), and Italy (Böhme et al., 1999; Heulin et al., 2000; Mayer et al., 2000). Ovoviviparous populations have a vast distribution from Central France, the British Isles to the North Cape in Scandinavia, eastwards as far as eastern Siberia, Sakhalin Island and Hokkaido Island, Japan (Ananjeva et al., 1998). Thus, most part of the area is on territory of the former USSR, where there occur only ovoviviparous populations. In spite of this the biology of viviparous lizards, including reproductive characteristics, is insufficiently studied, especially in the eastern part of the area (Orlova, 1975; Sedalishchev and Belimov, 1978; Kuranova, 1983, 1998; Korotkov and Levinskaja, 1978; Korotkov, 1985; Tagirova, 1997; Dujsebaeva and Orlova, 2002). In the western part of the area, the biology of *Z. vivipara* is known in detail (see references in: Dely and Böhme, 1984; Heulin, 1985, 1988; Khodadoost et al., 1987; Pilorge, 1987; etc.).

MATERIAL AND METHODS

The reproduction of viviparous lizard was studied in the Perm' Oblast', on the West Siberian plain (Tomsk Oblast') and in the Kuznetskii Alatau mountains, North and North-East Altai [up to about 1200 m above the sea level (a.s.l.)]. In addition, we used the materials of the Zoological Museum of the Moscow State University, collected in Southern Altai (Markakolskaya Depression, 1500 – 1600 m

a.s.l.; foothills of Kuruchumskii Ridge up to 800 m a.s.l.), Novgorod Oblast', and the Southern Urals. A total of 375 females from 13 populations, with eggs in oviducts at different stages of development including the last ones, were used to estimate the fecundity. The maturity was determined by the gonad status of females in spring, and by the presence of mature spermatozoa in testicles and epididymes of males. Skeletochronological technique was used for age determination of sexual maturity (Smirina, 1974). The statistical processing of material was conducted with the spreadsheets MS Excel 7.0 and the statistical package STATISTICA 6.0. Differences of means were estimated by criterion of Mann – Whitney (*U*-test), degree correlation of indexes was estimated with help of rank correlation of Sperman (r_s).

RESULTS AND DISCUSSION

The earliest appearing of lizards after the hibernation was noted in the second – third decades of April. Females appear in 6 – 9 days to 2 – 3 weeks after males, depending on physical and climatic environmental parameters. After the hibernation, there were mature spermatozoa in large testicles of males. The minimum body length of sexually matured males was 44 – 46.0 mm. The active spermatogenesis lasts until the beginning of June, when the size and weight of testicles are the highest. The degradation of testicles starts after the breeding season. By the end of August, testicles increase in size again (up to 3.8×1.8 mm), which is connected with the preparation for the next season of reproduction.

The gonad development in the females also starts at the end of the hibernation, because 7 – 13 large (2.3 – 4.2 mm) yellow oocytes were contained in ovaries at the very first days of activity. The right ovary functions more intensively than the left one. In the end of May – middle of June mature oocytes 6 – 7.5 mm in diameter enter the oviduct. The egg size in June increases to $(12 – 14) \times (9 – 10)$ mm, and in July they contain well-formed embryos 15 – 21 mm

¹ Zoological Museum of the Moscow State University, B. Nikitskaya str. 6, 103009 Moscow, Russia; E-mail: orlova@zmmu.msu.ru.

² Tomsk State University, Lenina pr. 36, 634050 Tomsk, Russia; E-mail: kuranova@bio.tsu.ru; bulakhova@bio.tsu.ru.

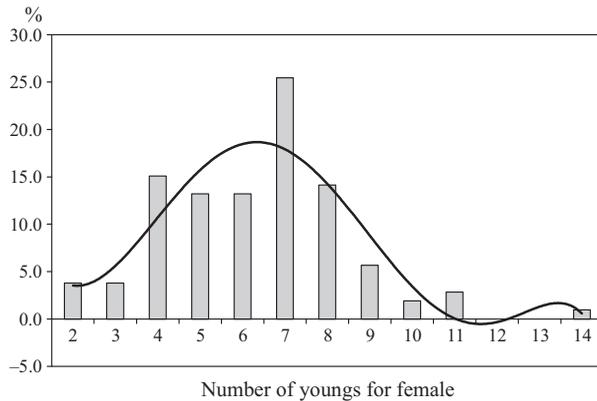


Fig. 1. Number of females (%) of viviparous lizard, *Zootoca vivipara* with the different brood size ($n = 106$, Tomsk area).

in length. After the birth of young animals (during July) the ovaries contain follicles 0.7 – 1.3 mm in diameter.

Reproductive parameters. The results obtained by the method of skeletochronology revealed that females have survived not less than 2 – 3 hibernations to participate in reproduction (Kuranova, 1998). Their body length was 45.6 – 77.3 mm (59.3 ± 0.49 ; $n = 227$), and the fecundity was 2 – 14 (6.1 ± 0.14) with mean values 3.6–8.6 embryos or young per 1 female in different populations. Progenies of 41.5% females consisted of 4 – 6 young, those in 25.5% — of 7 (Fig. 1). The most variable brood size

was observed in populations of the southern taiga plains (Tomsk) while less variable — in Chudnoe Lake population. A positive correlation was observed between fecundity and female size ($r_s = 0.68$; $df = 239$, $p < 0.001$). It was shown for oviparous and viviparous populations of the viviparous lizard from different parts of its range. Pregnant females have significantly more embryos in the right oviduct, than in the left one ($n = 100$; U -test, $p \leq 0.05$).

The average fecundity in Tomsk population was 5.6 – 7.1 (6.3 ± 0.21 ; $n = 106$) in different years. In two localities of Northeast Altai (Artybash and Kebezen) a similar situation was recorded (Table 1). It was demonstrated, that all females had survived three hibernations in Artybash population, i.e., had been born in 1997. It is likely, that small size of females and their low fecundity were caused by the drought during the second half of summer in 1998, which induced dramatic fall of water level in low-mountain rivers and, consequently, reduction food resources. Females from Markakolskaya depression were smaller than others and demonstrated the most variable body length. Their fecundity was less than the total mean. Their fecundity was less than the total mean, while the females from Kuruchum Mountains foothills was the largest. Different situation was observed in Kuznetskii Alatau Mountains, where the largest females with the maximal fecundity occurred in middle mountains (Chudnoe Lake, 1170 m a.s.l.). The lizards from the low mountains of Kuznetskii Alatau have smaller size and their fecundity is

TABLE 1. Populational Variability of Body Size and Fecundity of Females of *Zootoca vivipara* in Asian Part of Area

No.	Investigated regions	Years	Body length, mm			Number of embryos or young for one female		
			n	$x \pm m_x$	limits	n	$x \pm m_x$	limits
1	South Altai, Markakolskaya depression, Uspenka, 1500 – 1550 m a.s.l.	1988	28	54.3 ± 0.94	46.0 – 68.0	28	4.7 ± 0.21	2 – 7
2	South Altai, Markakolskaya depression, Urunhaika, 1500 – 1600 m a.s.l.	1988	11	54.7 ± 1.12	50.0 – 60.5	11	5.2 ± 0.40	4 – 8
3	South Altai, foothills of Kuruchumskii ridge, up to 800 m a.s.l.	1988	5	62.1 ± 1.03	59.5 – 64.5	5	8.2 ± 0.38	7 – 9
4	North Altai, 900 – 1100 m a.s.l.	2001	9	59.6 ± 1.80	46.8 – 64.8	9	7.7 ± 1.00	3 – 13
5	North-East Altai, Pryteletskii region, Artybash, 450 – 500 m a.s.l.	2000	7	55.9 ± 0.80	53.4 – 59.8	7	3.6 ± 0.30	3 – 5
6	North-East Altai, Kebezen, 450 – 550 m a.s.l.	2002	4	58.7 ± 1.74	55.3 – 63.4	4	5.8 ± 0.63	4 – 7
7	Kuznetskii Alatau, Gavrilovka, 550 – 600 m a.s.l.	1978	24	61.4 ± 0.70	52.0 – 67.6	24	6.0 ± 0.32	3 – 10
8	Kuznetskii Alatau, Chudnoe lake, 1170 m a.s.l.	2001	11	73.2 ± 1.08	64.6 – 77.3	11	8.6 ± 0.31	7 – 10
9	Tomsk Oblast', Prichuly'm'e, Teguldet, 350 m a.s.l.	2000	7	59.5 ± 1.11	55.4 – 63.2	7	5.1 ± 0.99	2 – 10
10	Suburbs of Tomsk, 90 — 160 m a.s.l.	1978 – 1989	58	58.9 ± 0.81	45.6 – 70.2	27	7.1 ± 0.41	4 – 11
		2001	15	60.5 ± 1.25	51.8 – 71.5	15	5.6 ± 0.77	2 – 14
		2002	64	59.9 ± 0.61	46.2 – 71.1	64	6.1 ± 0.23	2 – 10
11	Perm Oblast'	1962	25	61.4 ± 0.97	52.0 – 73.0	22	5.4 ± 0.31	3 – 9
12	Novgorod Oblast'	1977	8	57.6 ± 1.24	53.2 – 63.2	8	4.9 ± 0.52	4 – 8
13	South Urals	1938, 1982	5	62.8 ± 1.87	57.1 – 67.7	8	6.6 ± 0.68	5 – 9

by 1.4 times lower (Table 1). Thus our data did not show any clear correlation between the number of offsprings and the position of the population in respect to the sea level. But in two lizards (*Darevskia valentini* and *D. caucasica*) the body length in females and the size of clutch increased in populations from high altitudes (Darevsky, 1967). While the fecundity of *Lacerta strigata* is two times lower in high mountain population in comparison to plain population (Melkumyan, 1983). Additional investigations on representative samples are necessary to solve this question.

The comparison of obtained results with earlier published data did not reveal the connection between the size of the offspring and the climate. However, some authors (Terentjev and Chernov, 1949; Lazareva, 1999) indicate that the number of newborns decreases northward and eastward. According to our data, the average population and individual fecundity in the north is not lower than in southern populations as marginal populations of the Markakolskaya depression, where the average fecundity is 4.9 ± 0.25 (2 – 8). The fecundity values of the viviparous lizard in Yakutia do not differ from those in the females from the Western Siberia and Ural region, but higher, than in the populations from Novgorod and Perm' Oblast's (Sedalishchev and Belimov, 1978). In the north and east of the Asian part of the area, the values of individual fecundity range within the same limits as in the European part of the area.

Our data correspond well to the results obtained on the mountain population of viviparous lizard (Pilorge and Xavier, 1981), as well as to the population-ecological parameters of European Lacertidae (Bauwens, 1999). In these studies the inter-year variations result from fluctuations in weather and amount of food. Thus, the main reproductive characteristics (female size and fecundity, etc.) seem to be affected by ecological factors in habitats.

Acknowledgments. This work was supported by Russian Foundation for Basic Research (grant No. 02-04-48611) and Federal Objects Program "The Integration of Science and high education in Russia, 2002 – 2006."

REFERENCES

- Ananjeva N. B., Borkin L. Ya., Darevsky I. S., and Orlov N. L. (eds.) (1998), *Encyclopedia of Russian Nature. Amphibians and Reptiles*, ABF, Moscow [in Russian].
- Bauwens D. (1999), "Life-history variation in lacertid lizards," *Natura Croat.*, **8**, 3, 239 – 252.
- Böhme W., Heulin B., and Bischoff W. (1999), "First data on a second oviparous population of the viviparous lizard *Zootoca vivipara* Jacquin, 1787 (Squamata: Lacertidae) from NW Slovenia," in: *Abstrs. of the 10th Ord. Gen. Meet. Soc. Eur. Herpetol.*, Irakleio (Greece), pp. 34 – 35.
- Brana F. and Bea A. (1987), "Bimodalité de reproduction chez *Lacerta vivipara*," *Bull. Soc. Herpetol. Fr.*, **44**, 1 – 5.
- Darevsky I. S. (1967), *The Rock Lizards of Caucasus*, Nauka, Leningrad [in Russian].
- Dely O. and Böhme W. (1984), "*Lacerta vivipara* Jacquin, 1787 — Waldeidechse," in: Böhme W. (ed.), *Handbuch der Reptilien und Amphibien Europas. Band 2/1*, AULA, Wiesbaden, pp. 362 – 393.
- Dujsebaeva T. N. and Orlova V. F. (2002), "On distribution and ecology of viviparous lizard *Zootoca vivipara* (Jacquin, 1787) in Markakolskaya Depression (South Altai)," in: *Zool. Res. in Kazakhstan: Present Situation and Perspectives. Proc. of Int. Sci. Conf., March 19 – 21, 2002*, Almaty, pp. 165 – 166 [in Russian].
- Heulin B. (1985), "Maturité sexuelle et âge à la première reproduction dans une population de plaine de *Lacerta vivipara*," *Can. J. Zool.*, **63**(8), 1773 – 1777.
- Heulin B. (1988), "Données nouvelles sur les populations ovipares de *Lacerta vivipara*," *C. R. Acad. Sci. Paris*, **306**, 63 – 68.
- Heulin B., Guillaume C. P., Vogrin N., Surget-Groba Y., and Tadic Z. (2000), "Further evidence of the existence of oviparous populations of *Lacerta vivipara* in the NW of the Balkan Peninsula," *C. R. Acad. Sci. Paris*, **323**, 461 – 468.
- Khodadoost M., Pilorge T., and Ortega A. (1987), "Variations de la densité et de la taille corporelle en fonction de la longitude et de la composition du peuplement de proies dans trois populations de lézards vivipares du mont Lozère," *Rev. Ecol.*, **42**(2), 193 – 201.
- Korotkov J. M. (1985), *Terrestrial Reptiles of the Far East*, Dal'nevostochnoe Knizhnoe Izd., Vladivostok [in Russian].
- Korotkov J. M. and Levinskaja I. K. (1978), "Ecology of Amphibians and Reptiles of the Sakhalin island," in: *Ecology and Zoogeography of Some Vertebrates Terrestrial of the Far East*, Vladivostok, pp. 3 – 15 [in Russian].
- Kuranova V. N. (1983), "Some aspects of activity and behavior of *Lacerta vivipara* Jacq. in Tomsk Oblast'," in: *Ecology of Vertebrates at Siberia*, Izd. TGU, Tomsk, pp. 128 – 138 [in Russian].
- Kuranova V. N. (1998), *The Fauna and Ecology of Amphibians and Reptiles of the South-Eastern Part of Western Siberia. Author's Abstract of Candidate's Thesis*, Izd. TGU, Tomsk [in Russian].
- Lantz L. A. (1927), "Quelques observations nouvelles sur l'herpétologie des Pyrénées centrales," *Bull. Hist. Nat. Appl.*, **8**, 54 – 61.
- Lazareva O. G. (1999), "Reproductive biology of the common lizard, *Lacerta vivipara* (Sauria, Lacertidae) in reserves of the forest zone of Russia," in: *Abstrs. of the 2nd Conf. of Volga Region Herpetologists*, Tol'yatti, pp. 27 – 29 [in Russian].
- Mayer W., Böhme W., Tiedemann F., and Bischoff W. (2000), "On oviparous populations of *Zootoca vivipara* (Jacquin, 1787) in south-eastern Central Europe and their phylogenetic relationship to neighbouring viviparous and South-west

- European oviparous populations,” *Herpetozoa*, **13**(1/2): 59 – 69.
- Melkumyan L. S.** (1983), “The growth of *Lacerta strigata* in lowland and mountains,” *Zool. Zh.*, **62**(4), 580 – 584 [in Russian].
- Orlova V. F.** (1975), *Systematics and Some Morpho-Ecological Peculiarities of Lizards of the genus Lacerta. Author's Abstract of Candidate's Thesis*, Izd. MGU, Moscow [in Russian].
- Pilorge T.** (1987), “Density, size structure and reproductive characteristic of three populations of *Lacerta vivipara* (Sauria, Lacertidae),” *Herpetologica*, **43**(3), 345 – 356.
- Sedalishchev V. T. and Belimov G. T.** (1978), “Materials on ecology of the viviparous lizard (*Lacerta vivipara* Jacq.) in Yakutia,” *Biol. Nauki*, **10**, 59 – 66 [in Russian].
- Smirina E. M.** (1974), “Age determination,” in: Yablokov A. V. (ed.), *Sand Lizard*, Nauka, Moscow, pp. 239 – 245 [in Russian].
- Tagirova V. T.** (1997), *Reptilia of Khabarovsk Oblast'*, Khabarovsk [in Russian].
- Terentjev P. V. and Chernov S. A.** (1949), *Field Guide of Reptiles and Amphibians*, Sovetskaya Nauka, Moscow [in Russian].

CONSERVATION OF THE AGILE FROG — THE RAREST AMPHIBIAN IN THE BRITISH ISLES?

L. Racca¹

In Jersey, the agile frog (*Rana dalmatina*) has declined to a single small population that is displaying erratic breeding success. Survival of eggs from hatching to metamorphosis can vary from 0 to 20% per year. This gives rise to pulses in recruitment which are reflected in the age structures of adult frogs. Variations in recruitment — and the resulting population fluctuations — may be related to the predatory invertebrates in the breeding pools, which in turn are related to hydroperiod. Microsatellite DNA analyses were employed to evaluate genetic problems within the Jersey population of frogs. Samples collected from frog populations from mainland Europe were also been used, to compare the DNA profiles of frogs from Jersey and France. An indication of the level of population management that is likely to be needed in the future emerged from the genetic analyses and the analysis of field data. Captive breeding will have an essential role in the long term conservation of *Rana dalmatina* in Jersey.

Keywords: Agile frog, conservation, ecology, Jersey, microsatellite DNA, population dynamics, *Rana dalmatina*.

INTRODUCTION

The largest of the Channel Islands, Jersey (117 km²), situated in the Bay of Mont St. Michel, is little more than 20 km from the northwest coast of Normandy (France) and 128 km from the English coast. *Lacerta bilineata* (green lizard), *Podarcis muralis* (wall lizard, not found in the UK), *Natrix natrix* (grass snake) and *Anguis fragilis* (slow worm) are reptiles that can be found in Jersey, whereas the only amphibian species are *Bufo bufo* (common toad), *Triturus helveticus* (palmate newt) and, finally, the agile frog, *Rana dalmatina* (States of Jersey, 2003). Apart from Jersey, the agile frog is not found anywhere else in the British Isles (Grossenbacher, 1997). The Jersey population of agile frogs has been declining in both range and numbers since the early 1900s (Gibson and Freeman, 1997). In the 1970s, the frog could be found only in seven localities, and by the 1980s this had dropped to only two sites (Tonge, 1986). In 1987, a spill of agricultural pesticide in one of the sites caused the loss of one of the two remaining populations of frogs (Gibson and Freeman, 1997). Therefore, at the present, there is only one site in the island, a developing coastal heathland, which supports a wild population of *Rana dalmatina*. It is a nature reserve called Ouaisné Common. It presents a variety of habitats, such as wetland areas, blocks of gorse (*Ulex* spp.), dwarf shrub heath, grassland and areas of open sand. The agile frogs mainly breed in two ponds called North and South Slack. These, and the area around them, were intensely surveyed for three consecutive *Rana dalmatina* breeding seasons, from 2001 to 2003. The main aim of the study

was to shed light on the dynamics that regulate the last remaining wild population of agile frogs in Jersey, as such knowledge is essential in creating any long term plan for the management of a habitat and the conservation of the species living in it (Caughley and Gunn, 1996).

MATERIAL AND METHODS

Surveys were carried out, each year, from the end of January until the end of August. During each survey, the following variables were recorded: air temperature at time of visit, weather conditions, maximum, minimum, and current water temperature at about 20 cm depth and soil temperature at 20 cm depth. *Rana dalmatina* adults were caught, at night, when they returned to the water to breed and were marked using Trovan PIT tags (Heyer et al., 1994). In 2002 and 2003, each animal was also toe-clipped (Heyer et al., 1994) and the toes individually preserved in methylated spirit to be later used to age the frogs with the skeletochronology technique (Castanet and Smirina, 1990). Once the anurans started to lay eggs, day surveys were regularly carried out. Every time a new clump of eggs was found, approximately one tenth of the eggs in it were counted, and the total number of eggs was estimated by multiplying by ten. The percentage of live eggs was also recorded. In 2002 and 2003, all the frog eggs were enclosed in “bags” made with 1-mm gauge mesh that prevented newt predation. During each survey, the stage of the embryos (Gosner, 1960) and the date when hatching started were recorded. After hatching, the tadpole development was monitored and the start of metamorphosis’ emergence noted. Drift fences and pitfall traps (Heyer et al.,

¹ The Durrell Institute of Conservation and Ecology, University of Kent, Canterbury, Kent CT2 7NS, UK; E-mail: lauracca1@yahoo.it

1994) were used to try to catch froglets as soon as they started leaving the water. They were made with 1.5 m high plastic sheets supported by wooden poles. On the side facing the edges of the ponds, pitfall traps were placed, approximately one every 2 m. They were 25 cm high plastic shrub tubs, positioned in holes dug in the ground, covered by lids which had just a small circular opening, in order to stop the froglets from jumping out. In 2001, the fences encircled the ponds only partially, whereas in the two next years both water bodies were completely enclosed by them. The pitfall traps were checked every morning. In 2001, all the froglets caught in the traps or found walking along the fences were marked with waterproof ink scratched with the needle of a microfine syringe (1 ml, 0.33×13 mm) on the skin of one of their back legs or of their throat. In 2002 and 2003 marking was not necessary, as all the metamorphs captured in the traps and along the side of the fence facing the water were released on the other side of the fence, and therefore never caught again.

During a four month study carried out at Ouaisné (Racca, 2000), before starting the first year's fieldwork, the potential aquatic predators of *Rana dalmatina* tadpoles were identified as being palmate newts (*Triturus helveticus*) and various species of macroinvertebrates, such as water beetles, backswimmers, Odonata nymphs and water spiders. Therefore, during all three years of fieldwork, data about their presence and relative abundance were collected in both slacks at Ouaisné. Adult palmate newts were caught in the water, as they regularly came back to the ponds towards the end of the winter to breed, using funnel traps (Griffiths and Inns, 1998). Monthly standardized net-

ting sessions were performed in order to catch and identify macroinvertebrates. Also, as grass snakes (*Natrix natrix*), ducks and cats are terrestrial predators of agile frogs at Ouaisné, they were always recorded if seen.

Finally, microsatellite DNA loci, used as molecular markers (e.g., Zane et al., 2002), were employed to try to compare the level of genetic variation between the Jersey frog population and populations from France, Germany and Italy (Table 1). Microsatellite loci isolated in *Rana temporaria* [*Rtempμ1*, *Rtempμ5*, and *Rtempμ1*, see (Rowe and Beebee, 2001a)] and *Rana latastei* [*RlatCa17*, see (Garner and Tomio, 2001)] were used in the analyses, as they showed polymorphism in *Rana dalmatina*.

RESULTS

Data about the frog spawn development are summarized in Table 2.

Newt were significantly more abundant in 2002 and 2003 than in 2001 (ANOVA, $p < 0.001$) (Fig. 1). Moreover, in 2001 dragonfly and damselfly nymphs and backswimmers were more abundant than in the two following years (ANOVA, $p = 0.006$, $p = 0.008$, and $p < 0.001$, respectively), whereas damselfly nymphs were present in significantly lower numbers (ANOVA, $p = 0.008$) (Fig. 2). The results of the skeletochronology are reported in Fig. 3, which shows the percentage of 1, 2, 3, and 4 year old frogs breeding at the site in 2002 and 2003.

Finally, the results of the genetic tests showed that just 1 locus was polymorphic in the Jersey population, 2 in the Italian population and 3 in the animals from France. In the German population, 3 loci were polymorphic at Brühl and all 4 at Dieburg.

CONCLUSIONS

On the whole, a picture of Ouaisné as an adequate, sometime exceptionally successful, *Rana dalmatina*

TABLE 1. Details of *Rana dalmatina* Sampling

Population	N	Collected by	Date	Location
Jersey	40	L. Racca	2002	Ouaisné Common
France	40	L. Racca	2002	Bois d'Ardennes (Avranches)
Italy	39	Dr. E. Marzona	2003	Airasca (Torino)
Germany	30	Dr. A. Kupfer	2003	Brühl
	10	Dr. T. Bobbe		Dieburg

Note. N, number of samples collected at each location.

TABLE 2. Summary of Data about *Rana dalmatina* Spawn Development

Parameter	North Slack			South Slack		
	2001	2002	2003	2001	2002	2003
Number of clutches	4	6	12	2	3	4
Estimated number of live eggs	1750	4120	6500	750	1600	2500
Minimum embryonic developmental time, days	36	42	35	27	34	27
Minimum tadpole developmental time, days	66	56	55	78	59	58
Total number of froglets caught	42	553	484	26	427	195
Min percentage survival from egg to metamorphosis	2.4	13.4	7.4	3.5	26.7	7.8

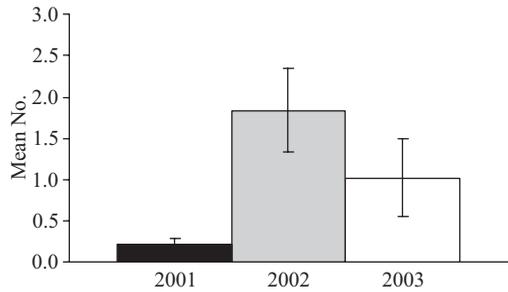


Fig. 1. Mean number of newts per trap and standard deviations, caught in North and South Slacks.

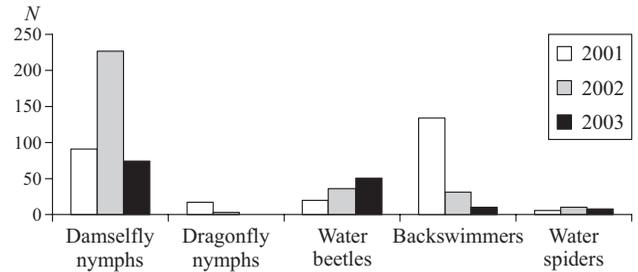


Fig. 2. Abundance of aquatic macroinvertebrates in *Rana dalmatina* breeding ponds at Ouaisné.

breeding site emerged from the results of the analyses of the data collected in 2001 – 2003. Low rates of embryonic and tadpole mortality were recorded during all three years of surveys and this resulted in at least 2.4% of the tadpoles completing their development successfully in 2001 (Table 1). This is a good percentage, when compared to the expected 1 – 2% (Beebee, 1985) for wild anuran populations. In 2002 and 2003, the situation improved even further, with an increased number of clumps laid, reflecting higher numbers of females breeding at Ouaisné, and an exceptionally high — 17.1 and 7.5%, respectively — recruitment of froglets (Table 1). Finally, once the metamorphs — the most vulnerable phase of a frog's life — emerged from the water and dispersed in the surrounding area, they most likely did not suffer from heavy predation, since grass snakes, their most dangerous predators, presently populate Ouaisné Common in extremely low numbers (McMillan, personal communication). However, in the past, the situation has not always been so successful, as data collected during the ten years preceding the start of the present research report years when no frog breeding occurred (Racca, 2002). Moreover, the results of the skeletochronology analysis showed that most of the frogs currently breeding at Ouaisné are young individuals, rarely older than 2 or 3 years old (Fig. 2). These results therefore suggest erratic recruitment of metamorphs at Ouaisné.

A much higher proportion of tadpoles successfully completed their development in 2002 and 2003 than in 2001 (Table 1), even though no apparent changes seemed to have occurred at the breeding site. The cause of such a difference may be found by analyzing the mechanisms that regulate the relationships between the various components of the ecosystem to which the agile frogs belong. From all the observations made during three years of surveys, the main factors influencing the frog aquatic phase are 1) water quality, 2) disturbance at the site, 3) competition, 4) predation, and 5) hydroperiod. As the four first factors did not vary significantly between years, the last two may

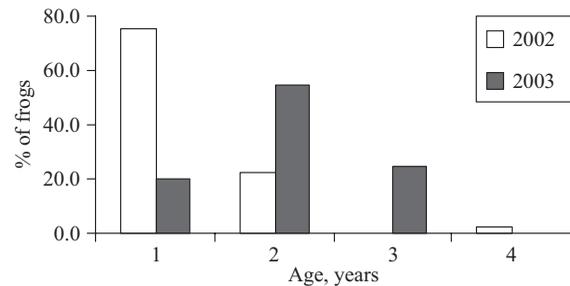


Fig. 3. Percentage of 1, 2, 3 and 4 year old frogs caught at Ouaisné in 2002 and 2003.

be responsible for the differences in recruitment observed. Each year's hydroperiod seems to have had a prominent role in regulating the composition of the frog tadpole predators' population. The fact that, due to an exceptionally dry winter, both *Rana dalmatina* breeding ponds did not hold any water from October 2001 to the following January was certainly the cause of the significant variations in abundances of some of the macroinvertebrates species (Loman, 2002). Moreover, protection of the spawn in 2002 and 2003 lowered the level of newt predation upon the eggs. Thus, protection of the spawn is a conservation measure that, as it does not require much effort, in terms of time and costs involved, should be adopted at Ouaisné every year. Moreover, the risk associated with the population inhabiting just a small area and mainly breeding in two ponds next to each other is another aspect to be taken into account when planning conservation actions. A single catastrophic event, such a spill of pollutants into the water during the reproductive season, could kill most of the population and make the habitat unsuitable for the frogs for a very long time, as was the case at Noirmont, historically a *Rana dalmatina* breeding site in Jersey (Racca, 2002). Furthermore, the results of genetic analyses have suggested that the *Rana dalmatina* population living at Ouaisné had low levels of genetic variation, possibly due to drift and inbreeding, when compared to agile frog popu-

lations from mainland Europe. Although this has not always been proved true (e.g., Rowe and Beebee, 2001b), low level of fitness may be correlated with low levels of genetic variation (Frankham, 1998; McAlpine, 1993; Newman and Pilson, 1997; Ralls et al., 1988; Saccheri et al., 1998). Therefore, more genetic analyses, accompanied by experimental tests, should be carried out in Jersey to measure the fitness of the frogs. Finally, whatever the reason for the low genetic variability detected in the frog population breeding at Ouaisné, either a result of their life history, or a consequence of being on an island (Frankham, 1998), any plans for its conservation have to aim to ultimately increase, or at least maintain, the current level of population genetic variation. First of all, the distribution range of the agile frogs in Jersey should be expanded, perhaps by creating a metapopulation at Ouaisné, through translocations. Finally, *Rana dalmatina* individuals from the captive sites (for details about the *Rana dalmatina* captive breeding program in Jersey, see: Partridge, 1995; Racca, 2002) should be introduced in new suitable sites across the island.

Acknowledgments. I wish to thank my supervisor, Dr. R. Griffiths. I am grateful to the Jersey Ecology Trust Fund, the Environmental Services Unit, the Jersey law firm Mourant de Feu & Jeune and the bank HSBC for sponsoring my research. Thanks also to the Durrell Wildlife Conservation Trust and to all the members of the Jersey Agile Frog Group for their constant support. I am greatly indebted to Prof. T. Beebee and Dr. F. Kraaijeveld-Smit for their help with the genetics analyses, to Dr. T. Garner for his primers and to Dr. E. Marzona, Dr. A. Kupfer and Dr. T. Bobbe for collecting samples for me. Finally, I very much appreciated Dr. C. Giacoma's and Dr. A. Rosso's assistance with the skeletochronology.

REFERENCES

- Beebee T. (1985), *Frogs and Toads*, Whitted Books, London.
- Castanet J. and Smirina E. (1990), "Introduction to the skeletochronology method in Amphibia and Reptilia," *Ann. Sci. Nat. Zool.*, **11**, 191 – 196.
- Caughley G. and Gunn A. (1996), *Conservation Biology in Theory and Practice*, Blackwell Science Ltd., Oxford.
- Frankham R. (1998), "Inbreeding and extinction: island populations," *Conserv. Biol.*, **12**(3), 665 – 675.
- Garner T. W. J. and Tomio G. (2001), "Microsatellites for use in studies of the Italian agile frog, *Rana latastei* (Boulenger)," *Conserv. Gen.*, **2**, 77 – 80.
- Gibson R. and Freeman M. (1997), "Conservation at home; recovery programme for the agile frog *Rana dalmatina* in Jersey," *Dodo. J. Wildl. Preserv. Trusts*, **33**, 91 – 104.
- Griffiths R. A. and Inns H. (1998), "Surveying," in: Gent A. H. and Gibson S. D. (eds.), *Herpetofauna Worker's Manual*, Joint Nature Conservation Committee, Peterborough, pp. 1 – 13.
- Grossenbacher K. (1997), "*Rana dalmatina*," in: Gasc J. P. et al. (eds.), *Atlas of Amphibians and Reptiles in Europe*, SHE and SPN-MNHN, Paris, pp. 135 – 136.
- Heyer W. L. et al. (1994), *Measuring and Monitoring Biological Biodiversity: Standard Methods for Amphibians*, Smithsonian Institution Press, Washington.
- Loman J. (2002), "*Rana temporaria* metamorph production and population dynamics in the field. Effects of tadpole density, predation and pond drying," *J. Nat. Conserv.*, **10**(2), 95 – 107.
- McAlpine S. (1993), "Genetic heterozygosity and reproductive success in the green treefrog, *Hyla cinerea*," *Heredity*, **70**, 553 – 558.
- Newman D. and Pilson D. (1997), "Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*," *Evolution*, **51**, 354 – 362.
- Partridge J. (1995), *Jersey Agile Frog Report. April to September 1995*, Countryside Services Department, States of Jersey Planning and Environment Committee (unpublished), Jersey.
- Racca L. (2000), *Agile Frog in Jersey: a Field Study (March – June 2000)*, Environmental Services Unit, States of Jersey Planning and Environment Committee (unpublished), Jersey.
- Racca L. (2002), "The conservation of the agile frog, *Rana dalmatina*, in Jersey (Channel Islands)," *Biota*, **3**(1–2), 141 – 147.
- Ralls K., Ballou J. D., and Templeton A. R. (1988), "Estimates of lethal equivalents and the cost of inbreeding in mammals," *Conserv. Biol.*, **2**, 185 – 193.
- Rowe G. and Beebee T. J. C. (2001a), "Primer note. Polymerase chain reaction primers for microsatellite loci in the common frog *Rana temporaria*," *Mol. Ecol. Notes*, **1**, 6 – 7.
- Rowe G. and Beebee T. J. C. (2001b), "Fitness and microsatellite estimates were not correlated in two outbred anuran populations," *Heredity*, **87**, 558 – 565.
- Saccheri I., Kuussaari M., Kankare M., Vikman P., Fortelius W., and Hansky I. (1998), "Inbreeding and extinction in a butterfly metapopulation," *Nature*, **392**, 491 – 494.
- States of Jersey (2003), <http://www.gov.je>, accessed July 2003.
- Tonge S. (1986), "The herpetofauna in Jersey," *Br. Herpetol. Soc. Bull.*, **17**, 18 – 19.
- Zane L., Bargelloni L., and Patarnello T. (2002), "Strategies for microsatellite isolation: a review," *Mol. Ecol.*, **11**, 1 – 16.

THE ROLE OF THE NATIVE POND ODOR IN ORIENTATION OF THE GREEN TOAD (*Bufo viridis* LAUR.) YOUNGS-OF-THE-YEAR

V. V. Shakhparonov¹ and S. V. Ogurtsov¹

Keywords: toadlets, orientation, native pond odor, buffer zone, periods of learning.

INTRODUCTION

It is known that froglets of the pool frog *Rana lessonae* keep near the native pond for 1 – 1.5 months after metamorphosis and this pattern of behavior is, at least partly, based on the preference for native pond odor that is learnt during larval development on stages 18 – 21 and 32 – 43 (stages are given by Gosner, 1960). Preference gradually changes to indifference or rejection with the onset of dispersal (Bastakov, 1986; Ogurtsov and Bastakov, 2001). Unlike the pool frog that belongs to a semiaquatic ecological group of Anura representatives of terrestrial ecological group, e.g., *Bufo viridis*, start to disperse from the native pond much earlier — within a few days. Does the dynamics of the reaction to the native pond odor in terrestrial species resemble that in *R. lessonae*? Does learning of the native pond odor in toads occur as a two-staged process as it is in the pool frog?

MATERIAL AND METHODS

The subject of our study was the green toad *B. viridis* obtained from the pond (~600 m²) that was located 60 km west from Moscow. Each day from the onset of metamorphosis in the pond we calculated the average density (mean calculated from minimum and maximum number of individuals per 1 m² observed at the place of the largest emergence of toadlets, total area of 10 m²) of tadpoles, toadlets near the water edge and toadlets at a distance of 5 – 8 m from the native pond in vegetation. The day when toadlets moved further than 50 m away from the pond was regarded as the start of dispersal (explanations in the text). To reveal the reaction of wild individuals (ind.) to native pond water we caught 10 groups 5 – 8 m away from the pond (5 groups, 179 ind., before and 5 groups, 148 ind. after the start of dispersal) and 5 groups (138 ind.) 50 – 500 m from the pond in the open field.

Juveniles were tested under pair choice conditions in a plastic chamber 76 × 12 × 15 cm divided into 5 sections. Tests were conducted under low illumination conditions. A pair of odorants (for natural toadlets - native pond water vs strange pond water, for artificially reared toadlets - tap water solution of chemical marker vs. tap water) was positioned in Petri dishes at the corners of the chamber. Each experimental group was divided into 2 or 4 subgroups of 6 – 10 individuals. Each subgroup was tested separately with an altered position of stimuli. At the start of a test a subgroup was placed in the center of chamber. For 40 min toadlets were left walking freely in a chamber and every 5 min we counted the number of individuals in sections. Then results of subgroups' tests were combined in accordance to the position of the familiar stimulus thus obtaining 8 consecutive observations on the distribution of a group as a whole. To analyze the repeated observations we compared our results with the model of random process using a distribution stability coefficient (*S*). It changes from 0 to 1. The more often toads locate themselves in a section with a native odor, the higher is the difference between the number of toads in the opposite sections the larger is the *S* for the section with the native odor (“+*S*”). If *S* obtained in a test is larger than 0.83 the distribution of toads is regarded as stable with $p < 0.05$. For “+*S*” distribution is interpreted as “preference” (Fig. 1A), for “–*S*” as “rejection.” If neither “+*S*,” nor “–*S*” exceed critical value of 0.83 the distribution is regarded as “indifference” (Fig. 1B) (for details of the method see Ogurtsov, 2005). In addition we used Wilcoxon matched pairs test to compare the number of individuals visiting the opposite sections during the test. From each group we obtained a pair of numbers, average numbers of toads that visited the opposite sections with native and strange stimuli during a test.

To reveal the learning period in larval development we reared tadpoles in the presence of a chemical marker (Table 1). After metamorphosis toads were tested with a chemical marker compared to dechlorinated tap water. For the tests we used morpholine in concentrations 10⁻⁸, 10⁻⁷, 10⁻⁶ mole/liter.

¹ Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Leninskie Gory 1/12, 119992 Moscow, Russia; E-mail: wshakh@yandex.ru, sk-ogurtsov@mtu-net.ru

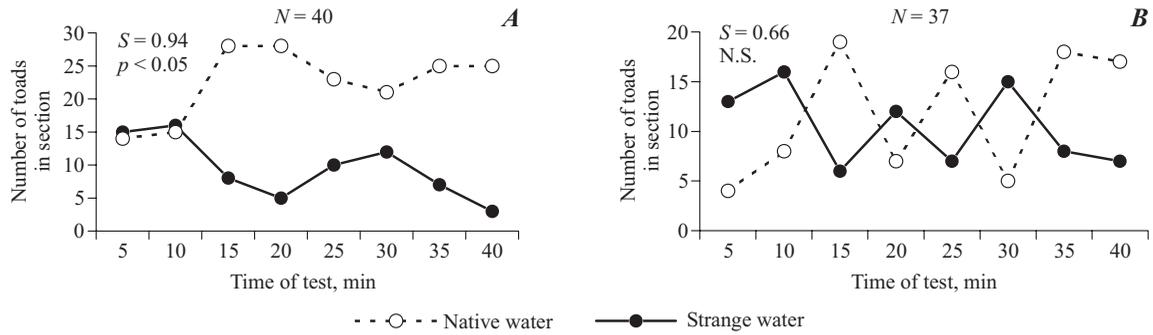


Fig. 1. Types of reaction to familiar water: **A**, preference (caught before start of disperse); **B**, indifference (caught after start of disperse).

RESULTS AND DISCUSSION

When moving from water to land, metamorphosing individuals did not stay long near the water edge, rather they all moved 5 – 8 m away to vegetation and formed a large aggregation there (Fig. 2). We called the zone of large aggregation a “buffer” zone as the density of aggregation there remains stable for a long time due to income of tailed toadlets and outcome of dispersing individuals. Zone with moist soil and plenty of shelters seems to be favorable for

toadlets to complete their physiological changes associated with metamorphosis. When toadlets start to leave the “buffer” zone we talk about the beginning of dispersal. After a week from the beginning of dispersal the majority of toadlets were found 500 m away from the pond.

Individuals from the “buffer” zone prefer the native pond water during a week before the beginning of dispersal (as described by a distribution stability coefficient *S*; Fig. 3). Soon after the start of dispersal some groups of toads caught in the “buffer” zone could still demonstrate preference to the native pond water, while others show indifference or rejection. The latter are, probably, individuals ready for dispersal. At the same time some groups of toads caught 50 m away from pond could also reveal preference to the native pond water. These could be individuals that make their first exploratory routes but have not decided to leave the pond yet. Later toadlets caught at far larger distances from the pond demonstrate indifference to the native pond water. Wilcoxon’s matched pairs test reveals significant differences between the number of individuals visiting native and strange stimuli only for toadlets caught in the “buffer” zone before dispersal (Fig. 4).

In the current study we investigate only temporal but not causal relations between toads behavior near the pond and behaviour in laboratory. Could toadlets simply forget the native pond odor with time thus revealing indifference

TABLE 1. Groups of Tadpoles Reared in Different Chemical Markers

Group	Exposition period, stages	Period characteristics	Marker (10 ⁻⁷ mole/liter)
1	19 – 21 (first sensitive period)	From hatching till the beginning of active feeding	Morpholine
2	31 – 41 (second sensitive period)	From formation of knee joint till appearance of forelimbs	Morpholine
3	31 – 41 (second sensitive period)	From formation of knee joint till appearance of forelimbs	Phenylethanol + isoamyl acetate + geraniol
Control	1 – 46		No marker

TABLE 2. Results of Tests with Chemical Markers

Group	N	Marker concentration, mole/liter	Distribution between sections with stimulus, median (min – max)		S for sections with stimulus		Random model p
			native	strange	native	strange	
Control	19	A10 ⁻⁸	8 (7 – 11)	8 (6 – 10)	0.55	0.39	N.S.
	32	B10 ⁻⁸	14 (6 – 18)	13 (10 – 19)	0.44	0.56	N.S.
1	18	A10 ⁻⁸	12 (9 – 14)	5 (2 – 8)	1.00	0.00	<0.01
2	20	A10 ⁻⁸	6 (3 – 7)	4 (3 – 6)	0.96	0.04	<0.01
	20	A10 ⁻⁶	4 (0 – 6)	6 (4 – 7)	0.03	0.97	<0.01
3	20	B10 ⁻⁸	5 (1 – 11)	2 (1 – 3)	0.94	0.06	<0.05

Note. A, morpholine; B, phenylethanol + isoamyl acetate + geraniol.

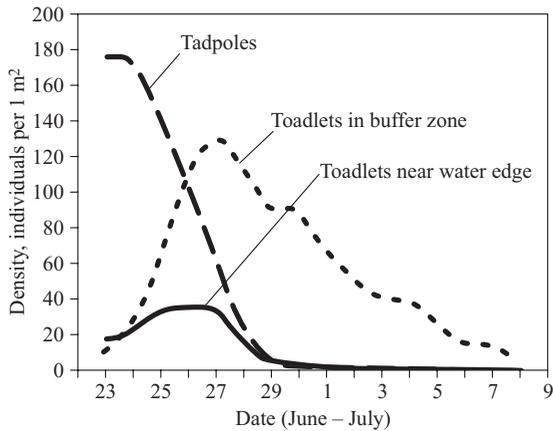


Fig. 2. Dynamics of toads' density.

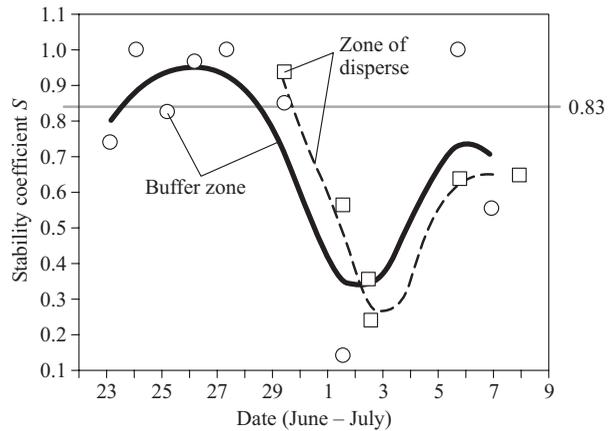


Fig. 3. Dynamics of stability coefficient S (least squares approximation).

to it during dispersal? A special study showed that the main parameters determining the type of reaction to the native pond odor in *B. viridis* and *R. temporaria* are the age of juveniles (but not the time spent on land after metamorphosis) and, probably, temperature and moisture conditions that determine the onset of dispersal (Arhipova et al., in press). Thus groups of wild toads hold in laboratory for 9 – 14 days still preferred the odour whereas newly caught individuals from natural conditions started to disperse and were indifferent to the same stimulus (unpublished data). So it seems that toads could switch the reaction off rather than forget it.

Toadlets that were reared in a chemical marker at first (Group 1) and at second exposition period (Groups 2, 3) showed preference to it (Table 2). Concentration of the marker more than that used during exposition caused rejection. One could assume that toadlets interpret high concentration of familiar odor as excessive proximity to native pond and try to keep some distance away from the odorant, as probably do wild toads from the “buffer” zone. Control group reacted indifferently to all chemical markers offered.

Like semiaquatic species, such as *R. lessonae*, *B. viridis* toadlets stay for a certain period near the native pond and prefer its odor. After the onset of dispersal they become indifferent or reject this odor. But unlike randomly distributed froglets of *R. lessonae*, green toads form dense aggregation in a “buffer” zone where they undergo physiological maturation. Like *R. lessonae* the green toad seems to have two periods of learning of chemical stimuli marking the native environment during larval development. The type of reaction to native chemical marker could depend on its concentration, the fact also known for the pool frog (Ogurtsov and Bastakov, 2001). Thus, representatives

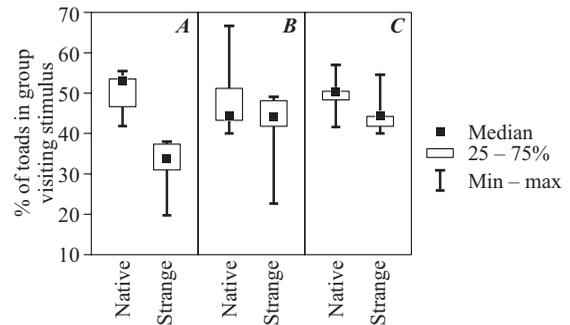


Fig. 4. Reaction to the native pond odor in different zones and in different stages of dispersal: *A*, buffer zone before dispersal, $p < 0.05$; *B*, buffer zone after starting of disperse; *C*, zone of disperse.

of two ecological groups of Anura (*R. lessonae* and *B. viridis*) have much in common concerning the periods of learning and dynamics of reaction to the native pond odor.

REFERENCES

Arhipova K. A., Borisov V. V., Van Yuanvan, Go Danian, Kazakov A. S., Mehova E. S., Minin K. V., Nilmaer O. V., Skvortsov T. A., Tretiakova J. A., Ukolova S. S., Shakhparonov V. V., and Ogurtsov S. V. (in press), “Parameters determining the type of reaction to the native pond odour in juveniles of the common grass frog (*Rana temporaria*) and of the green toad (*Bufo viridis*),” in: *Flora and Fauna of the Western Part of Moscow Region* [in Russian with English abstract].

Bastakov V. A. (1986), “Preference by young-of-the-year of the edible frog (*Rana esculenta* complex) for their own reservoir ground smell,” *Zool. Zh.*, **65**(12), 1864 – 1868 [in Russian with English abstract].

- Gosner K. L.** (1960), "A simplified table for staging anuran embryos and larvae with notes on identification," *Herpetologica*, **16**(2), 183 – 190.
- Grubb J. C.** (1973), "Olfactory orientation in the breeding Mexican toad, *Bufo valliceps*," *Copeia*, **1973**(3), 490 – 497.
- Ogurtsov S. V.** (2005), "Basis of native pond fidelity in anuran amphibians: the case of chemical learning," in: Ananjeva N. and Tsinenko O. (eds.), *Herpetologia Petropolitana. Proc. of the 12th Ord. Gen. Meeting of the Soc. Eur. Herpetol., August 12 – 16, 2003*, St. Petersburg, pp. 198 – 200 [this issue].
- Ogurtsov S. V. and Bastakov V. A.** (2001), "Imprinting on native pond odor in the pool frog, *Rana lessonae* Cam.," in: Marchlewska-Koj A., Lepri J. J., and Müller-Schwarze D. (eds.), *Chemical Signals in Vertebrates. 9*, Kluwer Academic/Plenum Publishers, New York, pp. 433 – 441.

NEW DATA ON REPRODUCTIVE BIOLOGY OF CAUCASIAN SPECIES OF THE GENUS *Vipera*

K. A. Shiryaev¹

Keywords: *Vipera*, Caucasian species, captive breeding, reproductive biology, comparative analysis.

INTRODUCTION

Numerous investigations on taxonomy, geographical distribution, ecology and phylogeny of Caucasian representatives of the genus *Vipera* Laurenti, 1768 were carried out for more than 100 years (for details see Nikolsky, 1913; Orlov and Tuniyev, 1986; Nilson et al., 1995). Modern investigations include the use of enzyme electrophoresis (Nilson et al., 1995) and sequencing of the mitochondrial cytochrome b gene (Joger, in press). At the same time, fragmentary data on the reproductive biology of Caucasian *Vipera* species were cited only for such taxa as *Vipera kaznakovi*, *V. dinniki*, *V. renardi*, *V. ammodytes transcaucasiana*, and *V. r. raddei* (Bozhansky, 1984; Orlov and Tuniyev, 1986; Darevsky and Orlov, 1988; Nilson et al., 1995; Trutnau, 1998). This may be due to the fact that the authors were not working with captive breeding groups of vipers, and field investigations were not involved with accumulating quantitative data on the biology of certain species.

An acute deficiency of data has not permitted a comparative analysis of various aspects of the reproductive biology of Caucasian vipers, or to reveal specific, subspecific and population differences. However, the results of

such analyses, especially involving research in hybridization, can be used to reveal the mechanisms of reproductive isolation, and also for solving controversial questions of the taxonomy of *Vipera* genus. On the other hand, working out methods for breeding reptiles under terrarium conditions is a necessary part of zoo activity focusing on nature conservation.

Some preliminary results of research on the breeding biology of *V. lotievi*, *V. eriwanensis*, *V. renardi*, and *V. a. transcaucasiana* have been published earlier (Shiryaev, 2002, 2003). This paper is the first attempt to compare some characteristic aspects of the reproductive biology of Caucasian representatives of the genus *Vipera*.

MATERIAL AND METHODS

Investigations were carried out in 1999 – 2003 at the Tula Exotarium with breeding groups (from 6 to 22 specimens in each group) of 9 taxa. The origin of all animals is given in Table 1. Detailed methods of keeping vipers of the genus *Vipera* at the Tula Exotarium have been described previously (Shiryaev, 2002). Annual simultaneous taking out in March from the hibernation the temperature of which was +2 – 12°C, the separate keeping of males and females, using in March – April as factors stimulating

¹ Tula Regional Exotarium, Oktyabr'skaya str. 26, 300002 Tula, Russia; E-mail: rept@tula.net.

TABLE 1. Origin of the Specimens Used in the Investigations of Reproductive Biology

Taxon	Sample locality	Altitude, m
<i>Vipera orlovi</i>	Russia, Krasnodar Krai: Praskoveevka, Mikhailovskii and Papaiskii passes, Aderbievka	70 – 360
<i>V. kaznakovi</i>	Russia, Krasnodar Krai, Adler	≤50
	Russia, Krasnodar Krai, Krasnaya Skala	400
<i>V. dinniki</i>	Russia, Krasnodar Krai, Achishkho Mountain	1200 – 1800
	Russia, Krasnodar Krai, Kardyvach Lake	1850 – 2000
<i>V. darevskii</i>	Armenia, Legli Mountain	2600 – 2800
<i>V. renardi</i>	Russia, Krasnodar Krai: Verkhnebakanskii, Goryachii Klyuch	150 – 250
	Russia, Volgograd Oblast', Kamyshevka	<200
<i>V. lotievi</i>	Russia, North Ossetia, Karmadon	1200 – 2000
<i>V. eriwanensis</i>	Armenia, Ara-Iler Mountain	1800 – 2300
<i>V. ammodytes transcaucasiana</i>	Georgia, 15 km to the west from Tbilisi	1100 – 1500
<i>V. r. raddei</i>	Armenia, Yerevan – Sevan region	>1500

breeding of every-day seances of ultraviolet radiation and gradual increasing of light day in the laboratory were necessary conditions of our work.

Introductions of males and females were initiated on the 7th day following the end of hibernation; introductions were repeated every day until all signs of sexual activity ceased in males. We noted the dates of matings for each specimen, the duration of copulations, dates of giving birth and the number of neonates for each female. Dates of giving birth for several females which were already gravid when caught were registered separately. The method of hybridization was used since 2002.

RESULTS AND DISCUSSION

Mating period. The main temporal characteristics (the maximum interval between the end of hibernation and the first mating, the interval between the first and last copulations during the spring, the duration of matings and the interval between copulations for males which mated repeatedly during the spring) noticeably vary in specimens within each taxon. But, between the taxa, there are clearly marked differences in these characteristics (Table 2). The ability of male vipers to mate with several females during one breeding season has already been noted for *V. r. raddei* (Trutnau, 1998), and was confirmed by our investigations for six Caucasian species. The following example is demonstrative. One male of *V. lotievi* during an 8-days period (from March 29 to April 6, 2002) mated in turn with four

females which gave birth in July to 60 viable neonates. Females of the species investigated by us copulated in terrarium during the spring, with a rare exception, one time

Period of gravidity of females. The period of gravidity (from mating till giving birth) in vipers which are kept in captivity under optimum temperature conditions and food supply is somewhat shorter than in specimens of the same species which occur in nature. Therefore, females of taxa which we investigated (data are lacking for *V. darevskii*) under laboratory conditions mated from the middle of March till the middle of April and gave birth in June, or much more rarely in July. In natural habitats, females of *V. renardi*, *V. kaznakovi*, *V. dinniki*, *V. a. transcaucasiana*, and *V. r. raddei* mate in March-May and give birth in August – September. Their gravidity, therefore, lasts more than 3 months (Bannikov et al., 1977; Bozhansky, 1984; Orlov and Tuniyev, 1986; Ananjeva et al., 1998). Our observations of females caught 1 – 1.5 months before giving birth confirm and supplement the literature data (Table 3). However, by keeping all species of the genus *Vipera* in Tula Exotarium, this permitted us to compare the relative period of gravidity of females belonging to different taxa (for details see **Comparative analysis**).

Potential of breeding. *V. lotievi*, *V. r. raddei*, and *V. orlovi* have the greatest productivity among Caucasian species of *Vipera* (Table 2). The maximum total number of newborns received from one female over 3-years period (females were breeding annually) was: *V. lotievi* — 41, *V. r. raddei* — 31, *V. orlovi* (Papaiskii pass) — 31, *V. re-*

TABLE 2. Reproductive Biology of Caucasian Representatives of *Vipera* Genus under Terrarium Conditions

Species subspecies	Minimum interval between the end of hibernation and first mating, days	Maximum duration of period of matings, days	Duration of copulation, h:min, min – max/x	Number of copulations during the spring (for males), max/x	Interval between matings (for males), days	Duration of gravidity (for females), days, min – max/x	Number of newborns (for females) min – max/x
<i>Vipera orlovi</i>	11	20	1:10 – 4:30/2:11 (n = 17)	2/1.36 (n = 14)	4 – 8 (n = 5)	70 – 85/77.6 (n = 14)	4 – 13/8.00 (n = 18)
<i>V. kaznakovi</i>	11	20	1:10 – 7:00/3:48 (n = 6)	2/1.50 (n = 6)	11 – 15 (n = 3)	72 – 83/77.0 (n = 5)	2 – 11/6.36 (n = 11)
<i>V. dinniki</i>	15	10	2:00 – 5:45/3:43 (n = 6)	2/1.50 (n = 4)	7 (n = 2)	68 – 89/75.0 (n = 6)	3 – 7/4.50 (n = 8)
<i>V. darevskii</i>	—	—	—	—	—	—	7 (n = 1)
<i>V. renardi</i>	18	7	1:35 – 2:20/1:55 (n = 4)	2/1.33 (n = 3)	3 (n = 1)	68 – 85/76.8 (n = 5)	3 – 15/7.33 (n = 12)
<i>V. lotievi</i>	15	10	0:50 – 3:00/1:59 (n = 15)	4/1.87 (n = 8)	1 – 5 (n = 7)	68 – 81/73.6 (n = 14)	2 – 20/10.37 (n = 19)
<i>V. eriwanensis</i>	—	—	—	—	—	80 (n = 1)	6/6.00 (n = 2)
<i>V. ammodytes transcaucasiana</i>	25	—	—	1/1 (n = 2)	—	83 (n = 1)	3 (n = 1)
<i>V. raddei raddei</i>	16	26	3:00 – 4:40/3:30 (n = 6)	2/2 (n = 3)	10 – 13 (n = 3)	92 – 104/96.6 (n = 5)	4 – 14/9.14 (n = 7)

vardi (Verkhnebakanskii) — 23, and *V. dinniki* (Achishkho Mountain) — 12. Captive-born females of *V. lotievi* bred for the first time at the age of 2 years, gave birth to 5 – 9 young neonates, while females of *V. kaznakovi* and *V. orlovi* gave birth to 2 – 4 young.

Laboratory hybridization. To investigate mechanisms of reproductive isolation between species of the subgenus *Pelias* Merrem 1808, in 2002 we produced interspecific hybrids in the laboratory between: 1) *V. lotievi* (Karmadon) and *V. renardi* (Kamyshevka), 2) *V. renardi* (Kamyshevka) and *V. eriwanensis* (Ara-Iler Mountain), 3) *V. kaznakovi* (Adler) and *V. dinniki* (Achishkho Mountain). All hybrid specimens are viable and are characterized by a rapid growth in comparison to the offspring produced from intraspecific breeding.

Comparative analysis. Analyzing the results of our investigations, we noted in the reproductive biology of *V. r. raddei* two characteristics which differentiate this representative of the subgenus *Montivipera* Nilson et al., 1995, from the species of the subgenus *Pelias*: (1) a more prolonged period of matings and (2) a longer period of female gravidity (Table 2). These characteristics, in our opinion, have a taxonomic significance. *V. r. raddei* is also characterized by a comparatively long average duration of copulation (more than three hours) and a larger average number of newborns per female (9.14).

Within the subgenus *Pelias* we distinguished for the species of the *V. kaznakovi* complex (*V. kaznakovi*, *V. dinniki*, *V. orlovi*) the following features which differentiate them from the representatives of the *V. ursinii* complex (*V. renardi*, *V. lotievi*): 1) the larger maximum and average values of duration of copulation; 2) the longer interval between matings in males; and 3) the smaller maximum number of newborns in females (Table 2). It is possible that these distinctions have a genetic basis, and also can be important for the taxonomy of the genus *Vipera*. The fact that the values of all three features considered above are rather close in *V. orlovi* and species of the *V. ursinii* complex is worth attention. In our opinion, this is a consequence of a natural hybridization between the taxa “*orlovi*” and “*renardi*” which occur sympatrically in the north-western foothills of the main Caucasus Ridge.

Between the ecological groups of lowland (*V. kaznakovi*, *V. orlovi*, *V. renardi*) and montane (*V. dinniki*, *V. lotievi*) species of the subgenus *Pelias* some differences in the average duration of the period of gravidity were observed. In the montane group, this duration is shorter. Evidently,

TABLE 3. Dates of Giving Birth of Vipers Caught at Last Stages of Gravidity

Taxon	Locality	Dates of giving birth
<i>V. orlovi</i>	Praskoveevka	08/1/2001 (<i>n</i> = 1)
<i>V. kaznakovi</i>	Krasnaya Skala	08/12/2000 (<i>n</i> = 1)
<i>V. dinniki</i>	Achishkho Mountain	09/18 – 10/3/1999 (<i>n</i> = 5)
<i>V. dinniki</i>	Kardyvach Lake	10/18/2002 (<i>n</i> = 1)
<i>V. darevskii</i>	Legli Mountain	09/4/2000 (<i>n</i> = 1)
<i>V. renardi</i>	Goryachii Klyuch	08/5/2002 (<i>n</i> = 1)
<i>V. lotievi</i>	Karmadon	08/13 – 08/19/2000 (<i>n</i> = 5)
<i>V. eriwanensis</i>	Ara-Iler Mountain	09/1/2001 (<i>n</i> = 1)
<i>V. r. raddei</i>	Yerevan — Sevan region	09/18/1999 (<i>n</i> = 2)

this peculiarity is an adaptation for living in the mountains where the warm season is very short.

REFERENCES

- Ananjeva N. B., Darevsky I. S., Borkin L. J., and Orlov N. L. (1998), “Amphibians and reptiles,” in: *Encyclopedia of Nature of Russia*, ABF, Moscow [in Russian].
- Bannikov A. G., Darevsky I. S., Ishenko V. G., Rustamov A. K., and N. N. Scherbak (1977), *Field Guide of the USSR Amphibians and Reptiles*, Prosveshchenie, Moscow [in Russian].
- Bozhansky A. T. (1984), “The Caucasus vipers,” *Priroda*, 41(6), 53 – 55 [in Russian].
- Darevsky I. S. and Orlov N. L. (1988), *Rare and Endangered Animals. Amphibians and Reptiles. A Handbook*, Vysshaya Shkola, Moscow [in Russian].
- Nikolsky A. M. (1913), *Reptiles and Amphibians of the Caucasus*, Izd. Kavkaz. Muz., Tiflis [in Russian].
- Nilson G., Tuniyev B. S., Orlov N. L., Höggren M., and Andrén C. (1995), “Systematics of the vipers of Caucasus: Polymorphism or sibling species?” *Asiatic Herpetol. Res.*, 6, 1 – 26.
- Orlov N. L. and Tuniyev B. S. (1986), “Present ranges, possible ways of their formation and phylogeny of the three species of vipers from the Eurosiberic group (*V. kaznakovi* complex) in the Caucasus,” *Trudy Zool. Inst. AN SSSR*, 157, 104 – 135 [in Russian].
- Shiryaev K. A. (2002), “Results of investigations of reproductive biology of Lotiev’s viper *Vipera lotievi* (Nilson, Tuniyev, Orlov, Höggren and Andrén, 1995),” *Nauch. Issl. Zooparkakh*, 14, 186 – 196 [in Russian].
- Shiryaev K. A. (2003), “Breeding of three subspecies of Long-nosed viper *Vipera ammodytes* Linnaeus, 1758,” *Nauch. Issl. Zooparkakh*, 16, 85 – 89 [in Russian].
- Trutnau L. (1998), *Schlangen im Terrarium. Band 2. Giftschlangen*, Verlag Eugen Ulmer, Stuttgart.

ECOLOGICAL PREFERENCES OF THE ITALIAN NEWT *Triturus italicus* (PERACCA, 1898) IN CALABRIA

E. Sperone¹ and S. Tripepi¹

Keywords: *Triturus italicus*, ecology, Calabria

INTRODUCTION

The Italian newt *Triturus italicus* (Peracca, 1898) is an endemic species of central-southern Italy, occurring only to the South of the Province of Frosinone (Latium) in the west coast and to the South of Ancona in the east one (Giacoma, 1988; Scillitani, 1996; Bologna, 2000). Calabria represents the southern limit of its distribution (S.H.I., 1996; Tripepi et al., 1999).

MATERIAL AND METHODS

Study area. The region of Calabria is the southern tip of the Italian peninsula. It has an area of 15,080 km² with a mainly mountainous (42%) and hill (49%) geography and an average elevation of about 556 m above sea level (a.s.l.). Plains are a small part (about 9%) of the total area of the region. The climate shows Mediterranean features with mild winters and hot and sultry summers, although it differs within the region according to exposure and altitude. Indeed, there are some important climatic differences between the two sides of Calabria: the Tyrrhenian side is more exposed to humid winds and has an ocean-

like climate with a large amount of precipitations; instead, the Ionian side is exposed to African influences and shows high yearly temperatures and short and intense precipitations (Versace et al., 1989; Caloiero et al., 1990).

Data collection and analysis. Data presented in this work were collected on field from 1983 to 2000. On capture some biometric measurements (total length, snout-vent length and weight) from each specimen were taken. Other important environmental information was collected: all information was registered in the herpetological database of the Department of Ecology, University of Calabria (Rossi et al., 1991).

RESULTS AND DISCUSSION

Biometry. The Italian newt is a small newt and in the study area adults rarely are larger than 7 – 8 cm including tail (longest total length recorded: 9.1 cm; specimen: adult female; place: Massif of Pollino; shortest total length recorded 2.4 cm; specimen: adult male; place: Massif of Pollino). Usually males are smaller than females: by sample of 1393 males and 1531 females, the formers reach a mean value of body length of about 3.009 cm ($s = 0.34$) (Fig. 1), the latter reveals of about 3.34 cm ($s = 0.42$) (Fig. 2). Processing biometric data reveals a relationship

¹ Department of Ecology, University of Calabria, 87036 Rende (CS), Italy; E-mail: s.tripepi@unical.it.

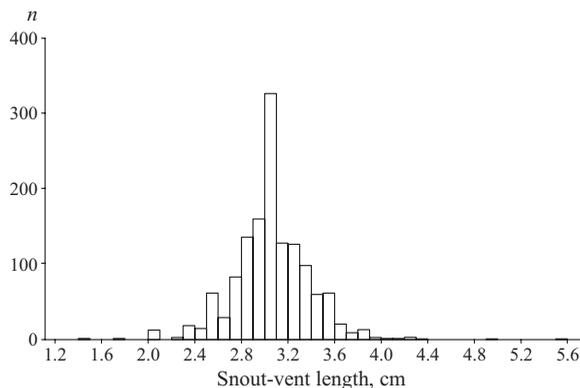


Fig. 1. Male body (snout-vent) length.

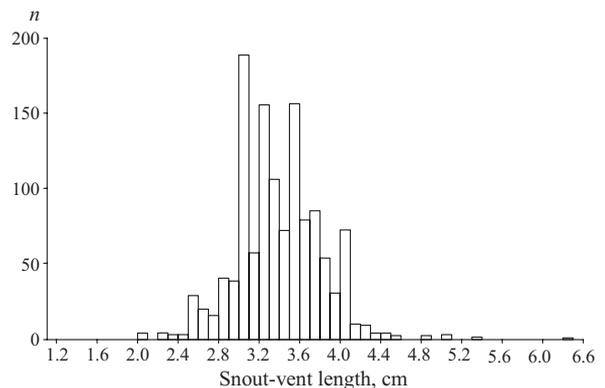


Fig. 2. Female body (snout-vent) length.

between average corporeal size of the populations of newts and the altitude of their location. The relationship is statistically significant and gives prominence how the average weight of the specimens considered (Fig. 3: males; $n = 1201$; correlation factor = 0.516) and the average snout-vent length (Fig. 4: females; $n = 1527$; correlation factor = 0.520) increase with the altitude. This relationship may be the result of the greater environmental stability of the biotopes of high altitude in comparison with the biotopes of lower altitude: in the presence of more stable environmental factors the newts of high altitudes can extend the permanence in water and the feeding period, increasing their sizes respect to the newts of lower altitudes. Probably populations at high altitude are more long-lived than those at lower altitude and have a smaller annual rate of growth (Giacoma et al., 2003).

Distribution. The Italian newt is the most frequently encountered salamander in Calabria (Giacoma et al., 1988). The 354 sampling resorts are placed rather uniformly in the study area (Fig. 5). In Calabria the Italian newt occurs from sea level to 1855 m a.s.l.: Sorgente Frido, in the Massif of Pollino, represents the highest locality know to be inhabited by *Triturus italicus* in its range (Lanza, 1977; Arnold and Burton, 1978; Tripepi et al., 1994;

Brunelli et al., 1996). This newt can be considered an euryhysic (*sensu* Brandmayr and Zetto-Brandmayr, 1979) tendentially eurytherme species. However, it is most frequent from sea level to 1000 m a.s.l. (Fig. 6).

Habitat. The Italian newt is an euryecious species with a high degree of flexibility and colonizes a lot of aquatic ecosystems (Fig. 7). However, it has a high preference for man-made tanks, used for irrigation, ponds and small seasonal pools.

Coexistence with other Amphibians. In the study area the Italian newt was founded in syntopy with: *Rana synklepton esculenta* (37.28%), *Rana italica* (29.66%), *Hyla intermedia* (25.70%), *Bufo bufo* (19.77%), *Rana dalmatina* (19.49%), *Bombina pachypus* (13.28%), *Triturus carnifex* (9.32%), *Bufo viridis* (8.19%), *Salamandrina terdigitata* (2.82%), *Triturus alpestris* (1.41%), and *Salamandra salamandra* (1.41%). *B. viridis*, *B. pachypus*, *S. terdigitata*, and *S. salamandra* live in habitats that do not meet Italian newt requirements: the green toad is usually found in sandy habitats, the yellow-bellied toad prefers shallow water habitats (small pools in marshes, wheel ruts and temporary puddles), the fire salamander and the

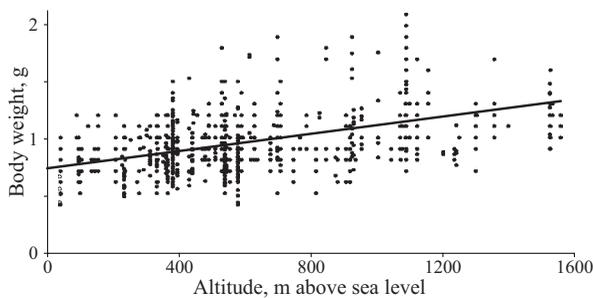


Fig. 3. Male body weight/altitude relationship.

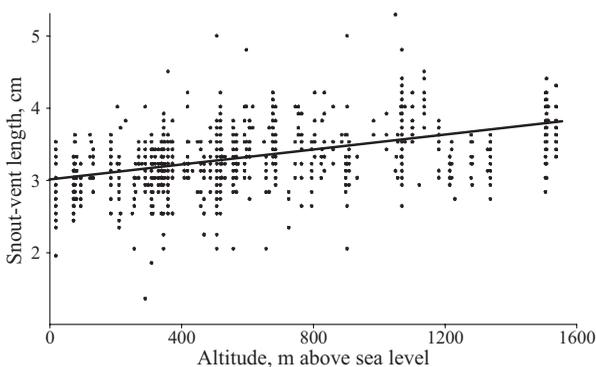


Fig. 4. Female snout-vent length/altitude relationship.

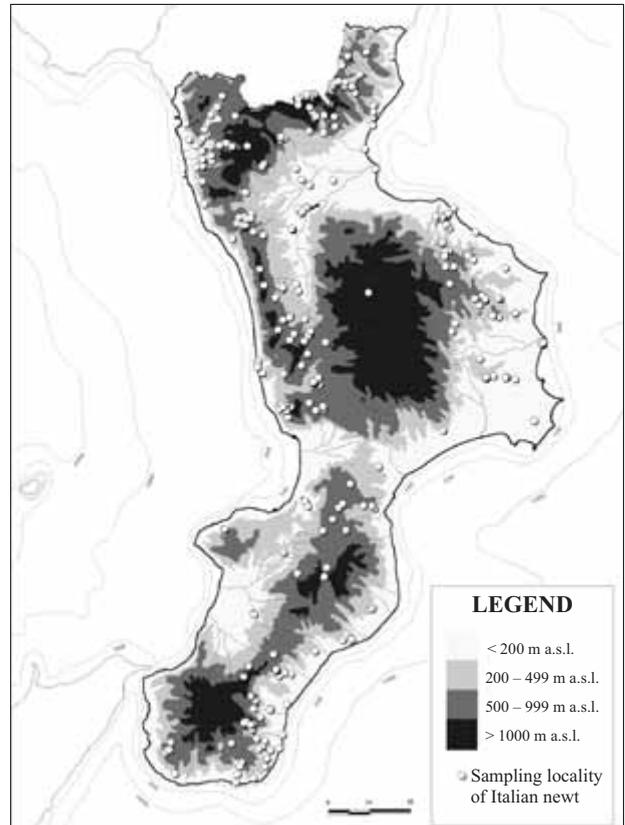


Fig. 5. Distribution of *T. italicus* in Calabria.

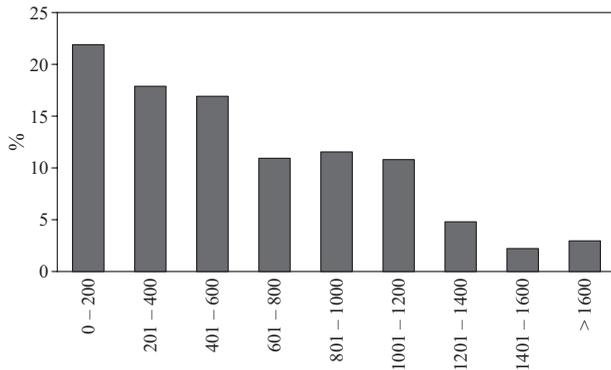


Fig. 6. Altitudinal preferences.

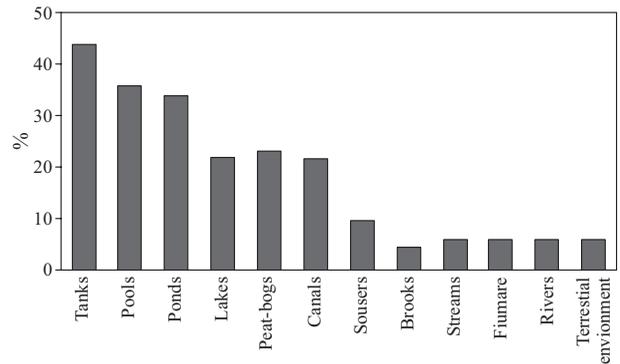


Fig. 7. Habitat preferences.

spectacled salamander have terrestrial habits and go to brooks and streams during the breeding season.

Low percentage values of syntopy between the Italian newt and the other newts of Calabria are not the result of the absence of relationships between them, but are influenced by the restricted distribution of Italian Warty newt and Alpine newt.

Acknowledgments. This study was supported by grant of the Aspromonte National Park (Calabria, Southern Italy) and it is included in a research project of the Doctorate in “Animal Biology” (University of Calabria).

REFERENCES

- Arnold E. N. and Burton J. A.** (1978), *A Field Guide to the Reptiles and Amphibians of Britain and Europe*, Collins, London, pp. 40 – 52.
- Bologna M. A.** (2000), “*Triturus italicus* (Peracca, 1889),” in: Bologna M. A., Capula M., and Carpaneto G. (eds.), *Anfibi e Rettili del Lazio*, Fratelli Palombi Editori, Roma, pp. 44 – 45.
- Brandmayr P. and Zetto-Brandmayr T.** (1979), “Contribution to ecology of an euryhypsic ground-beetle of eastern Alps and dinaric karst, *Carabus creutzeri* Fabr.,” *Zool. Jb. Syst.*, **106**, 50 – 64.
- Brunelli E., Rossi F., and Tripepi S.** (1996), “Gli anfibi della provincia di Cosenza: distribuzione ed ecologia,” *Rend. Sem. Fac. Sci. Univ. Cagliari*, **66**(1), 85 – 96.
- Caloiero D., Niccoli R., and Reali C.** (1990), “Le precipitazioni in Calabria (1921 – 1980),” in: *C.N.R.-I.R.P.I. Geodata*, pp. 1 – 36.
- Giacoma C.** (1988), “The ecology and distribution of newts in Italy,” *Ann. Inst. Mus. Zool. Napoli*, **26**, 49 – 84.
- Giacoma C., Picariello O., Puntillo D., Rossi F., and Tripepi S.** (1988), “The distribution and habitats of the newt (*Triturus*, Amphibia) in Calabria (Southern Italy),” *Monit. Zool. Ital. Nov. Ser.*, **22**, 449 – 464.
- Giacoma C., Marzona E., and Seglie D.** (2003), “Ambiente montano e modificazioni fenotipiche in un vertebrato picilotermino, *Triturus alpestris*,” in: *Proc. of the 64th UZI Congr., September 21 – 25, 2003*, Varese, p. 30.
- Lanza B.** (1977), “Sympatry and coexistence in the Italian *Triturus*, with notes on the ‘*Molge italica molisana*’ problem (Amphibia, Salamandridae),” *Monit. Zool. Ital. Nov. Ser.*, **11**, 113 – 118.
- Rossi F., Ripolo D., Scalzo A., Trecroci T., and Tripepi S.** (1991), “Banca dati dell’erpetofauna calabrese,” *S.I.E. Atti*, **12**, 977 – 981.
- Scillitani G.** (1996) “Tritone italico,” in: Scillitani G., Rizzi V., and Gioiosa M. (eds.), *Atlante degli Anfibi e dei Rettili della provincia di Foggia*, Monografie del Museo Provinciale di Storia Naturale di Foggia e del Centro Studi Naturalistici Vol. I, Gitto, Foggia, pp. 48 – 49.
- Societas Herpetologica Italica (S.H.I.)** (1996), “Atlante provvisorio degli Anfibi e dei Rettili italiani,” *Ann. Mus. Civ. Storia Nat. G. Doria*, **XCI**, 95 – 178.
- Tripepi S., Rossi F., Serroni P., and Brunelli E.** (1994), “Distribuzione altitudinale degli Anfibi in Calabria,” *Acta Biol.*, **71**, 67 – 101.
- Tripepi S., Carelli B., Giardinazzo E., Sperone E., and Serro-ni P.** (1999) “Distribuzione degli anfibi nella provincia di Reggio Calabria: dati preliminari,” *Riv. Idrobiol.*, **38**(1 – 3), 141 – 149.
- Versace P., Ferrari E., Gabriele S., and Rossi F.** (1989) “Valutazione delle piene in Calabria,” in: *C.N.R.-I.R.P.I. Geodata*, pp. 1 – 35.

MAPPING IMPORTANT HERPETOFAUNAL AREAS IN NORTHERN EUROPE

A. H. P. Stumpel¹ and K. F. Corbett²

Keywords: amphibians, areas, conservation, Europe, habitat assessment, mapping, reptiles, status.

INTRODUCTION

The “Important Herpetofaunal Areas in Europe” (IHA) project aims at producing a book that will include an overview of the most important areas for reptiles and amphibians in Europe. This is intended to provide information to planners, policy makers and conservationists and enable them to better protect reptiles and amphibians (herps) and their habitats. The project is divided into phases, with both species accounts and country accounts included. Similar books have been published by others on birds (Heath and Evans, 2000) and butterflies (van Swaay and Warren, 2003) and one on plants is in preparation.

Reptiles and amphibians are recognized as having many vulnerable and threatened taxa (species, sub- and super-species) and many countries have set up Red Data Books for herps. A modest selection of species is listed on the European Union’s Habitat Directive (Directive 92/43/EEG), under Annexes II and IV, and all species also figure on the Bern Convention (BC) under its Annexes II and III (Anonymous, 1979). All member states and contracting parties have legal obligations to protect these species and their habitats, except for the species of BC Annex III. The SEH Conservation Committee (CC) has played an important role in obtaining official Recommendations by the BC that relate to the conservation of specific habitats and, moreover, to demands for adequate habitat management. In practice, however, it has become clear that policy makers do not always know, or even care, which habitats need to be protected. Furthermore, protection on paper does not always equate to the physical protection of sites; i.e., implementation is sometimes problematic. During the 1980s and 1990s the CC conducted a series of habitat assessments for the most threatened species and, in conjunction with the IUCN SSC for European Herpetofauna, drafted a series of Species Action Plans that identified threats and proposed conservation measures. The methods employed are now being used as a basis for the identification of IHAs.

The IHA project started in 2002 within the framework of the Pan European Ecological Network (Nowicki, 1998). This project has no direct links to the designation of the Natura 2000 areas for the European Union, but is seen as a useful yardstick for this exercise. The IHA project is currently underway and should be finished by the end of 2004. The last obstacle to be tackled is the identification of IHAs in Northern Europe and, with this in mind, a poster presentation was given at the 12th SEH General Ordinary Meeting at St. Petersburg in order to raise interest and obtain cooperation from attendees.

METHODS

The two main information sources for the book are existing files containing previous CC habitat assessments and a questionnaire designed specifically for the IHA project. The CC habitat assessments involved the determination of key features in the field and the subsequent identification of actual and potential habitats. For the second approach, specialists have been invited to act as co-workers and to complete a questionnaire. This is in the form of a computer program that can be downloaded from the IHA web-site www.iha.alterra.nl. A manual with detailed instructions (Stumpel and Corbett, 2003) is also available on the web-site.

Although existing information could be used from species accounts, providing clear guidelines as to how select IHAs for countries proved to be difficult. A European Red Data Book for herps does not exist as yet, but preliminary rankings have been made by Honegger (1981), Corbett (1989), and Anonymous (2001). The latter two are similar and were drawn up in conjunction with the CC, a process involving many national and taxon experts. Corbett’s (1989) work resulted in a categorization of three classes: (a) taxa with a limited range that present a clear conservation problem; (b) taxa showing a decline across a significant part of their range; (c) taxa known to be at risk, but with insufficient information about their status. In Anonymous (2001), species are listed under five threat categories: critically endangered, endangered, vulnerable, lower risk and data deficient. The selection of taxa for the

¹ Alterra, Wageningen University and Research Centre, Postbus 47, 6700 AA Wageningen, The Netherlands; E-mail: anton.stumpel@wur.nl.

² The Herpetological Conservation Trust, 655a Christchurch Road, Boscombe, Bournemouth, Dorset BH1 4AP, UK; E-mail: paul.edgar@herpconstrust.org.uk.

IHA project was based on the same methods used for the butterfly project (van Swaay and Warren, 2003), i.e., IHA target taxa are those which meet at least two of the following criteria:

1. The taxon is restricted to or found primarily in Europe (listed according to the categories from above; Corbett, 1989; Corbett et al., 1990; Andrén et al., 1991, 1993; Anonymous, 2001).
2. The taxon is either on Appendix II of the Bern Convention and/or on Annexes II or IVa of the Habitat and Species Directive.
3. The taxon is globally threatened according to the IUCN Red List of Threatened Animals (Baillie and Groombridge, 1996).

This selection resulted in the identification of 59 target taxa (16 amphibians and 43 reptiles; Table 1). The most important habitats occupied by these taxa will qualify as IHAs. The IHAs are further divided into two types (Sites and Areas) to distinguish between taxa restricted to individual sites, where site based conservation measures are applicable, and widely distributed taxa, which are more suited to conservation initiatives that operate at a large scale (Stumpel and Corbett, 2003). IHAs can also be areas with rich assemblages of different taxa, including some fitting the threatened definitions above.

For the country accounts, areas can be selected using adapted criteria:

1. The habitat accommodates a species/taxon threatened across a significant part of its European range.
2. The area is one of the best habitats for the species/taxon in the country.
3. The area harbors a rich variety of reptiles and amphibians.
4. Data are sufficient for the proposal of an IHA.
5. Data are sufficient to identify specific conservation measures required.

CALL FOR HELP

The IHA project cannot be done without the help of herpetologists all over Europe. To date, 25 specialists have promised to provide data for the species accounts and the first completed questionnaires had already been received by the time of the 12th SEH General Ordinary Meeting at St. Petersburg.

During the early stages of the project it became clear that, on the basis of the target taxa selected, only countries in Southern Europe would have IHAs. The book, however, was meant to cover the whole of Europe so a second phase was initiated. This aimed to map IHAs in every European country and therefore interpose a national dimension on top of the broader international view. To supplement the information from Southern Europe already being obtained from the species accounts, additional data from Northern Europe had to be collected. Using the new criteria, national compilers were asked to identify the relevant species and select 5 – 25 IHAs per country (with the number related to the surface area of the country).

An extension of the network of contributors was needed in the short term and it was anticipated that the SEH congress would be an ideal venue for attracting co-workers. This has been the case and further co-workers have been found for a number of countries in the meantime. However, too many countries are still lacking contributors to the book and it will therefore be a race against time to finish the full book on schedule. The quest for cooperation is continuing and interested herpetologists remain invited to contact the project team (see the IHA web-site).

REFERENCES

- Andrén C., Balletto E., Bea A., Corbett K., Dolmen D., Grosenbacher K., Nilson G., Oliveira M., Podloucky R., and Stumpel A. (1991), *Threatened Amphibians in Europe Re-*

TABLE 1. Preliminary Selection of Target Species/Taxa

Salamanders	<i>Chioglossa lusitanica</i> , <i>Euproctus platycephalus</i> , <i>Mertensiella luschani</i> , <i>Proteus anguinus</i> , <i>Salamandra atra aurorae</i> , <i>S. lanzai</i> , <i>Speleomantes ambrosii</i> , <i>S. flavus</i> , <i>S. genei</i> , <i>S. imperialis</i> , <i>S. supramontis</i>
Frogs and toads	<i>Alytes dickhilleni</i> , <i>A. muletensis</i> , <i>Discoglossus montalentii</i> , <i>Pelobates fuscus insubricus</i> , <i>Rana latastei</i>
Turtles	<i>Caretta caretta</i> , <i>Chelonia mydas</i> (MED), <i>Testudo graeca nikolski</i> , <i>T. graeca</i> (W), <i>T. hermanni</i> (W), <i>Trionyx triunguis</i> (MED)
Lizards	<i>Algyroides marchi</i> , <i>Chalcides simonyi</i> , <i>Chamaeleo chamaeleon</i> (W), <i>Euleptes europaea</i> , <i>Gallotia galloti insulanagae</i> , <i>G. simonyi gomerana</i> , <i>G. s. intermedia</i> , <i>G. s. machadoi</i> , <i>Lacerta aranica</i> , <i>L. aurelioi</i> , <i>L. bedriagae</i> , <i>L. bonnali</i> , <i>L. clarkorum</i> , <i>L. dryada</i> , <i>Podarcis atrata</i> , <i>P. lilfordi</i> , <i>P. milensis</i> , <i>P. pityusensis</i> , <i>P. raffonei</i> , <i>Zootoca vivipara pannonica</i>
Snakes	<i>Coluber cypriensis</i> , <i>C. gyarosensis</i> , <i>Macrovipera schweizeri</i> , <i>Natrix megalcephala</i> , <i>Natrix natrix cetti</i> , <i>N. n. cypriaca</i> , <i>N. n. schweizeri</i> , <i>Vipera albizona</i> , <i>V. darevskii</i> , <i>V. dinniki</i> , <i>V. kaznakovi</i> , <i>V. nikolskii</i> , <i>V. pontica</i> , <i>Vipera ursinii moldavica</i> , <i>V. u. rakosiensis</i> , <i>V. u. ursinii</i> , <i>V. wagneri</i>

Note. W, Western population; MED, Mediterranean population.

- quiring *Special Habitat Protection Measures*, Report Conservation Committee, SEH, Bern.
- Andrén C., Balletto E., Corbett K., Darevsky I., Grossenbacher K., Korsós Z., Lescure J., Nilson G., Podloucky R., and Stumpel A.** (1993), *Threatened Amphibians and Reptiles of Eastern Europe Requiring Special Conservation Measures*, Report Conservation Committee, SEH, London.
- Anonymous** (1979), *Convention on the Conservation of European Wildlife and Natural Habitats*, European Treaty Series No. 104, Council of Europe, Strasbourg.
- Anonymous** (2001), *Preliminary European Red List of Vertebrates*, Documents T-PVS 31 and 32, Council of Europe, Strasbourg.
- Baillie J. and Groombridge B.** (eds.) (1996), *1996 IUCN Red List of Threatened Animals*, IUCN, Gland.
- Corbett K.** (ed.) (1989), *The Conservation of European Reptiles and Amphibians*, Christopher Helm, London.
- Corbett K., Andrén C., Balletto E., Bea A., Grillitsch H., Groombridge B., Grossenbacher K., Lescure J., Nilson G., Oliveira M., Podloucky R., and Stumpel A.** (1990), *Habitat Conservation Proposals for Threatened Reptiles in Europe. First Draft of a Report Prepared under Contract for the Council of Europe*, Report Conservation Committee, SEH, Bournemouth.
- Heath M. F. and Evans M. I.** (eds.) (2000), *Important Bird Areas in Europe: Priority Sites for Conservation*, Birdlife, Cambridge.
- Honegger R. E.** (1981), *Handbuch der Reptilien und Amphibien Europas. Suppl. Vol. Threatened Amphibians and Reptiles in Europe*, Akad. Verlagsgesellschaft, Wiesbaden.
- Nowicki P. L.** (ed.) (1998), "The green backbone of Central and Eastern Europe," in: *Proc. of the Conf., February 25 – 27, 1998, Cracow*, ECNC Publ. Ser. on Man and Nature, Tilburg, Vol. 3.
- Stumpel A. H. P. and Corbett K. F.** (2003), *Manual for Compilers. Identifying Important Herpetofaunal Areas in Europe. Phase 1. Species Accounts. Report No. 821*, Alterra, Wageningen.
- van Swaay C. A. M. and Warren M. S.** (eds.) (2003), *Prime Butterfly Areas in Europe. Priority Sites for Conservation*, National Reference Centre of the Ministry of Agriculture Nature and Food Quality, Ede.

AN ACCESS TO THE FEMALES AS A RESOURCE OF MALE'S TERRITORY IN *Lacerta saxicola*

A. Yu. Tsellarius¹ and E. Yu. Tsellarius¹

INTRODUCTION

Recent ideas of social interrelations of animals, territoriality included, are mainly based on the results of investigations of social insects, birds and mammals. These animals are characterized by great postnatal parental investment. Many features of mating systems and systems of resource monopolization are considered as direct results of optimization of this investment (Emlen and Oring, 1977; Davies, 1992). Amphibians and reptiles do not have such investment. However, social structure of their communities may have characteristics closely resemble social interrelations in birds and mammals. It is probable that a detailed analysis of formation of social structure of reptiles would be useful for solution of some problems of behavioral ecology.

MATERIAL AND METHOD

Since 1996, we have been observing a settlement of bisexual *Lacerta saxicola* in one of canyons of the Navagir mountains, in Northern Caucasus. The settlement is located in a little clearing in a dense hornbeam-beech forest. Such clearings that arise as a result of a fall of one or two trees are the most favorable habitat to *Lacerta saxicola* in studied region (Tsellarius and Tsellarius, 2001). The space under forest canopy outside the clearings is sparsely populated, and the majority of encountered individuals were the wanderers.

The observed settlement included 19 to 25 individuals in different years, sex ratio was nearly 1:1. Every spring, all settled members of the settlement were captured and measured, the color mark was renewed if required. The same operations were performed with the wanderers which visited the territory of the settlement. In every lizard we amputated in certain pattern up to three phalanges of fingers. It is a permanent mark, which permits to recognize the individual during all its life and, additionally, amputated phalanges are used to determine the age of lizards by bone layers. Every day, if the weather was favorable for activity of the lizards, the territory of the settlement was under surveillance during one to five hours. Recorded

were the movements over the space and the behavior of lizards during encounters with conspecifics. In total, by now, we have marked nearly two hundred lizards, and the overall duration of observations being more than 450 h. As an index of diversity of females in home range of a certain male we use the polydominance index ($1 \leq S_\lambda \leq 8$) (Pesenko, 1982). An intensity of a male patrolling was estimated as a ratio of the frequency of registration of patrolling to that of any other activities (Tsellarius and Tsellarius, 2005). The index named "aggression level" presents a stage of stereotyped agonistic behavioral pattern to which this pattern has been reduced in the average (Tsellarius and Tsellarius, 2004a). Detailed description of the observing technique and the data handling were published earlier (Tsellarius and Tsellarius, 2001, 2002).

RESULTS

Space Use in Males

During one or two years after a hatching the males of *Lacerta saxicola* wander over the vast space, that is up to 0.5 km². A switch to the settled mode of life occurs, as a rule, soon after reaching sexual maturity, i.e., after a second hibernation. Having settled, the male usually keeps fidelity to the selected site during all the life, i.e., up to 5 – 15 years. In the initial period of settled life the male does not display any aggression towards conspecifics, i.e., he has a non-territorial status. He uses the space of home range more or less evenly, there are neither area of concentrated use (the core area, in *sensu*: Samuel et al., 1985) nor centers of activity. In this stage of life male's home range is on average 118 ± 24.1 m² ($n = 5$) and broadly overlaps both the home ranges of the coevals and those of the territorial males. We term the males of such stage the poachers. The period of poacherism lasts 2 – 3 years, sometimes up to 5 years, certain individuals probably can be stuck in this status for all the life (Tsellarius and Tsellarius, 2002). The males that passed into the next stage of ontogenetic trajectory — that is territorial stage — were named the residents. The home range of resident is less than poacher's home range (75 ± 34.2 m², $n = 12$, $t = 2.94$), and always has strongly pronounced internal structure. The main elements of the home range are: an area of concentrated use

¹ Laboratory of Comparative Ethology, Severtsov Institute, Leninsky pr. 33, 199071 Moscow, Russia; E-mail: cellarius@land.ru.

or core area; the centers of activity; the patrol routes. The residents, unlike poachers, display an aggression towards other males. The aggressive initiatives are localized within the bounds of the area of concentrated use, i.e., there exists a territory. Size of the territory is on average $20.3 \pm 3.06 \text{ m}^2$. The home ranges of residents may broadly overlap. The core areas, however, are completely separate in all instances. The structure of resident's home range is strongly determined by the individual space patterns of the females inhabiting his home range (Tsellarius and Tsellarius, 2005).

The centers of activity are the sites where a male spent the most part (60 – 70%) of activity time. There may be from 2 to 7 such sites per home range. Centers of activity of a male always coincide with the centers of basking of certain (not all) females (Tsellarius and Tsellarius, 2005). In the event that preferred female alters a disposition of her basking centers, the disposition of male's activity centers will be altered too. Connection between male's centers of activity and female's basking centers remains close during all the year and is not restricted by the mating period only.

Patrolling is the particular mode of movement over home range. Patrolling male moves quickly, in relatively straight line, with frequent short stops. Additionally, the patrolling includes almost whole set of the behavioral acts typical of situation with shortage of prognostic information (Tsellarius and Tsellarius, 2005). It is probable that motivation of patrolling is an information deprivation. An intensity of patrolling is closely connected with diversity of females in resident's home range ($r = 0.81$, $n = 12$) and has no connection with the frequency of visitation of home range by other males. Patrolling is the main kind of activity of the majority of residents over almost the entire season. A system of patrol routes usually includes all the basking centers of all the females within the core area. Actually, movements of a male over the home range are the patrol runs, which start and finish in the basking centers of females. The core area that contains male's activity centers and a patrol routes network is formed just owing to intensive patrolling. A frequency and duration of male's excursions beyond the borders of his core area negatively associated with the diversity of females inhabiting core area ($r = -0.84$, $n = 15$).

Access to Females

The residents display aggression towards both the individuals of their own status and the poachers. The poachers, however, are able to avoid contacts with residents and exploit the ecological resources of resident's home range without significant limitation. But they have no regular ac-

cess to basking centers of females, where females spend more than 70% of the time of activity, since these centers are intensively patrolled by residents. As a result, the poachers are deprived of a long communication with females. It is significant that limitation concerns exactly communication, while an access to copulation remains almost unlimited.

Social status of copulated females and a rate of successful attempts at copulation are significantly different in poachers and in residents. However, a total number of accomplished copulations (per individual) may be about equal in males of different status. In average, the poachers takes more attempts at copulations and more frequently takes attempts at rape.

Ontogenetic Formation of Territoriality

In all instances, an appearance of aggressiveness and, consequently, establishing of a territory and forming of initial structure of home range, coincides with event when poacher obtains an access to basking centers of females. The access was always obtained as a result of either re-disposition of basking centers of females in such a way that new centers arise beyond the borders of resident's territories, or death of one of residents controlling these centers. Thus, a residence is always formed in place unoccupied by territorial males. We have never observed an usurpation of other's territory, and, probably, it is fundamentally impossible.

Having obtained an access to the basking center of female, the poacher begins to visit it regularly. As a result, a center of activity of the male is formed here and an area of concentrated use surrounds it. At the same time the male begins to display aggression towards other males, initially only in response to aggression of neighbors. Consequently, all-round passive defense zone is formed around female's basking center. As yet we cannot term this zone a territory, since territoriality demands active defense of space (Burt, 1943). Soon after, however, the male begins to take the aggressive initiatives, and, accordingly, he obtains resident status. As a result, in studied site the long-term "ownerless" female's basking centers have never been recorded. All long-term basking centers were disposed within the bounds of defended territories of residents. It must be taken into account that penetration of one resident into another's territory has for an object, as a rule, the basking centers of females disposed in this territory (Tsellarius and Tsellarius, 2005a). This tendency, as well as connection of male's activity centers with basking centers of females, takes place during the entire season and is not restricted within the mating period only.

Thus, an agonistic behavior of male and, accordingly, forming of defended space within home range are induced by regular communication with female, but not by copulation in itself. A possibility to communicate with female is under control of forces of social nature, whereas sexual activity and the possibility of its accomplishment (copulation) are not significantly affected by social circumstances. The given picture well corresponds with experimental data obtained in captivity (Sakata et al., 2002). Just the striving to communication with female, but not to copulation, determines the space use pattern of a male. It is a possibility to communicate, but not to copulate, is, in male's point of view, that the main resource of his home range. In other words, in ordinary conditions amicable communication is more strongly motivated than any other activity.

Motivation of Aggression and Resource Defense

Here it would be appropriate to say some words about aggression in general. Unambiguously desired effect of any actions referred to the direct aggression is — in aggressor's point of view — the removal of the circumstances hindering in realization of certain activity and/or circumstances increasing uncertainty of situation. In other words, an aggression is the forcible change of circumstances, which results in a renewal of mental comfort (in *sensu*: Ovsianikov and Badridze, 1989). In the case of *Lacerta saxicola*, only few particular residents — and in only particular circumstances — display aggression towards the conspecifics which hinder (or may hinder) in communication with female (Tsellarius and Tsellarius, 2005a). In ordinary resident, in the event that intruder courts female, an aggression level, the frequency and distance of aggressive actions are the same as in other situations (Table 1). Only an attempt of forced copulation always provokes very vigorous reaction of resident and is usually crowned by severe fight with following chase of violator. It is probable, however, that resident takes a rape as nothing but some aggression in his territory, since this pattern of copulation starts with male's behavior resembling vigorous attack most of all (Tsellarius and Tsellarius, 2005a, 2005b).

There exists another peculiarity characterizing male's aggression. A formal display of threat towards intruder is

turned into the strict, direct and unambiguous one not in the situation when intruder attempts to court female, but when his behavior is either non-complementary to behavior of territory owner or it threatens to become non-complementary (Tsellarius and Tsellarius, 2005a). For example, intruder does not run away or does not take submissive pose in response to resident's formal threat. If a familiar intruder runs or submits, the resident does not attack or chase him, with the exception of some particular situations. In other words, an aggression is directed towards circumstances, which hinder (or may hinder) in realization of resident's preference to accomplish certain forms of social behavior (in *sensu*: Maslow, 1936). In the case of *Lacerta saxicola* males these forms are essential behavioral traits of male's status in a system of male/male (but not male/female) relationships. Thus, a male defends not a resource, but its own social status.

DISCUSSION

Territoriality is usually considered as one of the ways of defense of some resources (Wynne-Edwards, 1962; Ever, 1968). It is supposed that the main resource of territory is an access to copulation (Emlen and Oring, 1977; Davies, 1992; Martins, 1994; etc.). In other words, defending of certain space inhabited by females, the male tries to secure here a priority for his own gametes. This conception permits to create a rather consistent explanation of the origin and evolution of territoriality. The fact that male in some extent renounces the copulation for the sake of amicable communication with female (Tsellarius and Tsellarius, 2005ab) and that communication is the most attractive resource of his home range, it is poorly corresponds with current view of a background of territoriality. Moreover, there is another paradox. Although aggressiveness of a male is stimulated by female and an access to communication with female is — in male's point of view — of utmost value, male's aggression, as a rule, is not used for the defense of access to communication itself. Aggressive male provides females inhabiting his territory for protection against forced copulation and for freedom of choosing of the sexual partner, but territory owner does not inevitably

TABLE 1. Reactions of Residents to Intruders in the Borders of Core Area

Conditions of the contact	Number of observed contacts	Frequency of agonistic interactions, %	Distance of aggressive initiative is taken, m	Aggression level
Intruder is at a distance of more than 1 m from female and does not strive for contact with her	220	74.1	1.2 ± 0.67 (n = 33)	3.9 ± 0.92 (n = 81)
Intruder is about female or courts her	34	82.4	1.5 ± 0.60 (n = 5)	3.7 ± 1.12 (n = 21)

become a preferred sexual partner (Tsellarius and Tsellarius, 2005a, 2005b).

On the other hand, female gives an access to communication, as a rule, to aggressive males only (Tsellarius and Tsellarius, 2002). Thus, male's aggressiveness (= territoriality) provides him for an access to the desirable resource, but does not guarantee for exclusive use of female. Moreover, in certain circumstances territoriality may — to a rather great extent — complicate a life of the male since it restricts an access to ecological and social resources of the habitat. In *Lacerta saxicola*, in any case, conservative males — which for term of life are fastened to their territories — are found in undoubted disadvantage (Tsellarius and Tsellarius, 2005).

Such situation is not unique. Similar state is well known of many species and, generally, is rather typical (Carpenter, 1987). As for *Lacerta saxicola*, it is quite possible that territoriality is nothing but side effect of male's ability to defend female. In itself, the territory, may have no adaptive significance. In our point of view, a much more interesting question is not the question of the origin of territoriality and its influence on fitness, but what a profit is got by male from communications with female, why he so strongly seeks for communication even though it does not provide him for preference for copulation. Mentioned profit must be rather large in order to compensate for damage from side effects of aggression, the territoriality including. However, we must be ready for that comprehension of the situation — in the framework of such notions as a benefit, a cost, a fitness and so forth — will be found impossible, since we have dealt with a behavior that is realization of certain mental state, i.e., motivation. Thus distant and indirect after effect of behavior as a fitness cannot be motivation in any case.

REFERENCES

- Burt W. H.** (1943), "Territoriality and home range concepts as applied to mammals," *J. Mammol.*, **24**, 346 – 352.
- Carpenter F. L.** (1987), "Food abundance and territoriality: to defend or not to defend?" *Am. Zoologist*, **27**, 327 – 336.
- Davies N. B.** (1992), "Mating systems," in: Krebs J. R. and Davies N. B. (eds.), *Behavioral Ecology: An Evolutionary Approach*, Blackwell Sci. Publ., Oxford, pp. 263 – 294.
- Emlen S. T. and Oring L. W.** (1977), "Ecology, sexual selection, and the evolution of mating systems," *Science*, **197**, 215 – 223.
- Ewer R. F.** (1968), *Ethology of Mammals*, Logos Press, London.
- Martins E. P.** (1994), "Phylogenetic perspectives on the evolution of the lizard territoriality," in: Vitt L. J. and Pianka E. R. (eds.), *Lizard Ecology*, Princeton Univ. Press, pp. 117 – 144.
- Maslow A. H.** (1936), "The role of dominance in the social and sexual behavior of infra-human primates," *J. Gen. Psychol.*, **48**, 310 – 338.
- Ovsianikov N. G. and Badridze Ya. K.** (1989), "The conception of mental comfort in the interpretation of motive powers of behavior," *Dokl. AN SSSR*, **306**, 1015 – 1018 [in Russian].
- Pesenko Yu. A.** (1982), *Foundations and Methods of Quantitative Analysis in Faunistical Research*, Nauka, Moscow [in Russian].
- Sakata J. T., Gupta A., Chuang Chien-Pei, and Crews D.** (2002), "Social experience affects territorial and reproductive behaviors in male leopard geckos, *Eublepharis macularius*," *Animal Behav.*, **63**, 487 – 493.
- Samuel M. D., Pierce D. J., and Garton E. O.** (1985), "Identifying areas of concentrated use within the home range," *J. Animal Ecol.*, **54**, 711 – 719.
- Tsellarius A. Yu. and Tsellarius E. Yu.** (2001), "Alteration of spacing pattern of population of *Lacerta saxicola* in broad-leaved forests of Navagir mountain ridge," *Zool. Zh.*, **80**, 1 – 8 [in Russian].
- Tsellarius A. Yu. and Tsellarius E. Yu.** (2005), "Formation and defense of the individual space in males of *Lacerta saxicola*. 1: Home range," *Zool. Zh.*, **84**, in press [in Russian].
- Tsellarius E. Yu. and Tsellarius A. Yu.** (2002), "Age change and probable function of the signaling coloration in males of *Lacerta saxicola*," *Zool. Zh.*, **81**, 970 – 977 [in Russian].
- Tsellarius E. Yu. and Tsellarius A. Yu.** (2005a), "Formation and defense of the individual space in males of *Lacerta saxicola*. 2: Territory and territorial relations," *Zool. Zh.*, **84**, in press [in Russian].
- Tsellarius E. Yu. and Tsellarius A. Yu.** (2005b), "Interrelations between sexes in *Lacerta saxicola*," in: Ananjeva N. and Tsinenko O. (eds.), *Herpetologia Petropolitana. Proc. of the 12th Ord. Gen. Meeting of the Soc. Eur. Herpetol., August 12 – 16, 2003*, St. Petersburg, pp. 226 – 229 [this issue].
- Wynne-Edwards V. C.** (1962), *Animal Dispersion in Relation to Social Behavior*, Oliver and Boyd, Edinburgh – London.

INTERRELATIONS BETWEEN SEXES IN *Lacerta saxicola*

E. Yu. Tsellarius¹ and A. Yu. Tsellarius¹

INTRODUCTION

Mating systems are considered as a result of the interdependent development of behavioral reproductive strategies of sexes (Davies, 1992; Govaty and Buschhaus, 1998). However, there exists no generally accepted hypothesis on the origin and evolution of these strategies. Investigations of intersexual social behavior of reptiles may be useful for solution of this problem. Rather advanced intersexual relations of reptiles are known (Bull, 1994; Tsellarius and Tsellarius, 1996; Panov and Zykova, 1999). However, detailed long-term observations on intersexual relations of reptiles are very scarce.

MATERIAL AND METHOD

In the article at hand we have briefly reported the results of our seven-year observations of a group of individuals of *Lacerta saxicola* in the deciduous forest of the Navagir mountains, in Northern Caucasus. Observed was the group of the lizards inhabiting a little clearing arisen as a result of a fall of two trees. All the members of the settlement were marked with personal color mark and their age was known. In total, more than 600 contacts between marked males and females have been described and analyzed. A detailed description of the studied region, the observation and the data handling technique have been published (Tsellarius and Tsellarius, 2001; 2005a; 2005b; Tsellarius and Tsellarius, 2002). In order to appreciate correctly the process of social life in the settlement it must be taken into account that age of *Lacerta saxicola* may attain probably 15 years. At present, the age of settled individuals, those constituting “backbone” of the settlement, amounts from 5 to 12 years.

RESULTS

Intersexual Behavior

Having examined intersexual behavior of lizards, we differentiate three main kinds of behavior of males. 1. Indifference. Male does not express visibly an interest in female. 2. Amicable communication. Curving the neck,

male touches by nose female's sacrum, back and nape, crawls over her, sometimes slightly bites her tail or neck. Male regularly and for a long time lies over female or near her, putting his legs on her back. Some of these actions are included in the courtship also, but we differentiate those as a particular set of behavioral acts since they are rather often performed without any connection with copulation and occur not during mating period only, but throughout the activity season with approximately equal frequency. 3. Aggression towards female. In all instances, it is the redirected aggression of non-ritualized nature. It has been rather rarely observed exclusively either in the course of, or immediately after the border conflict between territorial males, from one of the contestants.

In females, there are four kinds of non-sexual behavior. The first (1. Indifference) and the second (2. Amicable communication) are almost entirely similar to those of males. 3. Rejection of a bodily contact. Female dodges, but usually does not take to flight. This action may be accompanied by peculiar displays, that is rotation of forelegs, which is connected, in the event of maximum expression, with bending up the forepart of body. 4. Aggression towards male. Ritualized threat has been observed from time to time towards only unfamiliar non-territorial males. Females well recognizes settled males of the settlement personally. Direct vigorous non ritualized attack with strong bites was observed as a response to forced copulation only.

In the studied region we have observed three patterns of copulation. 1. Amicable copulation. Male takes a female by jaws by the sacrum and massages it, and at the same time often more or less vigorously scratches the base of tail with foreleg. Then he bends the hind part of body under female and inserts a hemipenis. The female usually appears to be rather indifferent or slightly bites the male, and never takes to flight after copulation. The copulation may be preceded and/or followed by amicable communication. 2. Initiation. It differs from amicable copulation mainly in that at the beginning the male firmly takes the female by the tip of tail and follows her at such a way for some time, before to take her by the sacrum. The scratching of female's tail base always takes place and it is always vigorous. During the massage, sometimes during coitus, female tries to tear herself away and may strongly bite the male. Amicable communication after copulation has never been observed. However, the female usually remains near

¹ Laboratory of Comparative Ethology, Severtsov Institute, Leninsky pr. 33, 199071 Moscow, Russia; E-mail: cellarius@land.ru.

the male. This pattern took place mainly in those instances when a territorial male mated a virgin or an unfamiliar young female. 3. Rape. A male rushes to the female, his behavior resembling an attack, and immediately seizes her by the side or the sacrum, and copulates with her after brief vigorous massage in spite of the violent resistance of female. The scratching may be absent. In the case of rape, a male pays no attention to any displays of female. After mating, female either takes to flight or attacks the male, cruelly bites and chases him. Strength of the massage is very different in different patterns of mating. In females undergone the initiation or the rape, on a sacrum and on a base of tail the bruises arise, which are distinctly observable even at a distance, with binoculars. There were no bruises after amicable copulation.

Intersexual Relationships

There are several quantitative indices found rather useful for analysis of dyad non sexual interrelations.

1. Degree of intimacy, $INT = (C_b - C_w)/(C_b + C_w)$, where C_b is amount of interactions, which include bodily contacts, C_w is amount of interactions without bodily contacts.
2. Degree of female's initiative, $FI = (I_f - I_m)/(I_f + I_m)$, where I_f is amount of bodily contacts on female's initiative and I_m is amount of bodily contacts on initiative of male.
3. Degree of female's amicability, $FA = (A_f - N_f)/(A_f + N_f)$, where A_f is amount of interactions in which female carries out behavior of amicable communication, N_f female is indifferent or rejects a bodily contact. All indices vary from -1 to $+1$. In observed settlement, in the case of long-term dyad interrelations, the absolute value of any index amounts to either about zero or more than 0.4. It permits to operate with only three values: positive, zero and negative. Six kinds of long term non sexual interrelations are most usual here (Table 1).

Different kinds of interrelations are the successive stages of progress of relationship between female and settled male. Female's switch to settled life follows a reaching of sexual maturity and takes place usually after third hibernation. Female-newsettler initially finds herself among unknown or unfamiliar males, and female's rela-

tions with all neighboring males are initially hostile. The males, from time to time, try to enter into amicable contact with encountered female, but female avoids any interactions with males. Hence, saying hostility we mean the hostile behavior of female, but not of male. The latter is characterized by mixed indifferent-amicable behavior. In this case, a copulation is always forced. Female actually has no possibility to select a sexual partner.

Almost simultaneously with forming of structure of her own home range, which may overlap territories of several males, female may begin to ingratiate herself with one of the males having the resident status. Ingratiating female does not look for meetings with the male. In any encounter, however, she takes an initiative for amicable communication. Female does not ingratiate herself with more than one resident at a time. In a certain case, however, she may switch from one subject to another. After 7 – 15 days of female's ingratiating, the resident includes her basking centers in his system of patrol routes (Tsellarius and Tsellarius, 2005a) and interrelations become amicable. From this point, female usually stops to take the initiatives in amicable communication, she begins to avoid, if possible, a bodily contact, but may amicably respond to male's initiatives.

Interrelations with other "overlapped" residents become amicable too, however on initiative of males (casual ingratiating, see Table 1), and this process takes 3 – 4 months. In this case, a male, from time to time, takes attempts to enter into amicable contact, and female, little by little, begins to take no avoidance and next begins to amicably respond to initiatives of the male. It appears that there occurs nothing but a habit. It is rather usual, that female-newsettler does not ingratiate herself with any resident; and amicable interrelations will be established in such opportunistic manner. It is significant that, however, amicable relations are established not with all residents. Interrelations with some individuals remain hostile for unlimitedly long time for unknown reason.

In almost all instances the relations with poachers were kept hostile. These relations may turn into amicability after only the poacher becomes resident. However,

TABLE 1. Characteristics of Different Kinds of Dyad Interrelations between Males and Females

Kind of interrelations	INT	FI	FA	Period of occurrence
Hostility	–	–	–	All the season
Female's ingratiating*	+	+	+	Before and/or after mating period
Male's casual ingratiating*	–	–	0	Before and/or after mating period
Male's obstinate ingratiating	0	–	0	All the season
Amicability	+	–	+	All the season
Partnerships	+	0	+	All the season

* During mating period, a reverting to the previous kind of interrelations (see below) usually takes place on female's initiative.

there are rare exceptions to this rule. We observed the incident of arising of amicable relations between an old female and a poacher. These rare exceptions are very significant since they are showing that there exists no insuperable impediment to amicability to any male.

Amicability is characterized by high frequency of interactions with bodily contacts and by amicable response of female to male's initiatives. The initiatives, however, are taken by male mainly. Amicable lizards may forage and bask about one another for a long time, but without bodily touch. Amicable relations may be continued for an indefinitely long time. A number of amicable partners are not limited both in males and in females. In the event that relations are amicable, female has an ability to suppress importunity of the male by means of either to assume posture of mate rejection or to crawl repeatedly over the male's back. It always leads to stopping the importunity of the male. Therefore, a forced copulation takes place, actually, in situation when female persistently refuses amicability with the male.

A partnership is the next stage after amicability. The conversion of the amicability into partnership, however, is not inevitable. There are approximately 80% of adult females, which have never had partnership status. Sometimes, a partnership is a result of male's initiatives (obstinate ingratiating). It demands hard efforts for a long time. The male persistently follows preferred female during two, three years. After all, the female surrenders, as it were. Much more often, however, the partnership is a result of female's efforts. Female begins to form partnership making efforts to enter into bodily contact in every encounter with amicable resident. The male begins to visit her basking centers more and more frequently. As a result, formed are the centers of male's activity in basking centers of that female (Tsellarius and Tsellarius, 2005b). Having converted amicability into partnership, female always kept a fidelity to the resident selected as an object of ingratiating, unlike conversion of hostility into amicability. Basking centers of female are disposed over the territories of several males. Usually, but not always, selected is the resi-

dent, in whose territory the majority of basking centers of given female have been formed.

When partnership relations have been established, partners spend much time together. The animals lie in bodily contact for a long time, crawl over one another, frequently return to the partner. Just the recumbent posture in mutual embrace is the specific difference between amicable and partnership pairs. A partnership can last for many years. In all observed pairs these relations last up to the death of one of the partners. It is important, that both kinds of intimate relations, amicability and partnership, are intense during all the year and are not restricted within the mating period only.

Actually, any female may be forced from time to time. The female, however, which has partnership status, is significantly more rarely forced (Table 2). The attempts to rape are broken off with aggression of her partner towards violator. Protection is very effective just because of the male spends much time in female's basking centers (Tsellarius and Tsellarius, 2005a, b) and intensively patrols them. During mating period, however, female copulates not only with the partner, but permits copulation with several amicable males.

DISCUSSION

One of the current ideas of the evolution of intersexual relations is that sexual aggression is the peculiar strategy of males. It is believed that this strategy to force females to resort to the protection of a certain male and to pay copulation for this protection (Govaty and Buschhaus, 1998). It is evident, however, that the sexual aggression of males of *Lacerta saxicola* is a result of social fastidiousness of female. In other words, a sexual aggression is an inevitable side effect of social fastidiousness of females. Another side effect is that females are forced to use various behavioral devices in order to compensate for the consequences of their fastidiousness. As a result, the situation is rather paradoxical. Really, establishing an amicability or, especially, a partnership, female choose not a sexual partner, but a male, which can be used as a protector, and which can be rejected as a sexual partner. Per se, she creates the surroundings in which she qualifies for freedom in choosing of a sexual partner. The reason that female does not establish these interrelations with each neighbor is unknown.

Poorly corresponds with current conceptions of behavioral ecology is the fact also, that there simultaneously exist two ways of forming of pairs, on initiative of male and on initiative of female. However, a more interesting

TABLE 2. Frequency of the Unsuccessful Attempts at Rape in Different Surroundings

Relations between female and the owner of the territory where attempt took place	Total number of observed attempts	Rate of attempts being unsuccessful because of aggression of territory owner, %
Hostility*	24	45.8
Amicability	37	78.4
Partnership	33	100

* Attempts of territory owner, which are successful in 58.6% of cases ($n = 29$), are not taken into consideration.

fact, in our point of view, is the motivation of male's behavior. The majority of evolutionary conceptions either explicitly or implicitly suggest that the evolution of male's behavior leads, first of all, towards acquisition of access to copulation, preferably to exclusive access (Wilson, 1975; Emlen and Oring, 1977). In the case of the *Lacerta saxicola* male, however, it is evident that a possibility to communicate with female is much more attractive than copulation in itself. Accordingly, female pays not a copulation, but communication for defense and for freedom in choosing of sexual partner.

Just owing to these eccentric, with relation to reproductive success, predilections of the males there is a possibility of reconciliation of the conflicting objectives of the sexes. In female's point of view, the main and rather complicated problem is that territories – and, consequently, resources — are randomly distributed among males. The poacher establish a residence either in initially empty space or on a place, which is emptied since death of previous owner. At a later time, redistribution of territories does not happen (Tsellarius and Tsellarius, 2004). A combination of good quality of territory and of its owner is a rare random event. As a result, female has no possibility to obtain simultaneously good ecological conditions and good sexual partner. Female has a possibility to slip out the situation just owing to the amicable communication, which is, in male's point of view, the most attractive resource for all year round. The resident pays protection for communication and renounces a copulation to some extent.

As a result, both the males and the females have a possibility to convert their main motivations into behavior. In other words, everybody is highly pleased. This harmony, however, has no connection with breeder fitness and offspring viability by no means. It is quite possible that situation cannot be interpreted in such terms as a cost, a benefit and reproductive success. It must be taken into account that behavior of animal is a realization of certain psychic state, that is motivation. A fitness cannot be the motivation in any case. It is beyond any doubt that the issue demands further investigation. Anyway, however, the situation appears to be essentially more complex than some socio-biological conceptions suggest.

REFERENCES

- Bull C. M.** (1994), "Population dynamics and pair fidelity in sleepy lizards," in: Vitt L. J. and Pianka E. R. (eds.), *Lizard Ecology*, Princeton Univ. Press, pp. 159 – 174, 335 – 387.
- Davies N. B.** (1992), "Mating systems," in: Krebs J. R. and Davies N. B. (eds.), *Behavioral Ecology: An Evolutionary Approach*, Blackwell Sci. Publ., Oxford, pp. 263 – 294.
- Emlen S. T. and Oring L. W.** (1977), "Ecology, sexual selection, and the evolution of mating systems," *Science*, **197**, 215 – 223.
- Govaty P. A. and Buschhaus N.** (1998), "Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy," *Am. Zoologist*, **38**, 207 – 225.
- Panov E. N. and Zykova L. Yu.** (1999), "Social behavior and communication in rock agama *Laudakia caucasica*," *Russ. J. Herpetol.*, **6**(3), 215 – 230.
- Tsellarius A. Yu. and Tsellarius E. Yu.** (1996), "Courtship and mating in *Varanus griseus* of Western Kyzylkum," *Russ. J. Herpetol.*, **3**(2), 122 – 129.
- Tsellarius A. Yu. and Tsellarius E. Yu.** (2001), "Alteration of spacing pattern of population of *Lacerta saxicola* in broad-leaved forests of Navagir mountain ridge," *Zool. Zh.*, **80**, 1 – 8 [in Russian].
- Tsellarius A. Yu. and Tsellarius E. Yu.** (2005a), "An access to the females as a resource of male's territory in *Lacerta saxicola*," Ananjeva N. and Tsinenko O. (eds.), *Herpetologia Petropolitana. Proc. of the 12th Ord. Gen. Meeting of the Soc. Eur. Herpetol.*, August 12 – 16, 2003, St. Petersburg, pp. 222 – 225 [this issue].
- Tsellarius A. Yu. and Tsellarius E. Yu.** (2005b), "Formation and defense of the individual space in males of *Lacerta saxicola*. 1: Home range," *Zool. Zh.*, **84**, in press [in Russian].
- Tsellarius E. Yu. and Tsellarius A. Yu.** (2002), "Age change and probable function of the signaling coloration in males of *Lacerta saxicola*," *Zool. Zh.*, **81**, 970 – 977 [in Russian].
- Tsellarius E. Yu. and Tsellarius A. Yu.** (2005), "Formation and defense of the individual space in males of *Lacerta saxicola*. 2: Territory and territorial relations," *Zool. Zh.*, **84**, in press [in Russian].
- Wilson E. O.** (1975), *Sociobiology: The New Synthesis*, Harvard Univ. Press, Cambridge.

RAPD MARKERS IN APPLICATION TO *Rana temporaria* GENOTYPE CHARACTERIZATION (PRELIMINARY DATA OF POPULATION STUDY)

O. V. Tsinenko¹

Keywords: DNA extraction, RAPD-PCR, molecular methods, *Rana temporaria*, skeletochronological method.

INTRODUCTION

Rana temporaria is a classical laboratory subject, one of the most common vertebrate species in the North-West of Russia. We present preliminary results of a comparative study of genetic polymorphism using randomly amplified polymorphic DNA as molecular markers. This study was conducted within the framework of the project “Comparative analysis of the structure of several geographically isolated populations of *Rana temporaria*.”

MATERIAL AND METHODS

Sampling and DNA Extraction

Samples consisting the fourth toe of 67 individuals were collected from the *Rana temporaria* population of in Leningradskaya Oblast', 14 km northward from Osmino town, in the middle stream of the Luga River. The sampling was carried out during the migration of frogs to their winter places in August 2002 ($n = 29$) and at the spawning places in April – May 2003 ($n = 38$). Samples were preserved in 96% ethanol.

We applied this toes to both skeletochronological and molecular studies at the same time (Gonser and Collura, 1996; Jehle and Arntzen, 2002). The second phalanges were used for age determination of individuals by the skeletochronological method (Smirina, 1972, 1994; Castanet et al., 1977); the third and fourth phalanges were used for DNA extraction. Dried fingers also were used for tissue sampling (without previous 4% formaldehyde treatment) but ethanol fixation was more suitable for DNA extraction. DNA was obtained by proteinase K digestion and phenol-chloroform, chloroform extractions followed by ethanol precipitation. Since DNA extracted from toes was more pure than DNA from testis or liver, phenol extraction (unlike the standard protocols of Sambrook et al., 1989) was omitted. The DNA was dissolved in 300 – 500 μ l TE buffer (10 mM Tris-HCl pH = 7.4; 1 mM EDTA; pH =

7.35) depending on the yield of DNA. The samples were then stored at -20°C prior to PCR with RAPD-primers.

RAPD Amplifications

To generate RAPD profiles from frogs' DNA we tested 20 ten base primers (A1 – A20) from the Operon Technologies Primer Kit A in PCR amplifications (Williams et al., 1990). Amplification reactions were performed in final volumes of 25 μ l containing 60 – 100 ng/ μ l of DNA, 1 μ l of primer, 4 μ l of 50 mM MgCl_2 , 0.6 μ l of 25 mM dNTP, 0.7 μ l of 5000 U/ml Taq polymerase (ROSNIIGenetika, Russia), 2.5 μ l of 10 \times reaction buffer for Taq polymerase and 15.2 μ l of water. Thermal cycling was conducted in a GeneAmp* Cyclor and consisted of an initial 96 $^{\circ}\text{C}$ for 6 min, followed by 40 cycles of 1:15 min at 96 $^{\circ}\text{C}$ (denaturation), 1:15 min at 33 $^{\circ}\text{C}$ (annealing) and 1:30 min at 72 $^{\circ}\text{C}$ (elongation). There was then an additional 10 min period for elongation at 72 $^{\circ}\text{C}$. Negative controls were used to verify for contamination.

PCR products were separated on 1.5% agarose gels that were run at 65 V for 3 h in 0.5 M TBE buffer. Amplified DNA was stained with ethidium bromide and photographed using a Vilber Lourmat camera and UV transilluminator. We used BioCaptMW 10.04 for Windows to record band patterns on disc.

The following RAPD primers: OPA 03 5'AGTCAG-CCAC3'; OPA 04 5'AATCGGGCTG3'; OPA 11 5'CAATCGCCGT3', were selected based on the polymorphism and reproducibility of the bands they generated. Amplifying the same samples on different days tested reproducibility. Primer OPA 10 (5'GTGATCGCAG3'), L15/AS19 (5'GAGGGTGGCGGCTAG3') and L45 (5'GTAACG-ACGGCCACT3') UP-primers (according to Bulat et al., 1993) might be used for RAPD-PCR of *Rana temporaria* as well. These primers were not used for preliminary results.

OPA 03 produced 8 scorable bands, OPA 04 — 9, OPA 11 — 11. Thus they produced a total of 30 polymorphic DNA fragments ranging in size from 300 to 1160 bp.

¹ Zoological Institute of Russian Academy of Sciences, Universitetskaya nab. 1, 199034 St. Petersburg, Russia; E-mail: 12SEH@zin.ru.

All gel photographs were scored for presence and absence of bands. They also were used to determine the RAPD genotypes of individuals based on RAPD-profiles. UPGMA cluster analysis was performed using Treecon 1.3b for Windows to detect genetic differentiation in the population.

RESULTS AND DISCUSSION

Skeletochronological Data

Age determination was carried out by counting annual layers (Smirina, 1994) in the bones of amphibians caught during only the autumn migration. We counted the number of lines of arrested growth (LAG) in the cross-sections of the diaphysis of toes' phalanges. The number of LAGs by the adults and immature individuals ranged from 0 to 5.

There is a large marrow cavity in the phalangeal cross-sections of the froglets of August 2002; metamorphosed line (ML) was destroyed by the resorption process. The marrow cavity diameter of adults is equal to or slightly larger than the diameter, limited by the resorption line. The diameter of the first visible LAG is not much larger than the diameter of the resorption line. Probably the first visible LAG is a sign of the second wintering, not the third one. In several cross-sections the first visible LAG was almost totally destroyed by the resorption. That diameter is equal to the diameter of the phalangeal cross-section of the froglets. Thus, the ML and LAG of the first wintering are destroyed by the resorption in the phalanges of older individuals.

It is known that common frogs mature and start breeding after their third wintering (Terentjev, 1950). In the case of two LAGs resorption we should find individuals with 0 visible LAGs. In other words, two LAGs are resorbed and the third one does not come off from the edge of the bone because the frogs do not grow during spawning. The absence of spawning individuals having 0 visible LAGs proves that only one LAG is destroyed by the resorption process.

It is necessary to clarify how many (one or two) LAGs are destroyed by the resorption. We suppose that individuals were caught between the 1st — 6th wintering (their age was 1 – 6 years). There were 4 individuals aged 1+, one aged 2+, fifteen — 3+, six — 4+, two — 5+ and one individual was at the age of 6+.

Molecular Data

The percentage of polymorphic bands, repeatabilities for fragments and differentiation of RAPD genotypes were used as indicators of genetic diversity.

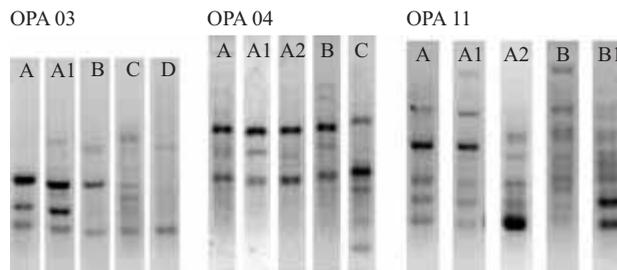


Fig. 1. RAPD genotypes.

Fragment repeatability ranged from 1.0 (all individuals were the same) to 0.0526 (two of 67 individuals showed this band). As a whole, fragment repeatability is, in general, medium; however the percentage of polymorphic fragments is rather high in both series of individuals.

Table 1 summarizes data on the number of fragments detected per primer and the number of polymorphic fragments (absent from at least one individual in all frogs used).

The mean repeatability of fragments from both series of individuals ($n = 67$) for three primers was $49.06 \pm 6.85\%$, while $93.34 \pm 0.01\%$ of these fragments were polymorphic. Thus, the polymorphism level is rather high in the population for chosen molecular markers (Table 1).

Differentiation of RAPD genotypes is based on absence/presence and different combinations of the bands in RAPD profiles. Figure 1 shows the determined groups of RAPD genotypes. Their determination was difficult for OPA 11 because of the quantity of combinations of the polymorphic bands.

Table 2 shows the repeatability of different RAPD genotypes, which ranged from 2.63 to 52.63%. Different RAPD genotypes prevailed in two different series of individuals, caught from different places at different times. Thus, for OPA 03 genotype A1 has maximal repeatability in the autumn group (41.39), while the genotype B has maximal repeatability in the spawning group (52.63). Genotype C has minimal repeatability in both series. Genotype B for OPA 04 repeats most often among all the genotypes in both the autumn and the spring series, while geno-

TABLE 1. Characteristics of RAPD Products

Primer	Number of scorable fragments	Mean % repeatability		% polymorphic fragments	
		Autumn 2002	Spring 2003	Autumn 2002	Spring 2003
OPA 03	8	50.54	55.92	87.50	90
OPA 04	9	41.76	43.57	100	88.89
OPA 11	13	55.70	46.16	100	100
Total	30	47.98	50.14	96.67	90

TABLE 2. Repeatability of RAPD Genotypes

Primer	RAPD genotype	Autumn 2002, %	Spring 2003, %
OPA 03	A	17.24	7.90
	A1	41.39	34.21
	B	24.14	52.63
	C	3.44	2.63
	D	13.79	2.63
OPA 04	A	3.44	10.53
	A1	20.69	10.53
	A2	24.14	21.05
	B	27.59	31.58
	C	24.14	26.31
OPA 11	A	24.14	18.42
	A1	3.44	15.79
	A2	20.69	36.84
	B	20.69	5.26
	B1	31.04	23.69

type A is the rarest among those two series. Genotypes A1 and B are the rarest, whereas B1 and A2 are the most common for OPA11.

Nei-Li UPGMA clustering, neighbor-joining bootstraps and their dendrograms produced results depending on the 30 random amplified polymorphic DNA markers (repeatability of bands). According to all dendrograms there were no significant differences at the level of RAPD-DNA polymorphism both among generations and among the individuals in different spawning places. Groups of RAPD profiles (RAPD genotypes) correspond neither to determined age groups, nor to sex, different spawning places, or feeding biotopes. In each series there are individuals of different age and different sampling sites. The absence of clear differentiation among series probably also reflects small series sizes of series and an insufficient quantity of DNA markers.

Moreover, the comparison with several individuals from an outgroup population in Pskov Oblast' (200 km southwestward) also does not show any differences. There must be further study in other populations to clarify if the low genetic variability is typical for the northern part of the distribution range as was shown for *Lacerta agilis* (Gullberg et al., 1998). We should use other molecular markers having relatively higher statistical power (micro-

satellite DNA for example) to estimate specific population polymorphism.

The combined application of collected samples for both skeletochronological and molecular studies let us use the population resources rationally, and to estimate the age and genetic population structure simultaneously.

Acknowledgments. I thank Vladimir G. Ishchenko (Institute of Plant and Animal Ecology, Russian Academy of Sciences, Ural Department) for help in age determination, Natalia B. Ananjeva (Zoological Institute, Russian Academy of Sciences) and Ella M. Smirina (N. K. Kol'tsov Institute of Developmental Biology, Russia) for their scientific consultations, and the staff of the laboratory "Taxon" (at the Zoological Institute, Russian Academy of Sciences, St. Petersburg) who supported this study. The work was supported by the Grant "Herpetological Scientific School" No. 1647.2003.4, The program of fundamental research of Russian Academy of Sciences "Dynamics of plant, animal and human gene pools," The program of presidium of Russian Academy of Sciences "Scientific fundamentals of biodiversity conservation in Russia."

REFERENCES

- Castanet J., Meunier F. J., and De Ricqles A. (1977), "L'energissement de la croissance cyclique, par le tissu osseux chez les vertebres poikilothermes: donnees comparatives et essai de synthese," *Bull. Biol. Fr. Belg.*, **111**(2), 183 – 202
- Gonser R. A. and Collura R. V. (1996), "Waste not-want not: toe clips as a source of DNA," *J. Herpetol.*, **30**, 445 – 447.
- Gullberg A., Olsson M., and Tegelström H. (1998) "Colonization, genetic diversity, and evolution in the Swedish sand lizard, *Lacerta agilis* (Reptilia, Squamata)," *Biol. J. Linn. Soc.*, **65**, 257 – 277.
- Jehle R. and Arntzen J. W. (2002), "Microsatellite markers in amphibian conservation genetics," *J. Herpetol.*, **12**, 1 – 9.
- Smirina E. M. (1972), "Annual layers in bones of *Rana temporaria*," *Zool. Zh.*, **51**, 1529 – 1534 [in Russian].
- Smirina E. M. (1994), "Age determination and longevity in amphibians," *Gerontology*, **40**, 133 – 146.
- Terentjev P. V. (1950), Frog, Sovetskaya Nauka, Moscow [in Russian].
- Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A., and Tingey S. V. (1990), "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucl. Acids Res.*, **18**(22), 6531 – 6535.

GENE CRYOBANKS FOR CONSERVATION OF ENDANGERED AMPHIBIAN SPECIES

V. Uteshev¹ and E. Gakhova¹

Keywords: genetic cryobank, amphibian cryocollection, cryopreservation of amphibian spermatozoa, cryopreservation of amphibian germinal cells.

INTRODUCTION

Conservation of genomes of endangered animal and plant species in genetic cryobanks now becomes one of the basic strategies of rescue of the nature from irreversible destruction. This strategy is based on the advanced achievements of biological science and biotechnology. The main purpose of genetic cryobanks is long-term preservation of biological material (reproductive and somatic cells) of representatives of endangered species. Genetic material conserved in cryobank provides a chance to restore animal and plant species that are completely lost in nature or faced with extinction.

Strategy of creation of genetic cryobanks has exceptional advantages. It should develop in close interaction with such traditional strategy as allocation of strictly protected territories and breeding of animal in zoos and nurseries.

GENETIC CRYOBANK IN INSTITUTE OF CELL BIOPHYSICS (ICB)

In the last decades genetic cryobanks have been created in a number of the countries of Europe and America. In the Institute of Cell Biophysics of the Russian Academy of Sciences (ICB) an experimental genetic cryobank of plants and animals (Gakhova, 1998) has been created including cryocollection of amphibian genomes (Uteshev and Gakhova, 1994). This genetic cryobank has the necessary facilities including a room for cryostorage with stationary and portable Dewar vessels, a room with installations for production of liquid nitrogen, aquaria complex adjusted for cultivation of fishes, amphibians and other aquatic animals.

AMPHIBIAN CRYOCOLLECTION

Objects used for preservation in cryobanks are usually spermatozoa, oocytes, and embryos at early stages of de-

velopment. For creation of cryocollection of amphibian genomes we have taken spermatozoa and germinal cells as genetic material. Spermatozoa were obtained by disintegration of testis and germinal cells, by dissociation of embryos at blastula stage (Kaurova et al., 1996, 1998; Uteshev et al., 2001). The choice of germinal cells as an object for genetic cryocollection of amphibians is based on the fact that by the present time the technologies allowing cryopreserving of amphibian oocytes and embryos still have not been created. Nuclei of germinal cells of amphibians at blastula stages have totipotent and can be used successfully for homo- and hetero-transplantation into enucleated oocytes for obtaining of the reconstructed zygotes capable of normal development (Nikitina, 1996). These data allow considering germinal cells of amphibians as essential genetic material for cryocollections and cryobanks.

Cryopreservation of Amphibian Spermatozoa

Optimum cryoprotective solution for cryopreservation of amphibian spermatozoa is a medium on the basis of simplified Ringer's solution for amphibians containing 15% DMCO, 10% of saccharose and 1% of bovine serum albumin. Cryopreserved spermatozoa were estimated by their mobility using the methods of fluorescent and colorimetric analysis, and also by their fertilizing ability (Kaurova et al., 1996, 1997; Uteshev et al., 1999). As a result of using of cryopreserved spermatozoa of *Rana temporaria* for artificial insemination normally developing embryos have been obtained. Then tadpoles with normal metamorphose and have developed up to maturity.

Cryopreservation of Amphibian Germinal Cells

Germinal cells were obtained by dissociation of embryos at blastula stage in calcium-free variant of Niu-Twiggi medium. The best results at cryopreservation of germinal cells have been obtained when we used a combination of 10% DMCO and 10% saccharose prepared on the basis of calcium-free variant of Niu-Twiggi medium with addition of bovine serum albumin (1 – 0.5%) (Kaurova et al., 1998). Freezing of suspension of germinal cells in cryoprotective medium was carried out by direct immersing of test tubes with material in liquid nitrogen. Speed of

¹ Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, 142290 Moscow Oblast, Russia; E-mail: gakhova@icb.psn.ru.

freezing in this case was 500 – 1000°C/min. Defreezing of test tubes with germinal cells was carried out on a water bath at 38 – 40°C prior to the defreezing of thawing a solution in a test tube. Qualitative analysis of the defrozed germinal cells was carried out by fluorochroming of such fluorescent dyes as ethidium bromide or fluorescein diacetate (Elnikova et al., 2003). The analysis of fluorochroming is conducted using modified luminescent microscope (LUMAM-I3, LOMO, St. Petersburg, Russia). Our study has shown that the use of the described methods allows obtaining up to 80% intact cryopreserved germinal cells of amphibians. In a separate preliminary series of experiences cryopreserved germinal cells of *Bufo bufo* have been used for homo-transplantation in enuclear zygotes. In a part of the reconstructed zygotes the initial development up to a blastula stage was observed (Uteshev et al., 2002).

Thus, it has been shown, that spermatozoa and germinal cells of amphibians successfully resist freezing and after defreezing can be used to obtain viable animals. Therefore, it is necessary to consider spermatozoa and germinal cells as high-grade material for creation of genetic cryocollections of amphibians. Now in genetic cryocollections in ICB cryobank is stored genetic material (spermatozoa and germinal cells of 6 amphibian species: common frog (*Rana temporaria*), pool frog (*R. lessonae*); moor frog (*R. arvalis*), smooth clawed frog (*Xenopus laevis*), Kenya smooth clawed frog (*X. borealis*), and common toad (*Bufo bufo*).

Acknowledgment. This work was supported by the “R 2004 Naukograd” (grant No. 04-04-97300).

REFERENCES

- Uteshev V. K. and Gakhova E. N.** (1994), “The prospects of amphibian gene cryobank creation,” *Biophys. Living Cell*, **6**, 28 – 34.
- Nikitina L. A.** (1996), “Nuclear heterotransplantation in fish and amphibians,” *Ontogenez*, **27**(5), 325 – 340 [in Russian].
- Kaurova S. A., Chekurova N. R., Melnikova E. V., Uteshev V. K., and Gakhova E. N.** Cryopreservation of frog (*Rana temporaria*) sperm without a loss of fertilizing capacity. Genetic resource conservation,” in: *Proc. of the XIV Working Meeting, May 28 – 30, 1996*, Pushchino, pp. 106 – 107 [in Russian].
- Kaurova S. A., Uteshev V. K., Chekurova N. R., and Gakhova E. N.** (1997), “Cryopreservation of testis of frog *Rana temporaria*. Infusionster. *Trasfusiosmed.*, **24**(5), 378.
- Gakhova E. N.** (1998), “Genetic cryobanks for conservation of biodiversity. The development and current status of this problem in Russia,” *Cryo-Lett.*, Suppl. 1, 57 – 64.
- Kaurova S. A., Nikitina L. A., Uteshev V. K., and Gakhova E. N.** (1998), “Cryopreservation of totipotent embryo cells and their use in reconstruction of enucleated eggs. Genetic resource conservation,” in: *Proc. of the XV Working Meeting, October 13 – 15, 1998*, Pushchino, pp. 206 – 208 [in Russian].
- Uteshev V. K., Kaurova S. A., Vrublevskaya V. V., and Gakhova E. N.** (1999), “Low-temperature conservation of sperm and totipotent embryo cells of amphibians,” *Tsitologiya*, **41**, 321 [in Russian].
- Uteshev V. K., Kaurova S. A., and Gakhova E. N.** (2001), “Creation of low-temperature collection of viable reproductive and somatic cells of amphibians,” in: *The Problems of Herpetology. Proc. of the 1st Meeting of the Nikolsky Herpetol. Soc., December 4 – 7, 2000*, Pushchino, Pushchino – Moscow, pp. 300 – 301.
- Uteshev V. K., Melnikova E. V., Kaurova S. A., Nikitin V. A., Gakhova E. N., and Karnaukhov V. N.** (2002), “Fluorescence analysis of cryopreserved totipotent cells of amphibian embryos,” *Biophysics*, **47**(3), 506 – 512.

MORPHOLOGICAL DEVIATIONS IN POPULATION *Rana arvalis* NILSS. ON URBANIZED TERRITORIES: SPECTRUM, TOPOGRAPHY, FREQUENCY

V. L. Vershinin¹

Keywords: morphological deviations, moor frog, urban ecology, morphogenesis.

INTRODUCTION

Decline of amphibian population together with their high sensitivity to changes in water and terrestrial habitats can be a serious warning about the beginning of a global ecological catastrophe (Halliday, 1998). Individuals with various morphological deviations often occur in amphibian populations being a part of their natural variability (Kovalenko and Kovalenko, 1996). The reasons of that are different: mutation process, parasites, inbreeding depression, developmental stress, abnormal regeneration etc. (Dubois, 1979; Borkin and Pikulik, 1986; Hebard and Brunson, 1963; Talvi, 1994). Many facts confirm the influence of environmental conditions on increase of the frequency of morphological anomalies (Cooke, 1981). The anomalies, arising as a result of developmental deviations and atypical regeneration, often can be determined by inhibition or activation of the thyroid function by pollutants, these may lead to suppression of proliferation and morphogenetic processes (Syuzumova, 1985) and influence the level of metabolic processes (Tokar' et al., 1991). That is the reason why morphological deviations as well as the process of morphogenesis in this group of vertebrates may be sensitive indicators of environmental changes.

MATERIAL AND METHODS

The data below is a result of generalization of long-term investigations on the areas of urban development (Yekaterinburg, Russia) from 1977 to 2001 (14691 specimens) on froglets and adult *R. arvalis* in this area. City territory was conventionally divided into areas with the different levels of urbanization: zone II, multistory buildings; zone III, areas of low buildings; zone IV, forest parks of the city. Control sites with natural amphibian population (C) were located in the area situated in 23 km from the city. The acceptability of the typification was confirmed by the data of hydrochemical analyses. Overlap of

the deviations spectrum was determined by the Morisita index:

$$C_M = 2 \frac{\sum a_i b_i}{\sum a_i^2 + \sum b_i^2} aN bN,$$

where a_i are numbers of deviation i in population A; b_i are the same for population B; aN are numbers of abnormal animals in population A; and bN are the same for population B (Hurlbert, 1978).

RESULTS AND DISCUSSION

The results indicate (Table 1) that *R. arvalis* froglets from populations in all zones differed significantly from each other ($\chi^2 = 15.8 - 86.0, p = 0.05 - 0.001$), but the differences between populations from zones II and III were not so significant as between the populations from urban territories and forest. Adult individuals from zone II significantly differed from those in the forest and forest park zones ($\chi^2 = 4.7, p < 0.05$; $\chi^2 = 6.7, p < 0.05$, respectively).

Among froglets, the deviation spectrum is significantly wider in zones IV and III. In population subject to the highest influence of urbanization (zone II) it increased to 13 types, which exceeds the control level. This definitely indicates qualitative difference of populations from zone II. Overlapping degrees in adult frogs were similar to those in froglets. The degree of overlap of the spectra of deviations calculated with Morisita index showed that age changes of deviation spectrum are much higher than similarity — in the forest population, 18.5%, in zone IV, 26.8%, in zone III, 17.3%. Only in zone II it is overlapped by 45.0%. The comparison shows that among adults there are no mandibular hypoplasia and non-flexible limbs which are lethal for the animal. Cluster analysis in juveniles (Fig. 1) showed that the greatest differences are recorded between animals from forest population and populations from zone II, III, IV. In adult frogs the spectrum of

¹ Institute of Plant and Animal ecology, Yekaterinburg, Russia;
E-mail: wow@ipae.uran.ru.

deviations in the forest population is close to that in zone IV and is far from that in zones III and II.

Percentage of individuals with iris depigmentation in froglets from urban populations was very significant (5.7% in zone II). Detailed studies of the frequency dynamics of this recessive mutation (Rostand and Darre, 1970) showed evidence for the presence of inbreeding depression in urban populations and for high mutagenesis in the urban environment (Vershinin, 2004). Having analyzed the nature and topography of abnormality frequencies, we noted that among juveniles there are differences between males and females. In comparison with females, general percentage of deviations in males was significantly higher ($\chi^2 = 4.07$, $p < 0.05$, $N = 6296$). I also found that the frequency of skeleton deviations in all males was significantly higher than in all females ($\chi^2 = 4.89$, $p < 0.05$). We suppose that this phenomenon is correlated with low general variability and ontogenetic stability of

females in comparison with males. The frequency of bilateral variants of deviations was 38.3%. The study of proliferating activity and some morphophysiological parameters allowed us to suppose high degree of equilibrium of the morphogenetic processes and a decrease of the frequency of morphological deviations in stress environmental conditions. We found that there is a significant (p fluctuated between 0.0012 – 0.046) correlation between liver index and the froglet mitotic index. That, in our opinion, indicates the presence of adaptive changes in populations of urban areas. We think that there is resemblance in the processes of urbanization and domestication. It is expressed as changes in direction of natural selection and disappearance of some factors of natural mortality. We found an increase of the frequency of “striata” morph which is determined by a monogenic dominant mutation in the city area. The comparative analysis of the excitability of the nervous tissue in *R. arvalis* demonstrated that

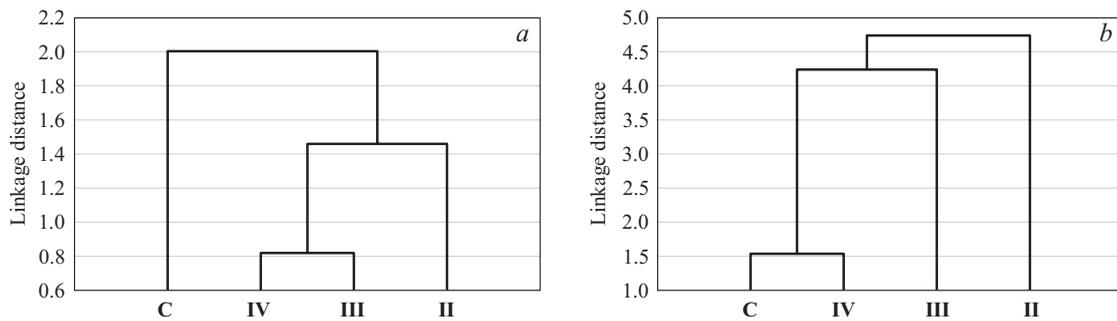


Fig. 1. Distances between groups of populations: a, juveniles; b, adults.

TABLE 1. Occurrence of Different Types of anomalies (%)

Type of anomalies	Zone							
	II		III		IV		C	
	adults	juveniles	adults	juveniles	adults	juveniles	adults	juveniles
Ectrodactyly	2.9	0.24	0	0.08	0.88	0.14	1.03	0.06
Sindactyly	0.96	0.03	0	0.16	0	0.03	0	0
Non-flexible limb	0	0.03	0	0	0	0	0	0
Hemimely	0	0.03	1.5	0.16	0.44	0.02	0	0.06
Brachymely	0.48	0.29	0	0.24	0	0.1	0	0.03
Ectromely	1.44	0.24	0	0.16	0.44	0.14	0	0
Eye defects	0	0.05	0	0	0.44	0	0	0.03
Iris depigmentation	0.48	1.65	0	1.8	0.44	1.2	1.0	0.34
Axial skeleton deformation	0.48	0.19	0	0.24	0.88	0.05	0	0.09
Mandibular hypoplasia	0	0.05	0	0.08	0	0.1	0	0.15
Pointed back pattern	4.3	1.46	3.0	0.47	0.44	0.14	1.0	0.03
Pigmentation defects	2.4	0.42	4.5	0.3	1.75	0.09	2.1	0.12
Edema	0	0.03	0	0.24	0.44	0.02	0	0
Total anomalies	28	177	6	50	14	119	5	30
N general	208	3766	66	1264	228	5835	97	3227
Total percentage	13.5	4.7	9.1	3.95	6.14	2.04	5.15	0.93

the excitation threshold of “*striata*” frogs (0.39 – 0.04; $N = 59$) was significantly ($F = 5.49$, $p = 0.02$) lower than in others (0.529 – 0.035). Environmental stress in urban conditions can influence ontogenetic process through nervous-hormonal axis which changes the spectrum of phenotypic variability. Thus the increase of phenotypical realizations in populations of the urban area is determined by high habitat heterogeneity, inbreeding depression under conditions of urban isolates and high mutagenesis, changes of the hormonal balance of morphogenetic processes by pollutants and selective survival of individuals with high stability of nervous system.

REFERENCES

- Borkin L. and Pikulik M. M.** (1986), “The occurrence of polymely and polydactyly in natural populations of anurans of the USSR,” *Amphibia-Reptilia*, **7**(3), 205 – 216.
- Cooke A. S.** (1981), “Tadpoles as indicators of harmful levels of pollution in the field,” *Environ. Pollut. Ser. A*, **25**, 123 – 133.
- Dubois A.** (1979), “Anomalies and mutations in natural populations of the *Rana* “*esculenta*” complex (Amphibia, Anura),” *Mitt. Zool. Mus. Berlin*, **55**(1), 59 – 87.
- Halliday T. R.** (1998), “Where have all the frogs gone?,” *People Planet*, **7**(4), 22 – 23.
- Hebard W. B. and Brunson R. B.** (1963), “Hind limb anomalies of a western Montana population of the Pacific tree frog, *Hyla regilla* Barid and Girard,” *Copeia*, **1963**(3), 570 – 572.
- Hurlbert S. H.** (1978), “The measurement of niche overlap and some relatives,” *Ecology*, **59**(1), 67 – 77.
- Kovalenko E. E. and Kovalenko Yu. I.** (1996), “Certain pelvic and sacral anomalies in Anura,” *Russ. J. Herpetol.*, **3**(2), 172 – 177.
- Rostand J. and Darre P.** (1970), “Une mutation de *Rana esculenta*: la grenouille aux yeux noirs,” *C. R. Acad. Sci. Ser. D*, **217**, 1414 – 1415.
- Syuzumova L. M.** (1985), “Influence of the ecological factors on development and limb regeneration of *Rana arvalis* larvae on the background of thyroid function inhibition by vitamin A,” in: *Ékologicheskie Aspekty Skorosti Rosta i Razvitiya Zhivotnykh [Ecological Aspects of the Growth and Developmental Rates of the Animals]*, Sverdlovsk, pp. 85 – 98 [in Russian].
- Talvi T.** (1993), “Naturally occurring limb abnormalities in anurans of the Estonia,” in: *Progr. and Abstrs. of the 7th Ord. Gen. Meet. Soc. Eur. Herpetol.*, September 15 – 19, 1993, Barcelona, p. 124.
- Tokar’ V. I., Zhavoronkov A. A., and Shcherbakov S. V.** (1991), *Fluoride and Endocrine System*, Nauka, Novosibirsk [in Russian].
- Vershinin V. L.** (2004), “Frequency of iris depigmentation in urban populations of *Rana arvalis* frogs,” *Ross. Zh. Ékol.*, **35**(1), 58 – 62 [in Russian].

EGG SIZE VERSUS CLUTCH SIZE: VARIATION AND TRADE-OFF IN REPRODUCTIVE OUTPUT OF *Rana dalmatina* AND *R. temporaria* IN A POND NEAR BONN (GERMANY)

K. Weddeling,¹ G. Bosbach,¹ M. Hachtel,¹
U. Sander,¹ P. Schmidt,¹ and D. Tarkhnishvili¹

Keywords: amphibians, *Rana dalmatina*, *Rana temporaria*, reproduction, egg size, clutch size, fecundity, trade-off, Germany.

INTRODUCTION

The common frog (*R. temporaria*) is a widespread and abundant species of woodland and agricultural landscapes in Europe and north-west Asia. In contrast, the distribution of the agile frog (*R. dalmatina*) is rather scattered and restricted mainly to deciduous and mixed forests in western, central and south-eastern Europe. Within their overlapping range, both species often breed in the same ponds. Although ecology of these brown frog species is well known in parts, many aspects of their biology possibly explaining niche differentiation and large and small scale distribution remain uncertain. Interspecific variation in reproductive output may be one key factor explaining local differences in their abundance and dispersal ability. Our study compares the reproductive output of both species in a single pond near Bonn (Germany) using data on egg size (diameter, egg mass), clutch size, body size, age, and body condition.

METHODS

The study pond is situated near Bonn (Germany) in an agricultural landscape with a distance of 200 m from a mixed forest. In spring 2001 and 2002 an overall sample of 29 females of the agile frog and 34 females of the common frog spawned under field conditions in plastic cages placed inside their breeding pond. For each female snout-vent length (SVL), weight before and after spawning, clutch size, and egg diameter were measured. Skeletochronology of finger bones was used to determine the age of the frogs (Kleinenberg and Smirina, 1969). Somatic condition (computed after Hemmer and Kadel, 1971), egg mass and relative investment in reproduction (clutch mass

spent/weight of spent females) were calculated from these data and used mainly for parametric linear regression analysis. Egg size parameter “egg mass” and “egg diameter” are only weakly correlated ($r^2 = 0.32$, $p < 0.001$); several correlations of egg size with other parameters are only significant with one of these measures, probably indicating a considerable error in measurement of egg diameter.

RESULTS

Clutch size differs significantly between species (Fig. 1, mean \pm standard deviation (S.D.): *R. temporaria*, 1766 ± 529 eggs; *R. dalmatina*, 950 ± 246 eggs) but egg diameter and egg mass do not (*R. temporaria*: egg diameter 2.08 ± 0.160 mm, egg mass 12.70 ± 5.123 mg; *R. dalmatina*: egg diameter 2.14 ± 0.141 mm, egg mass $12.70 \pm$

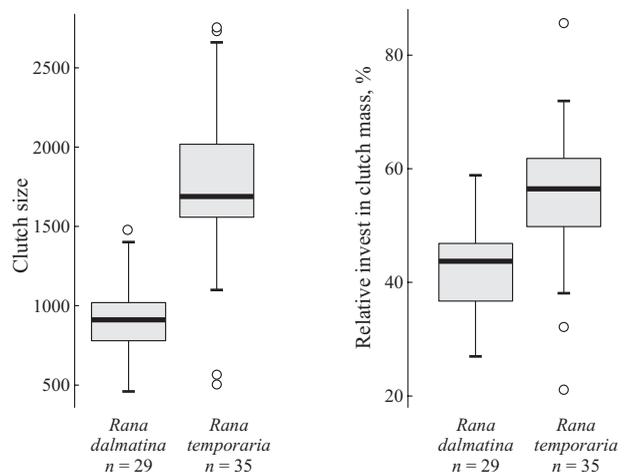


Fig. 1. Clutch size and relative investment in clutch mass ($100 \times$ clutch mass/weight of spent female); clutch size ($t = -8.364$, $p < 0.001$) and relative investment (arcsine transformed data, $t = -4.619$, $p < 0.001$) differ between species. Student's t -test.

¹ Zoologisches Forschungsinstitut und Museum Alexander Koenig, Sektion Herpetologie, Adenauerallee 160, D-53113 Bonn, Germany; E-mail: weddeling@web.de; www.amphibien-projekt.de.

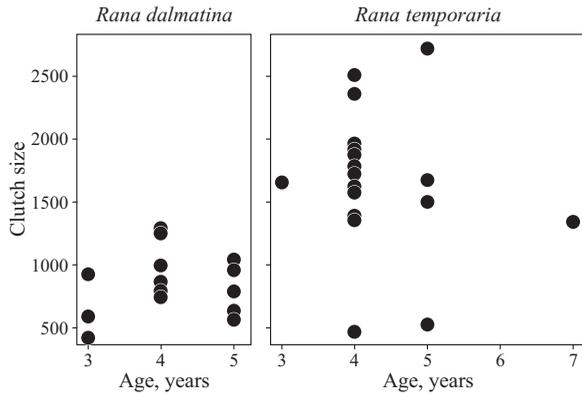


Fig. 2. Age and clutch size of females show no correlation (Spearman correlation, *R. dalmatina*: $r^2 = 0.02$, $p > 0.05$; *R. temporaria*: $r^2 = 0.04$, $p > 0.05$).

5.180 mg). Clutch size is not correlated with SVL. Relative investment in clutch mass differs significantly between species, the larger *R. temporaria* (0.557 ± 0.122) invests more than the smaller agile frog (0.421 ± 0.085), but in both species investment is not correlated significantly with SVL (Fig. 1). Mean age does not significantly differ between species (median for females of both species 4 years; Mann – Whitney *U*-test, $U = 153.5$; $p > 0.05$). Age is not correlated significantly with any clutch or egg parameter nor with body condition or relative investment in reproduction (Fig. 2). A significant trade-off between egg mass and clutch size could be shown for both species (log-log-transformed data, $y = \log[\text{egg mass}]$, $x = \log[\text{clutch size}]$: *R. temporaria*, $y = -0.593x + 2.983$, $r^2 = 0.30$, $p < 0.01$; *R. dalmatina*: $y = -0.925x + 3.801$, $r^2 = 0.63$, $p < 0.01$). Trade-off is more pronounced in *R. dalmatina* probably due to the smaller body size compared to *R. temporaria* (Fig. 3). Impact of somatic condition on egg diameter strongly differs between species: In *R. dalmatina* females with better somatic condition produce significantly bigger eggs, in *R. temporaria* in this case eggs are smaller (Fig. 4). Surprisingly condition does not affect clutch size.

DISCUSSION

Differences in clutch size and relative investment in reproduction are mainly due to the marked differences in SVL between species. When clutch size was compared accounting for SVL, mean egg number does not significantly differ between common frog and agile frog. Thus, one main difference in reproductive strategy and fecundity between species is the strongly enhanced growth rate in

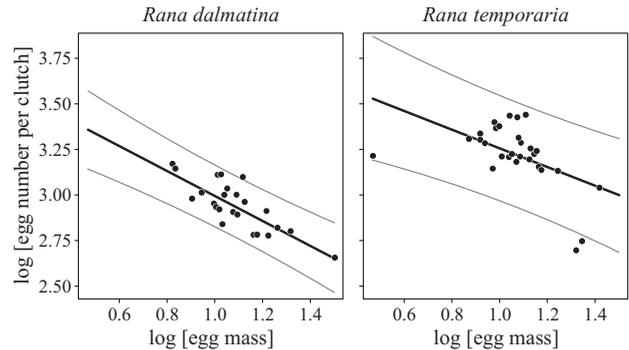


Fig. 3. Both species show a significant trade-off between egg size (measured as mean egg mass) and egg number (linear regression models and 95% confidence intervals, for statistics see the text).

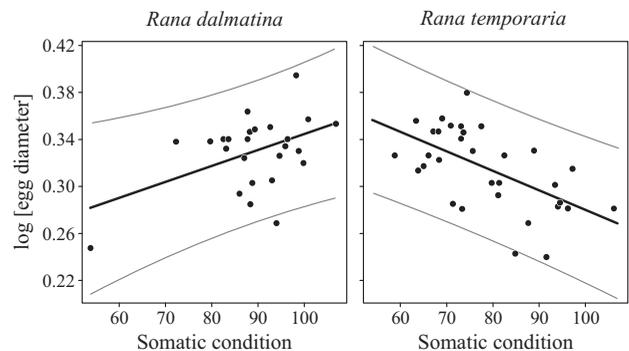


Fig. 4. Egg size response to different somatic conditions of females (linear regression models and 95% confidence intervals): *R. dalmatina* $b_{st} = +0.459$ ($F = 6.141$, $p < 0.05$); *R. temporaria* $b_{st} = -0.562$ ($F = 15.274$, $p < 0.001$).

R. temporaria, resulting in a nearly doubled mean egg number compared to *R. dalmatina*, since both species do not significantly differ in age. These data suggest that pre-suppositions for a high metamorphic output are generally better in *R. temporaria* than in *R. dalmatina*. However, our field census data on juvenile output of the species do not support this hypothesis, since a regular dominance of *R. temporaria* in ponds with *R. dalmatina* cannot be observed. Thus, other factors — probably pond specific survival rates of tadpoles and metamorphs — influence species dominance in the field.

In both species a marked trade-off between egg number and egg size is obvious. This indicates that frogs cannot optimize both, egg size and egg number simultaneously. Assuming that the number of eggs laid in spring is determined by body condition after spawning one year before, egg size is likely to depend on feeding conditions during the following summer and autumn (Kuhn, 1994;

Lüddecke, 2002). Terrestrial habitat quality (including weather conditions) one year before seems to adjust clutch size and egg size in the following season. Thus, maintenance of a considerable variation and a trade-off in reproductive output of *R. temporaria* and *R. dalmatina* in the studied pond might be regarded as a result of recent changes in environmental conditions.

Acknowledgments. This project was financially supported as a ‘testing and development project’ by the Federal Agency for Nature Conservation (BfN) and the Federal Environment Ministry (BMU). Many thanks to our colleagues Regine Damaschek, Anja Dissanayake, Ruth Rottscheidt, Anja Sampels, and Meike Thomas for their work in the field and discussions. Wolfgang Böhme we thank for leading the project.

REFERENCES

- Hemmer H. and Kadel K.** (1971), “Untersuchungen zur Laichgröße nebst Bemerkungen zur Populationsdynamik der Kreuzkröte (*Bufo calamita* Laur.) und der Wechselkröte (*Bufo viridis* Laur.),” *Zool. Beitr.*, **17**, 327 – 336.
- Kleinenberg S. E. and Smirina E. M.** (1969), “A contribution to the method of age determination in amphibians,” *Zool. Zh.*, **48**, 1090 – 1094 [in Russian].
- Kuhn J.** (1994), “Lebensgeschichte und Demographie von Erdkrötenweibchen *Bufo bufo bufo* (L.),” *Zeitsch. Feldherpetol.*, **1**(1/2), 3 – 89.
- Lüddecke H.** (2002), “Variation and trade-off in reproductive output of the Andean frog *Hyla labialis*,” *Oecologia*, **130**, 403 – 410.

SOME NOTES ON THE HERPETOFAUNA OF WESTERN BULGARIA

A. Westerström¹

This paper summarizes observations during ten visits to Bulgaria, during the period 1997 – 2003. New localities are described and taxonomical remarks on some species (*Triturus alpestris*, *Lacerta agilis*, and *Vipera berus*) are made. One winter-time observation of *Salamandra salamandra* and *P. muralis*, respectively as well as high altitude observations of *Bufo viridis* are described. The possible existence of *Rana kurtmuelleri* in extreme South-western Bulgaria is discussed and remarks on the altitudinal range extension of *L. agilis* are made.

Keywords: Bulgaria, distribution, taxonomical position, *Triturus alpestris*, *Rana kurtmuelleri*, *Lacerta agilis*, *Vipera berus*.

INTRODUCTION

Through the extensive efforts of Buresch and Zonkow (1932, 1933, 1934, 1941) comprehensive works on the herpetofauna of Bulgaria and the Balkan Peninsula emerged. Thereafter, contributions by Kabisch (1966), Beškov (1972), Beshkov (1984), and Beshkov and Nanev (2002) further added to the knowledge of the herpetofauna of Bulgaria.

The author summarizes the most interesting records made during several surveys in Western Bulgaria.

SPECIES ACCOUNT

Salamandra salamandra

Distribution. Detailed distribution of this species is given by Buresch and Zonkow (1941).

Observations. Two new localities were recorded for this species: Krusha and Godech. *S. salamandra* was also observed in the Osogovo mountain massif at an altitude of approximately 1600 m.

Remarks. An interesting observation of this species was made in winter 2000 just outside the town of Godech, where a specimen was encountered in a shaft. The unusually warm weather at the time implies that the salamander could have been active for some time before becoming trapped in the shaft. The single specimen was found together with other amphibian species namely, *Bufo bufo* and *Rana dalmatina*.

Triturus alpestris

Distribution. The alpine newt is mainly confined to mountainous areas in Western Bulgaria, where it has been

recorded from the Balkan and Rila mountains as well as from Osogovska Planina and Rodopi (Veselinov, 1993; Beshkov and Nanev, 2002).

Despite the existence of suitable habitat this species has never been reported from Vitosha (Beshkov, personal communication).

Observations. Observations of this species were made in the Osogovo mountain massif at approximately 1600 m elevation in spring 2002.

Remarks. Buresch and Zonkow (1941) preliminarily attributed the Bulgarian alpine newt to the nominate subspecies due to absence of sufficient collected material.

Veselinov (1993) stated (by referring to Herrero and Arano, 1987 — not seen by the author) that according to the nuclear DNA results obtained by Herrero and Arano (1987) the Bulgarian alpine newt could be attributed to *T. a. veluchiensis*.

However, using mtDNA the Bulgarian alpine newt does not turn out to be a part of *T. a. veluchiensis* but seems to cluster with *T. a. reiseri* instead (Alcobendas, personal communication).

A possible explanation could be that nuclear DNA of *T. a. reiseri* co-exists with mtDNA of *T. a. veluchiensis* (Alcobendas, personal communication). There is no contradiction herein, on the contrary evidence of population displacement; populations with mixed DNA reflect ancient secondary contact zone (García-Paris et al., 2003). In order to settle this issue a more thorough study of Bulgarian population is needed.

Bufo viridis

Distribution. *Bufo viridis* is common throughout the country and usually confined to altitudes below 1200 m, although an observation has been made in the Rila mountains at 2300 m elevation (Beshkov and Nanev, 2002).

¹ Skebokvarnsvägen 320, Bandhagen, S-124 50 Stockholm, Sweden;
E-mail: bosniensis@mail.bg.

Observations. In August 2001 corpses were found on the road in the Banderitsa valley, in the Pirin mountains at elevations between 1800 and 1960 m.

Remarks. Observations have been made of *B. bufo* and *Hyla arborea* at high altitudes (1960 and 2300 m, respectively) but it cannot be established whether or not these animals constitute naturally occurring populations.

Rana kurtmuelleri

Distribution. This species has been recorded at Novo Selo (Duharov, personal communication) in FYROM (Former Yugoslav Republic of Macedonia). Its existence in Bulgaria has not been verified.

Observations. In spring 2002 some sort of green frogs were observed in the border area of Bulgaria – FYROM at two localities, namely, Zlatarevo and Gega.

Remarks. There is some confusion as to which locality of Novo Selo was referred to. In FYROM three localities with this name were found. One locality is located in Western FYROM, north of Ohrid and two in close proximity of the FYROM – Bulgarian border. If any of the latter two was referred to, this species could well be found within the political boundaries of the Republic of Bulgaria. Specimens were photographed for documentation. It could not be established which species of green frogs was actually observed.

Lacerta agilis

Distribution. The sand lizard is mainly restricted to the western parts of the country with a sporadic distribution in Eastern Bulgaria (Beshkov and Nanev, 2002).

Observations. *Lacerta agilis* was encountered at several localities: Gintsi (Petrohan, Balkan mountains), Krusha, Yarema (Vitoshka), Rila mountains (southern slope), and Pirin mountains.

During a visit in August 2001 to the Pirin mountains a sand lizard was observed at 2500 m altitude. This record constitutes a significant altitudinal range extension — previous records do not exceed 2200 m (Buresch and Zonkow, 1933; Beshkov and Nanev, 2002).

At Krusha only a single specimen was ever observed during six years of observations in the area.

Remarks. The systematic position of *Lacerta agilis* in Bulgaria is insufficiently known.

The existence of two main morphotypes, *L. a. bosnica* and *L. a. chersonensis* in Bulgaria is presently accepted (Duharov, personal communication).

The “*bosnica*” morphotype is mainly restricted to mountainous areas whereas the “*chersonensis*” morphotype inhabits the plain around Sofia and adjacent areas.

The species was recorded from Krusha (650 m elevation) situated some 15 km south-west of the locality Chepan (900 m elevation — above Dragoman).

Both localities can be attributed to the “*chersonensis*” morphotype.

These morphotypes are not isolated from each other, on the contrary contact zones, e.g., in the foothills of Vitoshka exist, where the “*bosnica*” morphotype is rarer (Duharov, personal communication).

Podarcis muralis

Distribution. This species is widespread where suitable habitats exist throughout entire Bulgaria (Beshkov and Nanev, 2002).

Observations. A peculiar observation was made in winter on December 27, 2000 when a fully active, adult specimen was observed on rocky outcrops.

Remarks. This winter observation was made just outside the town of Godech (700 m elevation) in Western Bulgaria. Temperature at the time of observation was measured to be approximately 15°C.

Vipera berus

Distribution. Localities given by Buresch and Zonkow (1932, 1934) still present today include the main mountain ranges: Stara Planina, Sredna Gora, Vitoshka, Rila mountains, Pirin mountains, and Rodopi. The existence of *V. berus* on the Osogovo mountain massif was first reported by Beškov et al. (1967).

Observations. This species was recorded in Stara Planina, Vitoshka, Osogovska Planina, Rila and Pirin mountains.

Remarks. The Bulgarian adder has long been treated as belonging to two subspecies (Buresch and Zonkow, 1932, 1934; Beshkov and Nanev, 2002). The nominate subspecies is said to occur mainly in the Balkan mountains Buresch and Zonkow (1934) whereas *V. b. bosniensis* is said to occur in the Rila and Pirin mountains (Buresch and Zonkow, 1932, 1934).

This issue is rather complex from a morphological viewpoint.

Only rarely does any arbitrary specimen agree partly or fully with the brief description of Boettger (in Mojsisovic, 1889) and the diagnostics emphasized by Buresch and Zonkow (1934) with a double subocular row and split-up zigzag pattern.

Specimen from the Rila mountains and the Pirin mountains possessing characters of *V. b. bosniensis* have previously been reported as has been stated above. However, specimens from the region also share characters of the nominate subspecies (Beshkov and Nanev, 2002; own observations).

The ratio of specimens possessing two subocular scale rows is 38% for the Pirin and 29% for the Rila mountains, according to the numbers examined by Franzen and Heckes (2000).

Beškov et al. (1967) made no taxonomical remarks on the Osogovo adder. It however, agrees quite well with the criteria of *V. b. bosniensis* on a morphological basis (own observations).

Populations hitherto not examined and thus undetermined are those of Eastern Balkan mountains, Sredna Gora, and the Rodopi mountain massifs.

Specimens from the Rodopi massif however, seem to agree with the nominate subspecies (Hristov, personal communication)

Specimens deviant from the definition as stated above are not rare. A specimen from Vitoshka had a single subocular row on one side and a double on the other side (Beshkov, personal communication).

This scalation was also observed in a specimen from Ängskär, on the Baltic Sea Coast (“Upplandskusten”) in Sweden (own observation).

According to the figures of Saint Girons (1978) the double subocular scale row is not a very common character. In his material 4% of the nominate species possessed a double subocular scale row whereas the percentage was 6% in *V. b. bosniensis*.

The distinguishing character as given by Boettger (in Mojsisovic, 1889) and the split-up zigzag pattern alone are not sufficient to distinguish *V. b. bosniensis* from the nominate subspecies already mentioned by Sochurek (1953).

Recent genetical analyses (Ursenbacher et al., Joger, personal communication) have shown that the populations of the Balkan, Vitoshka, Rila, and the Pirin mountains are all part of *V. b. bosniensis*.

This combined with the facts stated above shows several inconsistencies in the definition of *V. b. bosniensis* and therefore it is my opinion that this subspecies needs to be re-defined morphologically.

Acknowledgments. Most of all I would like to thank my grandfather, Vlayko Grozdanov Dimitrov for invaluable help in undertaking numerous field-trips. I would like to extend my gratitude to my relatives in Godech for their hospitality during numerous stays. Sebastian Ljunggren Wiberg (Stockholm, Sweden), Mikael Norström (Stockholm, Sweden), and Jan Peterson

(Vadstena, Sweden) are thanked for most appreciated field assistance. I am much indebted to the family Sugarevi (Bansko, Bulgaria) for their generosity and hospitality during field trips in the Pirin mountains. I am most grateful to Vladimir Beschkov, Deyan Duhalov, and Krasimir Hristov for providing valuable information regarding localities. For valuable comments which significantly improved the quality of the manuscript I express my gratitude to László Krecsák, Mats Höggren, and Ulrich Joger. Thanks also goes to the two anonymous reviewers.

REFERENCES

- Beschkov V.** (1984), “Znatschenieto na Stara Planina za razprostranienieto na herpetofaunata v Balgaria,” *Acta Zool. Bulg.*, **25**, 9 – 14 [in Bulgarian].
- Beshkov V. and Nanev K.** (2002), *Zemnovodni i Vletshugi v Balgariya*, Pensoft, Sofiya – Moscow [in Bulgarian].
- Beškov V.** (1972), “Die Höhenverteilung der Schlangen in einer ihrer Artenvielfältigkeit nach spezifischen Region von Südostbulgarien,” *Bull. l’Inst. Zool. Mus.*, **55**, 167 – 173.
- Beškov V., Undshian E., Simeonov S., and Darakčiev** (1967), “Neue Angaben über die Verbreitung einiger Amphibien und Reptilien in Bulgarien,” *Bull. l’Inst. Zool. Mus.*, **25**, 5 – 10.
- Buresch I. and Zonkov J.** (1932), “Die Verbreitung der Giftschlangen (Viperidae) in Bulgarien und auf der Balkan-Halbinsel,” *Trav. Soc. Bulg. Sci. Nat.*, **15**(15), 189 – 206 [in Bulgarian].
- Buresch I. and Zonkov J.** (1933), “Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel I.,” *Mitt. Kgl. Naturw. Inst. Sofia*, **6**, 150 – 207.
- Buresch I. and Zonkov J.** (1934), “Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel II. Schlangen.,” *Mitt. Kgl. Naturw. Inst. Sofia*, **7**, 106 – 188.
- Buresch I. and Zonkov J.** (1941), “Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel III. Schwanzlurche.,” *Mitt. Kgl. Naturw. Inst. Sofia*, **14**, 171 – 237.
- Franzen M. and Heckes U.** (2000), “*Vipera barani* Böhme and Joger, 1983 aus dem östlichen Pontus — Gebirge, Türkei: Differentialmerkmale, Verbreitung, Habitate (Reptilia, Serpentes, Viperidae),” *Spixiana*, **23**(1), 61 – 70.
- García-París M., Alcobendas M., Buckley D., and Wake D. B.** (2003), “Dispersal of viviparity across contact zones in the Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits,” *Evolution*, **57**, 129 – 143.
- Herrero P. and Arano B.** (1987), “*Triturus alpestris*. Phylogenetic implications of C-wandering pattern in *Triturus alpestris* complex,” in: *Proc. of the 4th Ord. Gen. Meeting Soc. Eur. Herpetol.*, Nijmegen, pp. 191 – 194.
- Kabisch K.** (1966), “Herpetologische Exkursionen in die Umgebungen von Sofia,” *Aquarien Terrarien*, **4**, 131 – 135.

- Mojsisovic A.** (1889), “Über die geographische Verbreitung einiger westpalearktischer Schlangen,” *Mitt. Naturw. Ver. Steierm.*, 270 – 273.
- Saint Girons H.** (1978), “Morphologie externe compare et systématique des Vipères d’Europe,” *Rev. Suisse Zool.*, **85**(3), 565 – 595.
- Sochurek E.** (1953), “Irrtümer um *Vipera berus bosniensis* Boettger 1889,” *Carinthia II*, **143**(2), 103 – 104.
- Ursenbacher S., Carlsson M., Helfer V., Tegelström H., and Fumagalli L.** (2003) “Phylogeography and Pleistocene refugia of the Adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data,” in: Carlsson M (ed.), *Phylogeography of the Adder, Vipera berus. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the faculty of Science and Technology*, Uppsala, Vol. 849, p. 32.
- Veselinov D.** (1993), *Izsledvaniya Varho Razprostraneniето i Zashtitata na Alpiiskiya Triton (Triturus alpestris Laurenti, 1768) v Balgariya. Master’s Thesis*, Sofia University, Sofia [in Bulgarian].

ASSESSMENT OF REPRODUCTIVE FREQUENCY IN THE EUROPEAN POND TURTLE (*Emys orbicularis*) USING MANUAL PALPATION, ULTRASONOGRAPHY, AND RADIOGRAPHY

M. A. L. Zuffi,¹ S. Citi,² M. Giusti,¹ and A. Teti¹

Assessment of reproductive status in female European pond turtles has been investigated with three different methods. The comparative analysis of the obtained results revealed that all three methods are important in determining female reproductive status. Degree of efficiency is however different among them. Manual palpation is the easiest to perform but lacks of precision regarding determination of clutch and egg size; radiography is the most efficient in determining clutch and egg size but it is applicable only when egg shells are calcified; ultrasonography is almost certainly the most precise technique to understand if females are reproductive, while it can not have a complete insight over all the oviductal or follicular eggs. We strongly recommend the use of both three methods when studying reproductive plasticity and frequency in fresh water turtles.

Keywords: Palpation, Ultrasonography, Radiography, Reproduction, *Emys orbicularis*.

INTRODUCTION

A number of contributions on several populations of *Emys orbicularis* have recently showed that morphometric measures of adult reproductive females (i.e., carapace length, carapace height) positively correlate with clutch size (Zuffi et al., 1999). Despite of this, the range of variation of clutch size and clutch frequency (multiple deposition) within a single population has been recently studied only in few populations [Italy: Zuffi and Odetti, 1998; Spain: Keller, 1999; Ukraine: Kotenko, 2000; but see Kuzmin (2002) and reference herein]. Comparative analysis of available data on reproductive effort in European pond turtle reveals that most information are mainly anecdotal and descriptive. The assessment of actual reproductive effort in individual populations should be the basic goal, to understand any source of variation in reproductive patterns and phenotypic plasticity in *Emys orbicularis*. A deep and wide knowledge of biological characteristics is particularly necessary when studies are aimed towards protection and conservation plans. The use of manual and radiographic techniques to assess reproductive status has been widely used in most Chelonians (Wilbur, 1975; Gibbons and Greene, 1979; Keller, 1998; Zuffi et al., 1999), while ultrasonography is employed less frequently (Kuchling, 1989; Rostal et al., 1996). It is however rare the use of all the three methods when handling freshwater turtles.

MATERIAL AND METHODS

At capture, after standard measurements (Zuffi et al., 1999), each adult female was processed manually with palpation of the inguinal region in order to detect for any large follicular or oviductal egg, then analyzed with ultrasound techniques (ultrasound equipment: Toshiba Corevision with multifrequency microconvex probe 5 – 7 – 8-MHz probe). If eggs were very large, and easily detectable manually or if length was larger than 28 mm, we also performed an x-ray examination [15 mA, 55 kV, at 1 m distance, these values being much lower than those reported in by Hinton et al. (1997)]. Due to different research protocols used during a long term project started in 1996, we used palpation and x-rays in one target population, and palpation and ultra-sound procedure in an other target population. Each study section was performed during an individual season. Study areas lie within the Nature “Parco di Migliarino San Rossore Massaciuccoli” (Province of Pisa, coastal Tuscany, central Italy).

RESULTS

In a sample from Camp Darby we considered 53 individual adult *Emys orbicularis* females, all of them were palpated ($n = 26$, that is 49.1%, were reproductive); 19 out of them that carried well detectable eggs were also radiographed ($n = 15$, that is 28.3% of total sample and 78.9% of radiographed sample, showed calcified eggs). Even if

¹ Museum Natural History and Territory, University of Pisa, via Roma 79, I-56011 Calci (Pisa), Italy; E-mail: marcoz@museo.unipi.it.

² Department of Veterinary Clinical Sciences, University of Pisa, via Livornese, I-56010, S. Piero a Grado (Pisa), Italy.

manual vs. radiographic estimation was not significantly different, the observed trend indicates how manual palpation can allow precise information on reproductive status of sampled adult females. In the overall sample ($n = 53$), clutch size estimated under palpation was 3.73 ± 1.05 (1 SD; median = 4), while x-ray clutch size was 5.80 ± 1.21 (median = 6). Within the radiographed sample ($n = 19$), clutch size estimated under palpation was 3.16 ± 1.68 (1 SD; median = 4) while x-rays clutch size was 4.58 ± 2.65 (median = 5). Difference was significant in both cases (paired samples t -tests, $P < 0.005$). When taking into account reproductive females with enlarged oviductal eggs, we found no difference in the reproduction estimation between palpation and radiography (paired sample t -test = 1.0, $df = 18$, $P = 0.331$).

In a sample from San Rossore we considered 101 individual adult females during eight periods of two weeks each, from mid April to early August, all of them were palpated and then processed using ultrasonography. The number of estimated reproductive females per period was 6 ± 4.8 ($52.56 \pm 40.04\%$) using the palpation method and 8.6 ± 7.2 ($68.27 \pm 33.44\%$) using ultrasonography. In some periods, the estimated number of reproductive females was double if considering the ultrasound method (June 19 – July 2, $n = 14$, 85.7 vs. 42.9%; July 3 – July 18, $n = 32$, 68.7 vs. 33.3%). Despite this, difference between the two systems in the whole sample period was not statistically significant (Wilcoxon paired rank test, $Z = -1.826$, asymptotic $P = 0.068$), even if it indicates that ultrasonography is a relatively more valuable system than manual inspection alone. Females may often result negative to palpation (up to 16 – 18 days after vitellogenesis), but ultrasonography may show follicular vitellogenic eggs.

DISCUSSION

Manual palpation allows a quite valuable diagnosis about reproduction status already some days after vitellogenesis has occurred, but largely lacks of precision regarding clutch size and egg size. The ultrasound method enables the detection of vitellogenic follicles already at 3 to 25 mm length, even if negative to palpation and to x-rays. As a consequence, estimated body size of reproductive females may be very accurate and follicle maturation may be precisely assessed as well, and occurrence of multiple depositions may also clearly determined. Radiography is obviously the unique available method to precisely define both clutch size and diameters of eggs during or at the end of the shell calcification. Trying to establish a trend of in-

creasing efficiency in the involved compared methods, we can suggest i) radiography, ii) manual palpation, and iii) ultrasonography in determining reproductive status; regarding clutch size i) ultrasonography, ii) manual palpation, and iii) radiography; while the optimal method for egg size and egg maturation is much better evaluated with ultrasonography alone.

It is evident that the contemporary usage of all the three involved methods should be regularly applied to precisely access the plasticity and variability of the reproductive status of freshwater turtles.

REFERENCES

- Fritz U. (2001), “*Emys orbicularis* (Linnaeus, 1758). Europäische Sumpfschildkröte,” in: Fritz U. (ed.), *Handbuch der Reptilien und Amphibien Europas. Schildkröten I*, Aula Verlag, pp. 343 – 516.
- Gibbons J. W. and Greene J. L. (1979), “X-ray photography: a technique to determinate reproductive patterns of freshwater turtles,” *Herpetologica*, **35**, 86 – 89.
- Hinton T. G., Fleddermann P. D., Lovich J. E., Congdon J. D., and Gibbons J. W. (1997), “Radiographic determination of fecundity: is the technique safe for developing turtle embryos?” *Chelonian Conserv. Biol.*, **2**(3), 409 – 414.
- Keller C. (1998), “Assessment of reproductive status in the turtle *Mauremys leprosa*: a comparison between inguinal palpation and radiography,” *Wildlife Res.*, **25**, 527 – 531.
- Keller C. (1999), “Reproductive ecology of *Emys orbicularis* in southwestern Spain and comparisons with other populations,” in: *Progr. and Abstrs. of the 2nd Int. Symp. on Emys orbicularis*, 25 – 27 June 1999, Le Blanc (France), p. 15.
- Kotenko T. (2000), “The European pond turtle (*Emys orbicularis*) in the steppe zone of the Ukraine,” in: Hödl W. and Rössler M. (eds.), *Die Europäische Sumpfschildkröte. Stapfia 69*, Vol. 149, pp. 87 – 106.
- Kuchling G. (1989), “Assessment of ovarian follicles and oviductal eggs by ultra-sound scanning in live fresh water turtles, *Chelodina oblonga*,” *Herpetologica*, **45**, 89 – 94.
- Kuzmin S. L. (2002), *The Turtles of Russia and Other Ex-Soviet Republics*, Chimaira Editions, Frankfurt am Main.
- Rostal D. C., Paladino F. V., Patterson R. M., and Spotila J. R. (1996), “Reproductive physiology of nesting leatherback turtles (*Dermochelys coriacea*) at Las Baulas National Park, Costa Rica,” *Chelonian Conserv. Biol.*, **2**(2), 230 – 236.
- Wilbur H. M. (1975), “A growth model for the turtle *Chrysemys picta*,” *Copeia*, **1975**, 337 – 343.
- Zuffi M. A. L. and Odetti F. (1998), “Double egg deposition in the European pond turtle, *Emys orbicularis*, from central Italy,” *Ital. J. Zool.*, **65**, 187 – 189.
- Zuffi M. A. L., Odetti F., and Meozzi P. (1999), “Body size and clutch size in the European pond turtle, *Emys orbicularis*, from central Italy,” *J. Zool. Lond.*, **247**, 139 – 143.

**MORPHOLOGY,
DEVELOPMENTAL
BIOLOGY,
PARAZITOLOGY**

STRUCTURE AND ULTRASTRUCTURE OF THE SEXUAL SEGMENT OF THE KIDNEY IN THE DIURNAL SAHARAN LIZARD *Uromastix acanthinurus*, BELL 1825

M. Bahiani,¹ T. Gernigon-Spychalowicz,¹ and J.-M. Exbrayat²

The lizard *Uromastix acanthinurus* living in the Algerian Sahara has the spring reproductive cycle when the epididymis and the sexual segment of kidney undergoes seasonal structural and ultrastructural variations. During breeding period (spring), the tubules of sexual segment of kidney are hypertrophied and actively secretive. They are lined with high cells in which both proteic and glycoproteic secretion granules are accumulated in the apical pole. These serous cells contain a euchromatic nucleus, an active nucleolus with granular and dense fibrillar components, and a fibrillar center; the cytoplasm contains a vesicular RER, several Golgi areas, multivesicular bodies, numerous mitochondria and secretion granules. During the resting period (summer, autumn, and winter), the tubules of sexual segment of kidney are surrounded by a dense connective tissue. Epithelial cells have now mucous secretion. These cells present a basal euchromatic nucleus with an active nucleolus. The apical cytoplasm contains mucigenous vesicles. Mitochondria with lamellar crests are concentrated in the basal part of the cells and the Golgi apparatus is supra nuclear. The plasma membrane develops lateral and basal folds.

Keywords: lizard, the sexual segment of the kidney, seasonal variations.

INTRODUCTION

Lizards and snakes are remarkably diversified in the Algerian Sahara. The seasonal variations in photoperiod and the alternation of hot and cold seasons determine a period of breeding, more or less obligatory winter latency and act on the growth of the animals (Grenot and Vernet, 1973). A portion of the preterminal segment of the urinary tube presents some morphological and physiological differences according to the sex of the animal. To emphasize this difference, this segment was named: “sexual segment of the kidney” (Régaud and Policard, 1903). During breeding of the adult male, this segment is hypertrophied; its diameter is broader than that of the preceding segment.

The diurnal lizard *Uromastix acanthinurus* has an effective strategy of reproduction in arid environment of Sahara. Its breeding cycle was studied by Courrier (1929) and Kehl (1935, 1944). This species constitutes a good model for the study of these adaptations. The present study is a contribution to the knowledge of structural and ultrastructural aspects of the seasonal variations of the sexual segment of the kidney in the male *Uromastix acanthinurus*.

MATERIAL AND METHODS

The animals were captured in the area of Béni-Abbès of the Algerian Sahara (30°7' N and 2°10' W). Located at 250 km of Béchar, Béni-Abbès presents some individualized spaces which confer a typically desert profile.

The kidneys of 25 adult males *Uromastix acanthinurus* were preserved in Bouin's fluid. After dehydration and paraffin embedding, 5 µm thick sections obtained with a vertical “Leitz” microtome, were stained with Masson's trichrome, hemalum-picro-indigo-carmin. The histochemical stains used were periodic acid and Schiff (PAS), toluidine blue-periodic acid and Schiff, toluidine blue-ninhydrine and Schiff, nuclear fast red-alcian-blue.

For TEM, fragments of kidneys were fixed in 2.5% glutaraldehyde, and post-fixed in 1% osmium tetroxide, dehydrated with ethanol and embedded in Epon or Epon — Araldite. The semi-thin sections were stained with toluidine blue. The ultra-thin sections were contrasted with uranyl acetate and lead citrate and observed with a Zeiss TEM (service of Electron Microscopy, U.S.T.H.B., Algiers).

RESULTS AND DISCUSSION

Observations with Light Microscopy

During the period of reproduction (spring, May), the tubules of renal sexual segment are developed. Their epithelium is hypertrophied with narrow high cells, overloaded with numerous secretion granules (Fig. 1). These

¹ Laboratoire de Recherche sur les Zones Arides, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediène (U.S.T.H.B.), B.P. No. 32 DZ 16111 El-Alia, Algiers, Algérie; E-mail: mbahiani@yahoo.fr (M. Bahiani), tgerigon@yahoo.com (T. Gernigon-Spychalowicz).

² Laboratoire de Biologie générale, Université catholique et Laboratoire de Reproduction et développement des Vertébrés, Ecole Pratique des Hautes Etudes, 25 rue du Plat, 69288 Cedex 02, France; E-mail: jmexbrayat@univ-catholyon.fr.

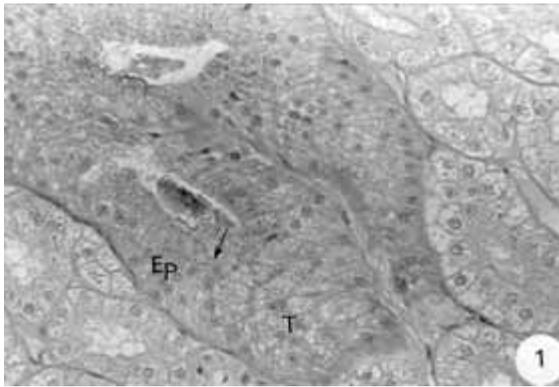


Fig. 1. The sexual segment of the kidney in the breeding period. Photonic microscopy ($\times 160$, Coloration: Hémalum Picro-indigo-carminé). The tubules (T) are developed. The cells of the epithelium (Ep) are overloaded with numerous secretions granules (\rightarrow).

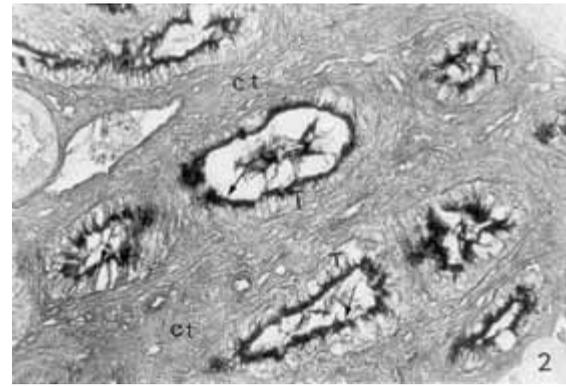


Fig. 2. The sexual segment of the kidney in the resting period. Photonic microscopy ($\times 160$, Coloration: nuclear fast red-alcian blue). The tubules (T) are surrounded by a dense connective tissue (ct), the secretion (\rightarrow) is mucous.

secretions are PAS and alloxane-Schiff positive, indicating their proteic and glycoproteic nature. Such a proteic and glycoproteic secretion has been described in the renal sexual segment of *Vipera aspis* (Gabe, 1959) and *Crotalus adamanteus* (Burtner and al., 1965).

During the sexual quiescence (summer, autumn, and winter), the tubules of sexual segment are surrounded by a dense connective tissue (Fig. 2). The epithelial cells notably decrease and elaborate a mucous secretion, containing acidic mucopolysaccharids, stained with the alcian blue. Several cells without mucous are disposed between the mucous cells. These cells without mucous are darker than the mucous cells after staining with the toluidine blue. Such dark cells have been described in *Thamnophis sirtalis* (Bishop, 1959) and in *Acanthodactylus erythrurus lineomaculatus* (Bons, 1970). They can be mucous cells having released their mucus.

Observations with TEM

Serous epithelial cell: in period of reproduction.

The tubules of sexual segment of kidney are hypertrophied and actively secretive. Their epithelium is composed of serous high cells in which secretion granules are accumulated at the apical pole. Each serous epithelial cell possesses a euchromatic nucleus. One or two nucleoli have a tripartite structure with a granular component, a dense fibrillar component and a fibrillar center. The cytoplasm contains a network of vesicular RER, numerous Golgi areas, multivesicular bodies, numerous mitochondria and many secretion granules (Fig. 3). Two types of granules can be observed: granules with a dense and homogeneous contents and granules with a dense, eccentric or central content, surrounded by a clear vacuole.

The aspect of serous epithelial cells testify to some intense proteic and glycoproteic synthesis. The characteristics of these cells are rather close to that of the main cell of mammalian seminal vesicle and prostate. These observations may confirm the function of an additional gland given to the sexual segment of saurophidiens. It is currently indicated that the sexual segment of saurophidiens is the equivalent of the seminal vesicle and the prostate of the mammals (Crews, 1979; Faure 1991).

Mucous epithelial cell: period of sexual rest. Each mucous cell presents a basal euchromatic nucleus, containing a nucleolus with a tripartite structure; the cytoplasm contains a supra-nuclear Golgi apparatus, several cisternae of RER and many mitochondria with lamellate peaks, concentrated at the base of the cell. The cytoplasm is filled with mucigen vesicles. The cell membrane develops an important network of lateral and basal folds. The non-mucous cells present a closed apical pole with small clear vesicles, a basal irregularly-shaped euchromatic nucleus and many mitochondria with lamellate peaks occupying almost the totality of the cytoplasm (Fig. 4).

During sexual quiescence, the sexual segment secretes a mucous, reminiscent of both immature females and males. This mucous secretion constitutes a protective material used to lubricate the walls of the urinary tubes (Gabri, 1983) and to facilitate the advance of the urine (which is pasty-consistent in the lizards). The basal and lateral folds of membrane, the great number of mitochondria at the base of mucous cell, testify the active absorption of which consequence is the production of an urine rich in uric acid that would minimize the water lost.

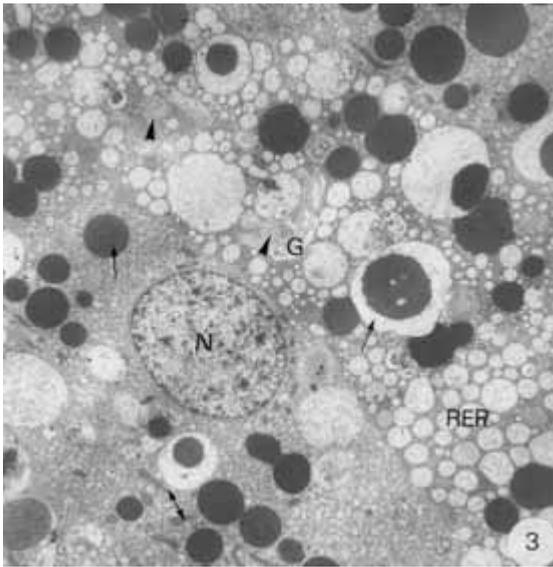


Fig. 3. The sexual segment of the kidney in the breeding period. Transmission electron microscopy ($\times 2400$). The serous epithelial cell presents an euchromatic nucleus (N). The cytoplasm contains rough endoplasmic reticulum with a vesicular configuration (RER), some mitochondria (\leftrightarrow), many Golgi areas (G), multivesicular bodies (∇) and numerous granules (\rightarrow).

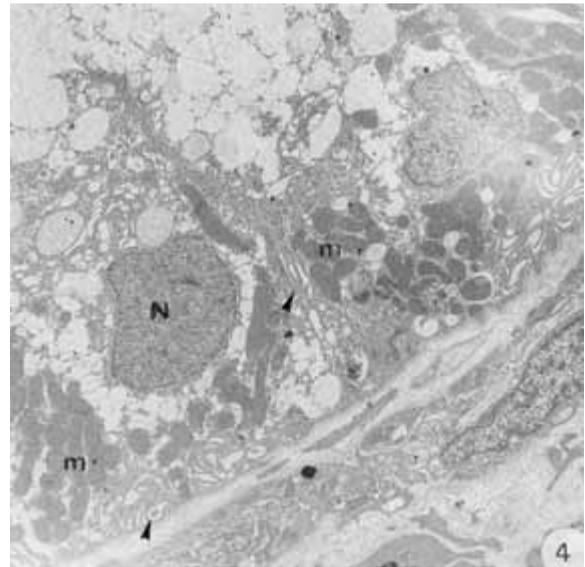


Fig. 4. The sexual segment of the kidney in the resting period. Transmission electron microscopy ($\times 2400$). The mucous epithelial cell presents a basal euchromatic nucleus (N), many mitochondria (m) with lamellate peaks and important network of side and basal folds (∇) of the cell membrane.

CONCLUSIONS

This study confirms the seasonal cyclic variations of the sexual segment of the kidney in *Uromastix acanthinurus* with the alternative elaboration of a serous secretion during the breeding period and a mucous secretion during the sexual rest. This change in the nature of secretion implies two different functions. During the period of reproduction, the serous sexual segment of the kidney ensures the role of an additional gland as well as the seminal vesicle and the prostate in mammals. In the period of sexual rest, the mucous sexual segment of the kidney is implied in the urinary functions.

Acknowledgments. We thank Beni-Abbes' population for participating in the animal capture.

REFERENCES

- Bons N.** (1970), "Le cycle sexuel du mâle chez *Acanthodactylus erythrurus lineomaculatus* Dum. et Bibr. (Sauria, Lacertidae)," *Bull. Soc. Sci. Nat. Phys. Maroc*, **49**, 161 – 197.
- Bishop J. E.** (1959), "A Histological and histochemical study of the kidney tubule of the common garter snake, *Thamnophis sirtalis*, with special reference to the sexual segment in the male," *J. Morphol.*, **104**, 307 – 357.
- Burtner H. J., Floyd A. D., and Lo, gley, J. B.** (1965), "Histochemistry of the 'sexuel segment' granules of the male rattlesnake kidney," *J. Morphol.*, **116**, 189 – 195.
- Courrier R.** (1929), "Les modifications saisonnières de l'appareil uro-génital chez *Uromastix acanthinurus* (Bell)," *Arch. Anat. Microsc.*, **25**, 388 – 394.
- Crews D.** (1979), "La commande hormonale du comportement chez un lézard," *Pour Sci.*, **10**, 35 – 42.
- Faure J.** (1991), *Les cellules épithéliales de l'épididyme: données structurales et ultrastructurales sur les activités géniques régulées par la testostérone. Thèse de Doctorat*, Université Blaise Pascal, Clermont Ferrand II.
- Gabe M.** (1959), "Données histochimiques sur le rein de *Vipera aspis* (L.)," *Ann. Histochem.*, **4**, 23 – 31.
- Gabri M. S.** (1983), "Seasonal changes in the ultrastructure of the kidney collecting tubule in the lizard *Podarcis taurica*," *J. Morphol.*, **175**, 143 – 152.
- Grenot C.** (1976), "Ecophysiologie du lézard saharien *Uromastix acanthinurus* Bell, 1825 (Agamidae, herbivore)," *Publ. Lab. Zool. E.N.S.*, **7**, 1 – 323.
- Grenot C. and Vernet R.** (1973), "Les lézards héliophiles du Sahara: facteurs écologiques et conditions d'élevage," *Bull. Soc. Hist. Nat. Afr. Nord.*, **64**, 53 – 78.
- Kehl R.** (1935), "Etude préliminaire du cycle sexuel de quelques reptiles sahariens," *Bull. Soc. Hist. Nat. Alger*, **26**, 61 – 67.
- Kehl R.** (1944), "Etude de quelques problèmes d'endocrinologie génitale chez certains reptiles du sud algérien," *Rev. Can. Biol.*, **3**, 131 – 219.
- Le Berre M.** (1989), *Faune du Sahara. 1. Poissons, Amphibiens, Reptiles*, Lechevalier – R. Chabaud, Paris.
- Regaud C. and Policard A.** (1903), "Variations sexuelles de structure dans le segment préterminal du tube urinifère de quelques ophidiens," *C. r. Soc. Biol.*, **55**, 216 – 218.

HELMINTHS AND TROPHIC RELATIONS OF COLUBRID SNAKES (COLUBRIDAE) IN THE VOLGA – KAMA REGION

A. G. Bakiev¹

Keywords: helminths, consumers, Colubridae, Volga – Kama Region.

INTRODUCTION

The Volga – Kama Region is a region with an area of more than 500 km² situated between 52 and 59° N and 43 – 52° E. The fauna of colubrid snakes in Volga – Kama Region includes 4 species: *Natrix natrix*, *N. tessellata*, *Coronella austriaca*, and *Elaphe dione*. These snakes are intermediate and supplementary hosts of helminths; they serve as food for mammals and birds. This paper is aimed to give a more detailed data on helminths of colubrid snakes and their trophic relations within Volga – Kama Region.

MATERIAL AND METHODS

The material was collected in 1995 – 2002. Snakes were narcotized by ether to reveal helminths in accordance with Skryabin's method (1928). 62 individuals were examined: *N. natrix* — 35, *N. tessellata* — 18, *C. austriaca* — 7, *E. dione* — 2. The published data (Garanin, 1976) and present data were used to prepare a list of Colubridae consumers of the Volga – Kama Region.

¹ Institute of Ecology of the Volga River Basin Russian Academy of Sciences, Komzin St., 10, 445003 Tol'yatti, Russia;
E-mail: ecology@avtograd.ru.

RESULTS AND DISCUSSION

Twenty species of helminths were found in *N. natrix*, in *N. tessellata*, seven, in *C. austriaca*, three, in *E. dione*, there were no helminths (Table 1). The data have extended the known species list of the snakes helminths (Sharpilo, 1976; Kirin, 2002).

Relative number of snakes with unjured tail in population may be used as indirect index of pressure of predators (Bakiev, 1999). The part of collected snakes had an injured tail: *N. natrix* — 10.8% (26 of 241), *N. tessellata* — 5.7% (5 of 87), *C. austriaca* — 6.9% (2 of 29), *E. dione* — 3.2% (1 of 31).

The data from the Samara region allow to add the published report (Garanin, 1976) of consumers of Colubridae in Volga – Kama region with 2 species of vipers, in particular, to add to the consumers of *N. natrix* the *Vipera renardi*, and to the consumers of *N. tessellata* the *V. berus* (Table 2).

I can conclude that not less than 22 species of helminths were found in Colubridae snakes of Volga – Kama Region, and at least 56 species of vertebrate animals are noted as Colubridae consumers. Colubridae snakes play an important part in helminth's circulation and helminth's transmission to animals of higher trophic levels.

TABLE 1. Helminths of colubrid snakes in the Volga – Kama region

Helminths		Colubridae		
Classes	Species	<i>N. natrix</i>	<i>N. tessellata</i>	<i>C. austriaca</i>
Trematoda	<i>Astioterma monticelli</i> , <i>Diplodiscus subclavatus</i> , <i>Encyclometra colubrimurorum</i> , <i>Leptophallus nigrovenosus</i> , <i>Plagiorchis elegans</i> , <i>Pleurogenes claviger</i> , <i>Metaleptophallus gracillimus</i> , <i>Opisthoglyphe ranae</i> , <i>Paralepoderma cloacicola</i>	+	—	—
	<i>Macrodera longicollis</i> , <i>Telorchis assula</i> , <i>Pharyngostomum cordatum</i> , larvae,	+	+	—
	<i>Strigea strigis</i> , larvae	+	—	+
	<i>Alaria alata</i> , larvae	+	+	+
Cestoda	<i>Ophiotaenia europaea</i>	+	+	—
Acanthocephala	<i>Acanthocephalus lucii</i>	+	—	—
	<i>Sphaerirostris teres</i>	—	—	+
Nematoda	<i>Cammallanus truncatus</i>	—	+	—
	<i>Rhabdias fuscovenosus</i>	+	+	—
	<i>Strongyloides mirzai</i> , <i>Physaloptera clausa</i>	+	—	—

TABLE 2. Trophic Relations of Colubrid Snakes in Volga – Kama Region

Predators		Colubridae as food items				
Classes	Species	<i>N. natrix</i>	<i>N. tessellata</i>	<i>C. austriaca</i>	<i>E. dione</i>	
Pisces	<i>Salmo trutta, Lucioperca lucioperca</i>	+	—	—	—	
	<i>Silurus glanis</i>	—	+	—	—	
Amphibia	<i>Bufo bufo, Rana ridibunda, R. lessonae</i>	+	—	—	—	
Reptilia	<i>Anguis fragilis, Coronella austriaca</i>	+	—	—	—	
	<i>Elaphe dione</i>	—	—	—	+	
	<i>Vipera berus</i>	—	+	—	—	
	<i>Vipera renardi</i>	+	—	—	—	
Aves	<i>Podiceps cristatus, Ciconia ciconia, Ciconia nigra, Falco subbuteo, Falco tinnunculus, Milvus milvus, Haliaeetus albicilla, Aquila chrysaetus, Circus pygargus, C. aeruginosus, Aquila clanga, A. pomarina, Pandion haliaetus, Lanius excubitor, L. cristatus, Turdus merula, Passer domesticus, Pica pica</i>	+	—	—	—	
	<i>Ardea cinerea</i>	+	+	—	—	
	<i>Mergus merganser, Plegadis falcinellus</i>	—	+	—	—	
	<i>Milvus korschun</i>	+	+	—	+	
	<i>Aquila heliaca, Aquila rapax</i>	—	—	—	+	
	<i>Buteo buteo</i>	+	—	+	—	
	<i>Circaetus gallicus</i>	+	+	+	+	
	<i>Bubo bubo</i>	+	—	—	+	
	<i>Strix lauco</i>	+	—	+	—	
	<i>Corvus frugilegus</i>	+	+	—	—	
	Mammalia	<i>Erinaceus europaeus, Desmana moschata, Rattus norvegicus, Nyctereutini procyonoides, Mustela nivalis, Mustela eversmanni, Mustela vison, Martes martes, Sus scrofa</i>	+	—	—	—
		<i>Ondatra zibethica, Lutra lutra</i>	—	+	—	—
		<i>Canis lupus</i>	—	—	—	+
<i>Vulpes vulpes</i>		+	+	—	+	
<i>Mustela putorius, Martes foina</i>		+	—	+	—	
<i>Meles meles</i>		+	+	+	+	

REFERENCES

- Bakiev A. G.** (1999), “The indicator of pressure of predation on snakes,” in: *Abstrs. of the 2nd Conf. of the Volga Reagion Herpetologists*, Tol’yatti, pp. 3 – 4 [in Russian].
- Garanin V. I.** (1976), “Amphibians and reptiles in the nutrition of vertebrates,” in: *Prirodnye Resursy Volzhsko-Kamskogo Kraja. Zhivotnyi Mir [Natural Resources of Volga-Kama Region. Animal World]* Vol. 4, Izd. KGU, Kazan, pp. 86 – 111 [in Russian].
- Kirin D.** (2002), “New records of the helminth fauna from Gras Snake, *Natrix natrix* L., 1758 and Dice Snake, *Natrix tessellata* Laurenti, 1768 (Colubridae: Reptilia) in South Bulgaria,” *Acta Zool. Bulg.*, **54**(1), 49 – 53.
- Sharpilo V. P.** (1976), *Helminthes of Reptiles of the USSR Fauna*, Naukova dumka, Kiev [in Russian].
- Skryabin K. I.** (1928), *Method of Perfect Helminth’s Autopsies of Animals, Including Man*, Izd. MGU, Moscow [in Russian].

SOME ASPECTS OF THE ONTOGENESIS OF THE IMMUNE SYSTEM ORGANS OF *Typhlonectes compressicauda* (DUMÉRIL ET BIBRON, 1841), AMPHIBIA, GYMNOPHIONA

P. Bleyzac¹ and J.-M. Exbrayat¹

Keywords: Gymnophiona, *Typhlonectes compressicauda*, development.

INTRODUCTION

Gymnophiona are still little known semi-aquatic and burrowing apodan Amphibians with a tropical distribution. Their immune system is poorly known. In accordance with work previously published (Paillot et al., 1997a, 1997b; Zapata et al., 1982; Welsch and Starck, 1984), the present study is devoted to the main morphological aspects of the development of the immune system in *Typhlonectes compressicauda*, a viviparous species living in the marshes of Kaw in French Guyana. The developmental stages have been determined after the table of Sammouri et al. (1990). We found worthwhile to describe the organization of the immune system throughout the embryonic development.

MATERIAL AND METHODS

The animals were captured in French Guyana during several missions. 29 individuals (Table 1) which stage of development was determined according to Sammouri et

¹ Laboratoire de Biologie Générale, Université Catholique de Lyon et Laboratoire de Reproduction et Développement des Vertébrés, Ecole Pratique des Hautes Etudes, 25, rue du Plat, F-69288 Lyon Cedex 02, France; E-mail: pbleyzac@univ-catholyon.fr.

TABLE 1. Stages of Development, Numbers, and Length of the Embryos of *Typhlonectes compressicauda* Observed

Phase	Stage	Number	Length, mm
Embryonic phases	21 – 22	1	7 – 8
	23	3	9
	24	5	10
	25	4	11
Fetal phases	26 – 27	3	13 – 14
	28	2	24
	29	3	30
	30	2	33
	31	2	40
Larval phases	32	4	63
	33	2	100

al. (1990). The stages of development are comprised between the stage 21 and the stage 34 (new-born). The animals were preserved with Bouin's fluid, dehydrated, and embedded in paraffin. The serial sections (5 µm thick) have been done with a vertical Minot's microtome. The sections were observed with a light microscope. The general organization was studied starting from the transverse, sagittal or parasagittal sections stained with the hemalum-eosine, Romeis's azan, or with Masson – Goldner's trichroma. In order to observe particularly the blood cells tissues were stained with the Pappenheim's method.

OBSERVATIONS AND RESULTS

In *Typhlonectes compressicauda* no bone marrow has been found. At stage 24, just before hatching, thymus and spleen develop from the fourth, fifth, and sixth visceral pouches. Spleen develop from a bud situated in mesentery

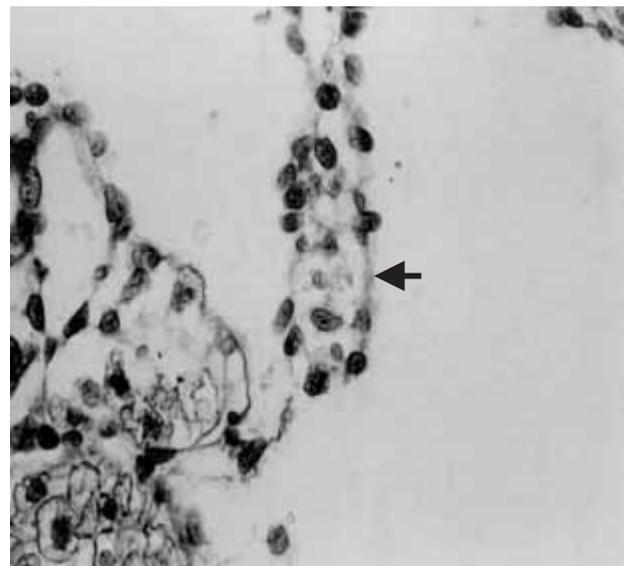


Fig. 1. Spleen. Stage 24: thickening of the mesentery (arrow) visible between the visceral cavity and the vitellin mass. ×200.

between stomach and vitellin mass. The thymus is composed with a medulla and a cortex (Figs. 5 – 8). At stage 28, between hatching and metamorphosis Hassal’s corpuscles like can be observed in the thymus (Fig. 7). They will be found throughout the life of animal.

At stage 30, just before metamorphosis, spleen is organized in a white and red pulp when blood islets disappear. Lymphoblast move between thymus and spleen through blood vessels. Mesonephros is not clearly hematopoietic in both embryos and adult. Liver develops earlier

than thymus and spleen. At stage 30, it becomes granulocytotoxic and monocytotoxic. It is equivalent to the bone marrow according to previous works (Paillot et al., 1997b).

The main events of development of immune system in *Typhlonectes compressicauda* are summarized in Table 2.

DISCUSSION AND CONCLUSIONS

The development of the immune organs of *Typhlonectes compressicauda* shows that, some characters bring them close to the other Amphibians [similar localization of thymic buds (Fig. 5)], and other characters bring them close to the amniotes (presence of structures resembling

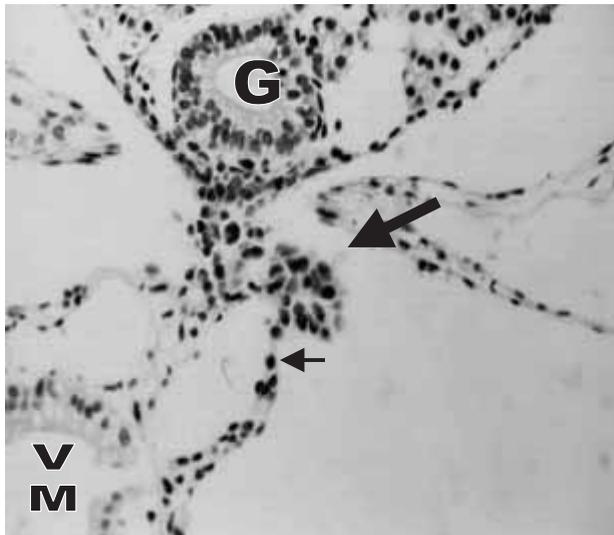


Fig. 2. Stage 25: budding of the spleen (big arrow) starting from a mesenteric thickening (small arrow). VM, vitellin mass; G, gut. ×40.

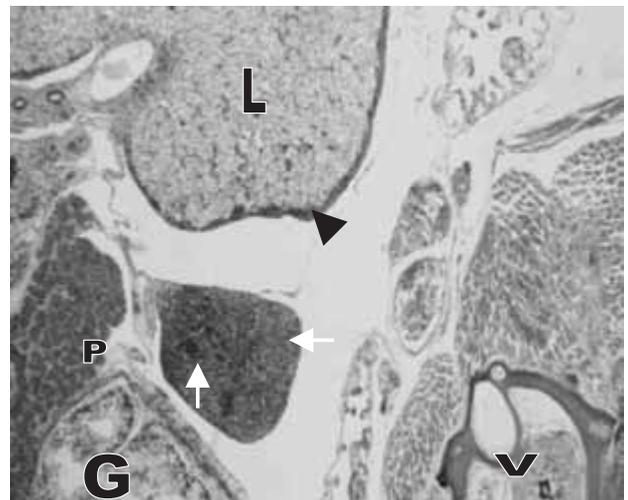


Fig. 4. The spleen and its environment at the stage 33: liver (L) and its cortical hematopoietic layer (head arrow), pancreas (P), digestive tract (G), spleen with white pulp (white arrow), and red pulp (black arrow) well delimited; vertebra (V). ×40.

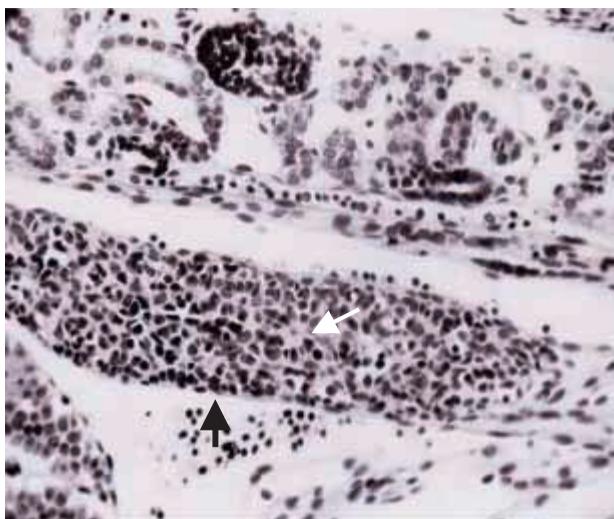


Fig. 3. Stage 30: the spleen starts to be organized in white pulp (white arrow) and in red pulp (black arrow). ×50.



Fig. 5. Stage 24 thymic buds (arrows): NT, neural tube. ×100.

REFERENCES

- Beaumont A., Truchot J.-P., and Du Pasquier L.** (1995), *Respiration-Circulation-Système immunitaire*, Dunod.
- Charlemagne J.** (1977), "Thymus development in amphibians: colonization of thymic endodermal rudiments by lymphoid stem-cells of mesenchymal origin in the Urodele *Pleurodeles waltii* Michah," *Ann. Immunol. Inst. Pasteur*, **128C**, 897 – 904.
- Paillot R., Estabel J., and Exbrayat J.-M.** (1997a), "Organes hématopoïétiques et cellules sanguines chez *Typhlonectes compressicaudus* et *T. natans* (Amphibiens, Gymnophione)," *Bull. Mens. Soc. Linn. Lyon*, **66**(5), 124 – 134
- Paillot R., Estabel J., Keller E., and Exbrayat J.-M.** (1997b), "Premières observations sur le foie hématopoïétique chez les Gymnophiones," *Bull. Soc. Herpetol. Fr.*, **82 – 83**, 29 – 40.
- Sammouri R., Renous S., Exbrayat J.-M., and Lescure J.** (1990), "Développement embryonnaire de *Typhlonectes compressicaudus* (Amphibia, Gymnophiona)," *Ann. Sci. Nat. Zool. Paris. 13^{ème} Sér.*, **11**, 135 – 163.
- Tournefier A.** (1973), "Développement des organes lymphoïdes chez l'amphibien Urodèle *Triturus alpestris* Laur.: tolérance des allogreffes après la thymectomie larvaire," *J. Embryol. Exp. Morphol.*, **29**(2), 383 – 396.
- Tournefier A., Lesourd M., and Gounon P.** (1990), "The axolotl thymus: cell types of the microenvironment, a scanning and transmission electron-microscopic study," *Cell Tissue Res.*, **262**, 387 – 396.
- Welsch U. and Starck M.** (1984), "Morphological observations on blood cells and blood cell forming tissues of gymnophiona," *Mém. Soc. Zool. Fr.*, **43**, 107 – 115.
- Zapata A. and Amemiya C. T.** (2000), "Phylogeny of lower vertebrates and their immunological structures," in: Du Pasquier L. and Litman G. W. (eds.), *Evolution of the Vertebrate Immune System*, Springer.
- Zapata A., Gomariz R.-P., Garrido E., and Cooper E.-L.** (1982), "Lymphoid organs and blood cells of the caecilian *Ichthyophis kotoensis*," *Acta Zool.*, **63**, 11 – 16.

STRUCTURE AND FUNCTIONAL SIGNIFICANCE OF THE NUPTIAL THUMB PADS IN *Rana esculenta* AND *R. perezi*

R. Brizzi,¹ G. Delfino,¹ and S. Jantra¹

The nuptial thumb pads of *Rana esculenta* and *R. perezi* are cutaneous specialisations including rows of glands, defined nuptial glands, embedded in the pad dermis. The pads are covered by a black, strongly keratinised epidermis, where high papillae alternate with gland pores. The mucous nature of the above glands is confirmed both under light and transmission electron microscopes. The nuptial pads function in promoting male's ability to retain the female during amplexus. The nuptial glands release their product onto the keratinised pads, which indicates an adhesive function of the secretion. Such performance is synergic with the complex ridge apparatus of the pad surface, capable of working like a multi-lamellar sucker. Secretions of these glands may also prevent sexual interference by rival males.

Keywords: *Rana*, thumb pad glands.

INTRODUCTION

Cutaneous glands play a key role during social activity of many amphibians (Duellman and Trueb, 1994; Houck and Sever, 1994). This is the case for many anurans which join under a variety of conditions and in response both to abiotic and biotic pressures. Usual aggregations of adult frogs and toads are those that form during reproduction, especially in species that display explosive breeding patterns (Wells, 1977). In this connection, the males of many anurans develop special skin glands and on this account these glands are usually defined as breeding glands (Thomas et al., 1993). These structures may occur in different body regions, in form of well visible glandular heaps or single glands dispersed in the skin (Brizzi et al., 2003). In many frogs and toads breeding glands lie in form of keratinised patches on the thumbs and forearms and are specifically termed nuptial pads. In this paper we describe the male thumb pads in *Rana esculenta* and *R. perezi*. The purpose of this study is to provide indications about structural and ultrastructural characteristics of these specialised glands and to discuss the nature of their secretory product in the light of its role during the amplexus.

MATERIAL AND METHODS

Nuptial thumb pads were removed from 3 males of *Rana esculenta* (Florence, Tuscany, Italy) and *R. perezi* (Mellid, Galicia, Spain) collected during the breeding season. A pad of each male was processed for light microscopy (LM), the other for transmission (TEM) or scanning (SEM) electron microscopies according to routinary pro-

cedures. For LM observations sections 8 µm thick were stained with hematoxylin-eosin or Mallory-Galgano trichrome method for general cytology. Selected sections from each specimen were tested with the following histochemical stains: periodic acid/Schiff's (PAS), alcian blue at pH 2.5 and ninhydrin/Schiff. For TEM observations ultrathin sections were treated with uranyl acetate and lead citrate. Entire nuptial pads, previously fixed in formalin, were submitted to the usual SEM procedures.

RESULTS

In *Rana esculenta* and *R. perezi* the nuptial pads occur on the inner fingers of their forelimbs and are covered by a black, strongly keratinised epidermis (Fig. 1A). These cutaneous specializations also include glands embedded in the dermis and opening on the pad surface. SEM observations provided further details of the external feature of the pads, particularly of its highly papillate epidermis and nuptial gland pores (Fig. 1B – D). Observed under LM, the thumb pad epidermis consists of some layers of cells, with the external one flattened in shape as effect of its horny evolution (Fig. 2A – B). In *R. esculenta* the pad surface shows hill-like protuberance (Fig. 2A, C), whereas in *R. perezi* it is covered by stumped papillae (Fig. 1B – D; 2B, D). The main structure of the glands embedded in the pad loose dermis is similar to that of the other cutaneous mucous glands, although some traits appear noticeable (compare Fig. 2A – D with Fig. 2E – F). The thumb pad glands are unusually large and closely packed together, with the major axis orthogonal to the epidermal layers. As a sectioning effect, in many cases it seems that two – three gland layers are stratified beneath the epidermis

¹ Department of Animal Biology and Genetics, University of Florence, Via Romana 17, I-50125 Firenze, Italia; E-mail: brizzi@dbag.unifi.it.

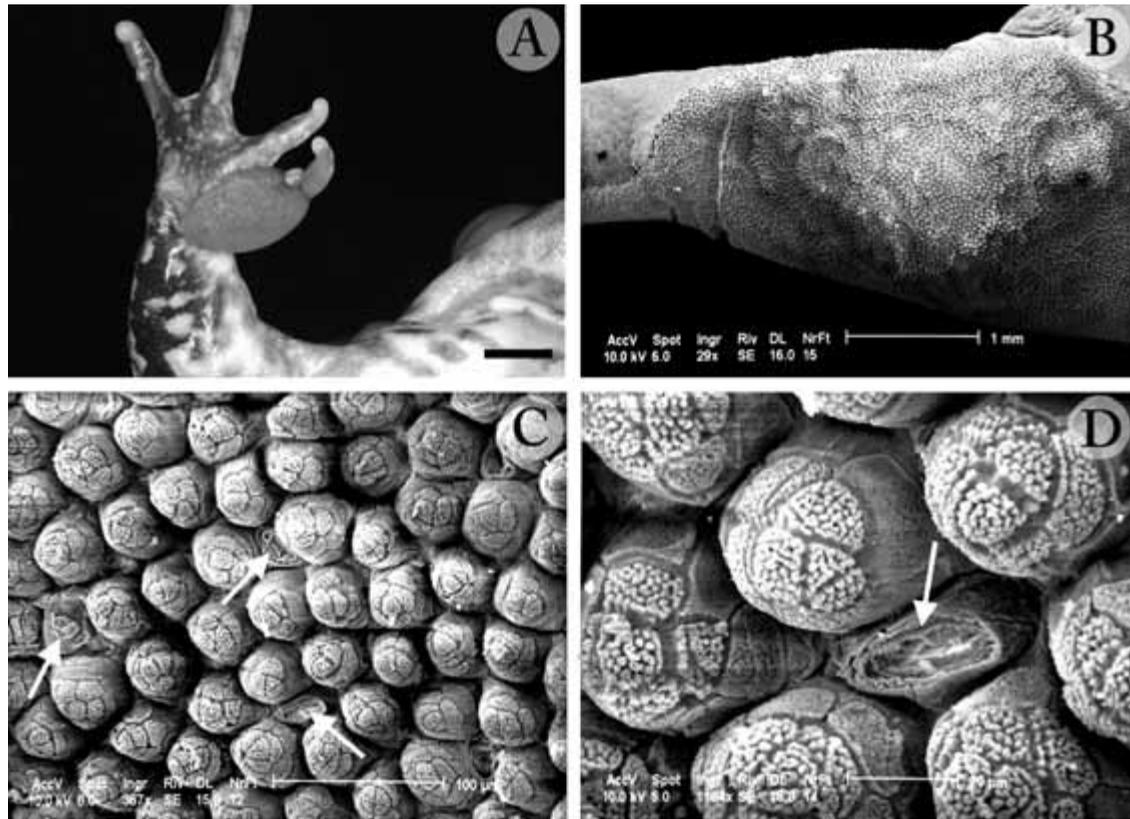


Fig. 1. Feature of nuptial thumb pads in *Rana perezi*. Note the papillate epidermis and the nuptial gland pores occurring among the adhesive protuberances (arrows in C – D). Bar in A is 2 mm.

(Fig. 2A, C). Mucocytes are tall and orderly arranged around a wide lumen (Fig. 2B). Most nuclei lay basally, according to an obvious functional polarisation. The cytoplasm contains large amounts of secretory material, that in some cases is also visible inside the gland lumina. Both cytoplasmic and luminal secretions are vesicular, basophilic and pale blue with the Mallory – Galgano method. Histochemical trials reveal the main mucous nature of the above product, which reacts positively to PAS and alcian blue. A moderate positivity is also evident towards the ninhydrin/Schiff test, which, on the whole, indicates proteins joined with neutral carbohydrates and carboxylated acidic glycosaminoglycans.

TEM observations confirm the specialised features of the thumb pads. The keratinocytes are polyhedral, with ellipsoidal nuclei (Fig. 3A) and a rich supply of mitochondria characterised by light content. A discontinuous, contractile sheath is obvious around the thumb pad glands (Fig. 3B). This allows cytoplasmic projections of the adenocytes to contact the dermal environment and, possibly, to increase trophic exchanges. Although repeated observations on several specimens, axonal profiles were not

found. A biosynthesis machinery, typical of mucocytes, includes abundant rER cisternae (Fig. 3B – C) and stacked saccules of the Golgi apparatus (Fig. 3D). Secretory material shows typical features of a mucous product. It consists of granules with various densities and closely packed together, which leads them to acquire a polyhedral shape (Fig. 3C). However, an obvious fluidification characterizes the late maturation process of the mucous granules, which are discharged in the gland lumen as a structureless, rather transparent material (Fig. 3E).

DISCUSSION

In the amphibians, typical examples of sexually dimorphic skin glands are the glandular nuptial pads occurring in the males of many frogs and toads. These glands release a glue-like substance that allows the male to adhere to the female during amplexus. (Duellman and Trueb, 1986). Secretions of these glands may also prevent sexual interference by rival males in attempts to remove the glued male from the female. Our study on thumb pads in *Rana*

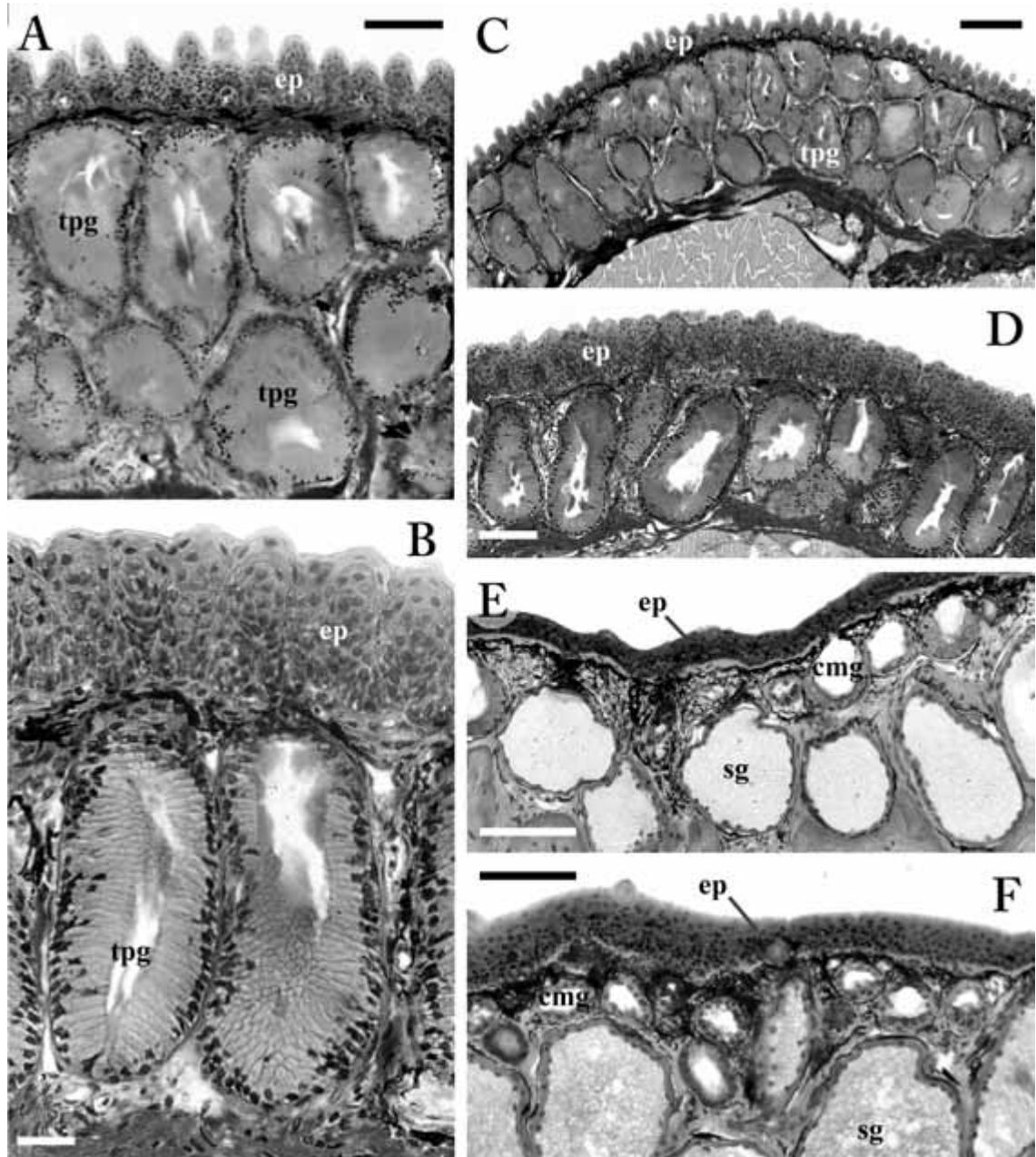


Fig. 2. Comparison between thumb pad glands and common cutaneous glands observed under LM. A, C, thumb pad glands in *R. esculenta*; B, D, the same in *R. perezi*; E – F, common serous and mucous glands in *R. esculenta* (E) and *R. perezi* (F). The mucous units are small, roundish and consist of low prismatic mucocytes. Labels: cmg, common mucous glands; ep, epidermis; sg, serous glands; tpg, thumb pad glands. Scale bars are 100 µm.

emphasizes the mechanical role of these structures, related to the epidermal specialisations (namely hillocks and ridges) and the adhesive properties of the secretory product acting as a glue on the thumb surface. Reports on the nature of the nuptial pad glands are rather contradictory. From the wide literature on frog skin glands (e.g., Dapson, 1970; Dapson et al., 1973; Hostetler and Cannon, 1974;

Cannon and Hostetler, 1976), it is known that the ordinary mucous glands contain neutral and acidic mucosubstances and lack proteinaceous and lipid secretions. In contrast, serous glands possess bound lipids, proteins and sometimes biogenic amines. In the past, some authors indicated the mucous appearance of the nuptial pad glands secretion (revised by Brizzi et al., 2003). More recently, Thomas

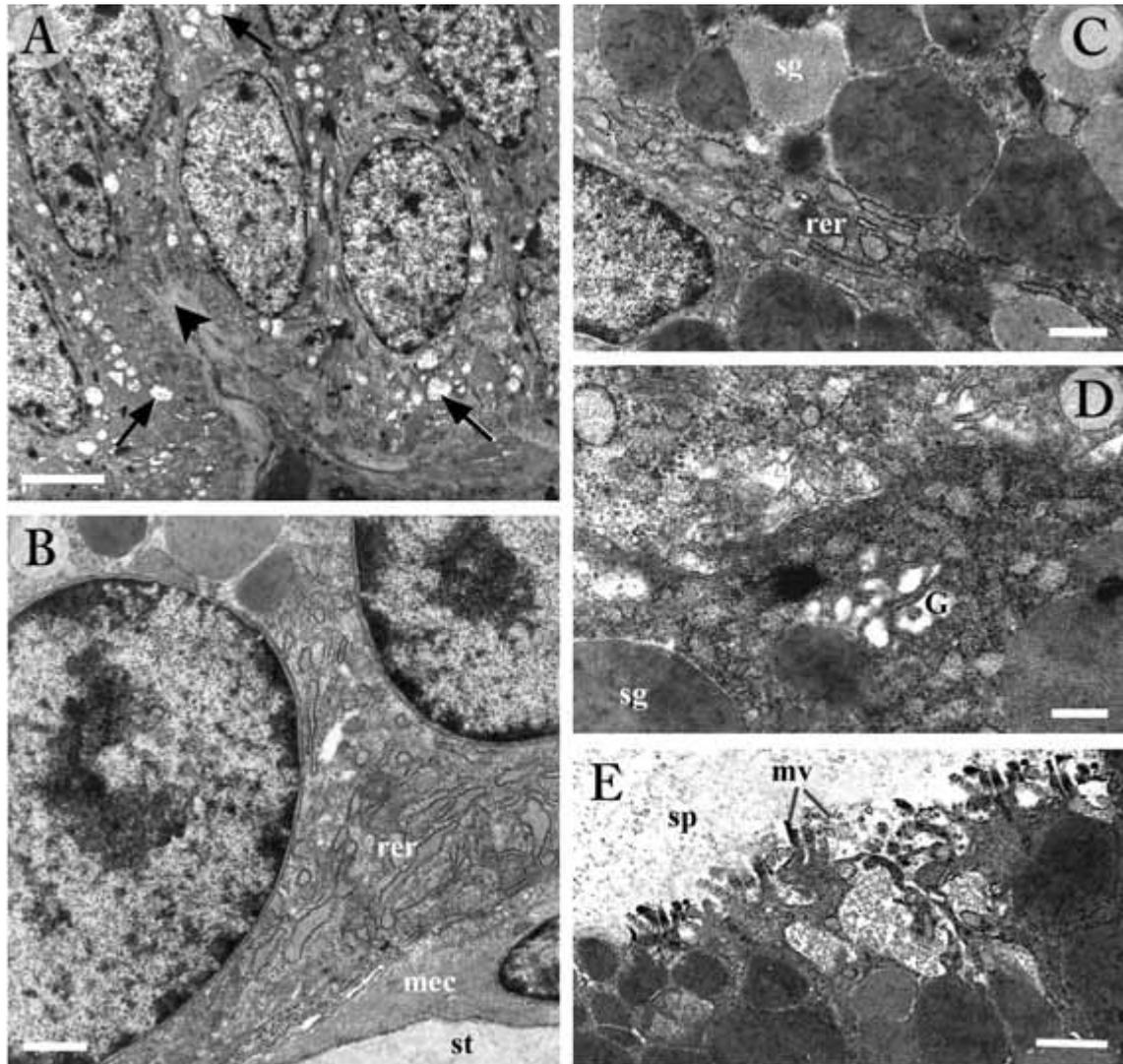


Fig. 3. Ultrastructural patterns of thumb pad epidermis and glands. **A:** *Rana esculenta*. Elongated nuclei of epidermal cells. Arrows point to mitochondria with light inner chambers. Arrow-head points to the dermal-epidermal boundary; **B:** *R. perezi*. Basal portion of a thumb pad gland. A mucocyte is visible near to the stromal environment, in a gap-area of the discontinuous myoepithelium. Notice rer profiles with variable shapes inside the mucocyte; **C:** *R. esculenta*. Mucous granules with different electron density and polyhedral shape; **D:** *R. perezi*. Sacculi of a Golgi stack with light compartments; **E:** *R. esculenta*. Secretory granules crowded at the cell apices before release. The gland lumen contains structureless material deriving by thinning of mucous product followed by merocrine release. Labels: G, Golgi stack; lu, gland lumen; mv, microvilli; mec, myoepithelial cell; rer, rough endoplasmic reticulum; sg, secretory granules; sp, secretory product; st, stroma. Scale bars are 5 μm (A), 1 μm (B – C, E), and 500 nm (D).

and Licht (1993) reported slightly differential staining characteristics in the nuptial glands and other sexually dimorphic skin glands of 14 frog species in comparison to either mucous or serous glands. On the basis of general criteria, Thomas et al. (1993) suggested that the nuptial pads and the other sexually dimorphic glands should be classified as a unique gland type, apart from mucous and serous glands.

The histochemical trials we performed on the nuptial pad glands of *Rana esculenta* and *R. perezi* reveal a wide

similarity between these cutaneous glands and the ordinary mucous ones. Other features resembling morphological traits usual in the mucous glands derive from LM and TEM observations. The biosynthetic machinery of the secretory cells, the features in the maturation steps, and the morphological traits of the secretory granules indicate that these glands belong to the mucous gland line. In addition, the myocytes, lacking any direct innervation, strongly resemble the myoepithelial cells of skin mucous glands. In contrast to specialised mucous glands detected in the skin

of some *Rana* species (Brizzi et al., 2002), mucocytes in thumb pad glands do not exhibit any lipid droplets; therefore it seems that these secretory organs are not engaged in the production of steroid compounds. Actually, the specialization degree of the thumb pad glands appears moderate. The most impressive traits of these glands consist of their peculiar localization and remarkable large size. However, a wide comparison of sexually dimorphic skin glands in anurans and urodeles (Brizzi et al., 2002) suggests that in both these amphibian orders the production of chemosignals for social and reproductive interactions is mainly a prerogative of the mucous gland line.

REFERENCES

- Brizzi R., Delfino G., and Pellegrini R.** (2002), "Specialized mucous glands and their possible adaptive role in the males of some species of *Rana*," *J. Morphol.*, **254**, 328 – 341.
- Brizzi R., Delfino G., and Jantra S.** (2003), "An overview of breeding glands," Jamieson B. G. M. (ed.), in: *Reproductive Biology and Phylogeny of Anura*. Vol. 2, Science Publishers Inc., Enfield (NH, USA), pp. 253 – 317.
- Cannon M. S. and Hostetler J. R.** (1976), "The anatomy of the parotoid gland in Bufonidae with some histochemical findings," *J. Morphol.*, **148**, 137 – 160.
- Dapson R. W.** (1970), "Histochemistry of mucus in the skin of the frog *Rana pipiens*," *Anat. Rec.*, **166**, 615 – 626.
- Dapson R. W., Feldman A. T., and Wright O. L.** (1973), "Histochemistry of the granular (poison) secretion in the skin of the frog, *Rana pipiens*," *Anat. Rec.*, **177**, 549 – 560.
- Duellman W. E. and Trueb L.** (1986), *Biology of Amphibians*, McGraw-Hill, New York; reprint, Johns Hopkins Univ. Press, 1994.
- Hostetler J. R. and Cannon M. S.** (1974), "The anatomy of the parotoid gland in Bufonidae with some histochemical findings. I. *Bufo marinus*," *J. Morphol.*, **142**, 225 – 240.
- Houck L. D. and Sever D. M.** (1994), "Role of the skin in reproduction and behaviour," in: Heatwole H. and Bartholomus G. T. (eds.), *Amphibian Biology*, Vol. I, Surrey Beatty and Sons, New South Wales (Australia), pp. 351 – 381.
- Thomas E. O. and Licht P.** (1993), "Testicular and androgen dependence of skin gland morphology in the anurans, *Xenopus laevis* and *Rana pipiens*," *J. Morphol.*, **215**, 195 – 200.
- Thomas E. O., Tsang L., and Licht P.** (1993), "Comparative histochemistry of the sexually dimorphic skin glands of anuran amphibians," *Copeia*, **1993**, 133 – 143.
- Wells K. D.** (1977), "The social behaviour of anuran amphibians," *Animal Behav.*, **25**, 666 – 693.

REPRODUCTIVE STRUCTURES IN *Mertensiella caucasica* AND *M. luschani* (AMPHIBIA: SALAMANDRIDAE)

R. Brizzi,¹ M. Sevinç,² and G. Tanteri¹

Although different in some aspects of their sexual biology, *Mertensiella caucasica* and *M. luschani* share various anatomical characters related to their reproduction. Males of *Mertensiella* have a tubercle on the dorsal surface of the tail. This structure plays a stimulating role on the female cloaca in the preliminary steps of the mating, namely before the male deposits a spermatophore and the female picks it up in her cloaca. A surprising finding is that females of *M. luschani* possess a rudiment of tail tubercle, which discloses new perspectives on the original function of this structure. Males of *Mertensiella* exhibit four types of cloacal glands (Kingsbury's, pelvic, ventral and dorsal glands), whereas females possess spermathecae for sperm storage and internal fertilization.

Keywords: *Mertensiella*, reproductive structures.

INTRODUCTION

The genus *Mertensiella* includes two species: *M. caucasica* and *M. luschani*. The Caucasian salamander occurs in North-East Turkey bordering the south-east edge of the Black Sea and West Georgia. Luschan's salamander is present in South-west Turkey and the south-east Aegean Islands. Apart from peculiar morphological and ecological characteristics (Griffiths, 1996), the two species exhibit some differences in their reproductive mode (Schultschik, 1994a, 1994b). In Luschan's salamander mating takes place on land, whereas in the Caucasian salamander it may occur on land or in water. In addition, the first species is oviparous, the second viviparous, namely it gives birth to fully developed young (Özeti, 1979). Nonetheless, both *Mertensiella* species exhibit similar courtship patterns, based upon mating embrace (the male holding the female from below). The courtship culminates with the male depositing a spermatophore on the substrate and manoeuvring the female so that it adheres to her cloaca. A peculiar reproductive character of *Mertensiella* is the male tubercle occurring at the boundary between tail and back. This structure stimulates the female cloaca directly before spermatophore pick up and may also release courtship odours (Arnold, 1987).

In this paper we report morphological observations on the tail tubercle and provide a main pattern of the cloacal glands in both sexes.

MATERIAL AND METHODS

Three adult males and three females of both *Mertensiella* species were collected between late spring and early

summer, corresponding to the mating season. *M. caucasica* at Sarikaya Yaylasi — Çaykara (Trabzon), *M. luschani* at Antalya, south-west Anatolia. Male tail tubercles were removed and processed for light microscopy (LM) or scanning electron microscopy (SEM) observations following standard methods. The cutaneous areas corresponding to those occupied by the male tubercles were excised from the females and processed using the same procedures. In addition, the cloacal regions of all the samples were processed for LM studies. Sections 10 µm thick were stained with hematoxylin-eosin or Mallory – Galgano trichrome method.

RESULTS

Males of *Mertensiella caucasica* and *M. luschani* possess a sexually dimorphic trait consisting in a conspicuous tubercle projecting from the skin of the dorsal tail base (Fig. 1A, B). Indicatively, the tubercle is about 2 mm in height and 2 – 2.5 mm in length. It bends anteriorly, with the apex mainly knob-like in *M. caucasica* and more or less pointed in *M. luschani*, although notable differences may occur also among males of the same species. The tail tubercle is covered by a keratinised epidermis similar to that of the contiguous regions and, likewise, it shows pores of the cutaneous glands occurring in the below dermis (Fig. 1C). Examination of the dorsal tail base in females of *M. caucasica* does not reveal presence of tubercle or modified epidermal texture. Nonetheless, a different pattern is visible in females of *M. luschani*. In these latter, observations under SEM revealed a tail swelling, just in the position where the tubercle occurs in males (Fig. 1D). The female rudiment shows a slight elevation but a length similar to that of the male tubercle that allows its recognition also under light stereomicroscope.

¹ Department of Animal Biology and Genetics, University of Florence, Via Romana 17, I-50125 Firenze, Italia; E-mail: brizzi@dbag.unifi.it

² Uludag University, Science and Art Faculty, Department of Biology Bursa, Turkey.

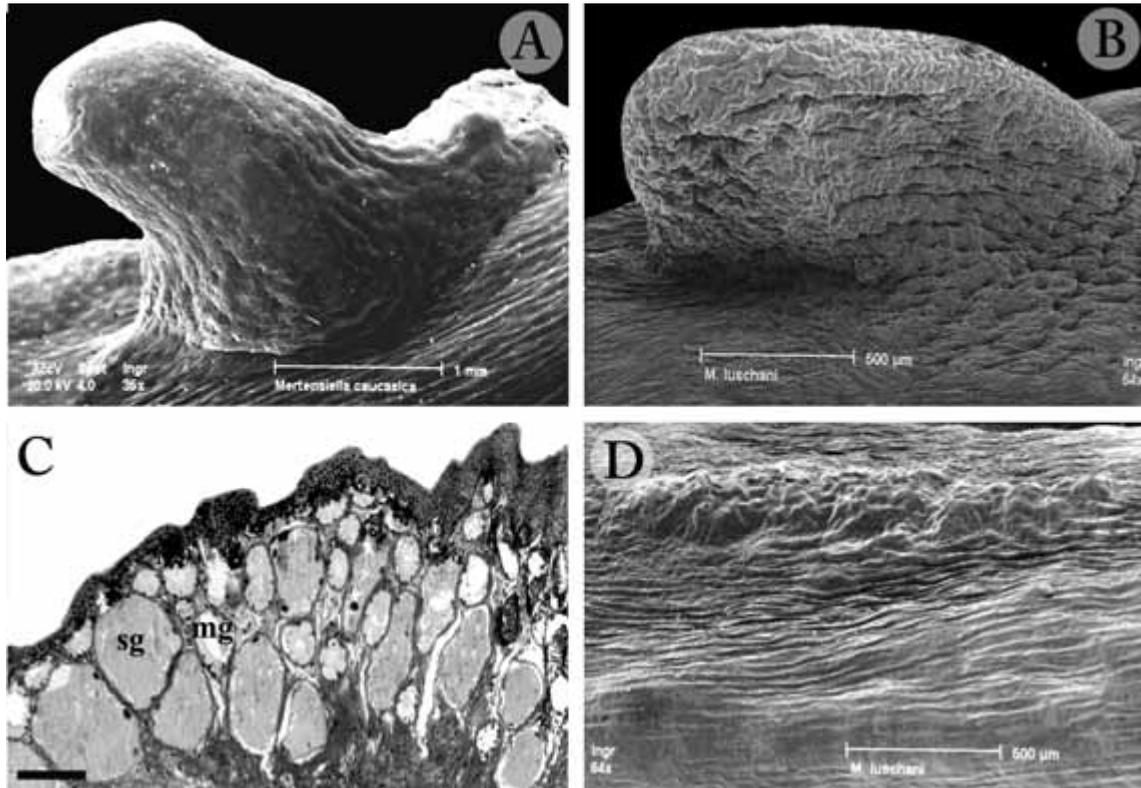


Fig. 1. Male tail tubercle of *Mertensiella* observed under SEM. **A:** male *Mertensiella caucasica*; **B:** male *M. luschani*; **C:** tangential section of the male tail tubercle of *M. luschani*. Note the rich supply of serous (sg) and mucous glands (mg) in the connective tissue; **D:** rudiment of tail tubercle in a female of *M. luschani*. Scale bar in C is 250 μm .

The male cloaca of *M. caucasica* and *M. luschani* consists in a small cloacal tube, posterior to the junction of the Wolffian ducts with the hindgut, and a proper cloacal chamber which opens onto the exterior. Spermatophore assemblage derives from the gradual mixing of sperm with secretory products from various cloacal glands (Kingsbury's, pelvic and ventral glands), whose tubules lie in the connective tissue around the cloaca. The outlets of these glands are visible at different levels on the cloacal walls (Fig. 2A – F). In addition, secretory pores of other cloacal glands (dorsal glands) occur along the cloacal border (Fig. 3A – B). Owing to their almost external position, the function of these glands is involved in pheromone production rather than in spermatophore formation.

Females of *Mertensiella* have sperm storage organs, the spermathecae, which allow internal fertilization. The spermathecae are simple tubular glands around the dorsal cloacal walls, each with a narrow neck opening into the cloaca and an expanded distal portion surrounded by a connective tissue sheath (Fig. 3C – D). After spermatophore collection by the female cloaca, sperm migrate into the spermathecae, where an epithelial secretion contrib-

utes to their survival until ovoposition. In females of *M. luschani* we found bundles of sperm inside the spermathecae (Fig. 3D). In contrast, most spermathecal tubules of *M. caucasica* were empty, which indicates animals in post-mating condition.

DISCUSSION

Within the Salamandridae, the genus *Mertensiella* belongs to the group of the “true” salamanders (*Chioglossa*, *Mertensiella*, and *Salamandra*), characterised by an advanced terrestrial way of life and reproductive mode base upon a close physical contact between male and female (Sever, 1992; Griffiths, 1996). In this connection may be regarded the occurrence of the male tail tubercle that characterises the genus *Mertensiella*. This tubercle is inserted into the female cloaca during amplexus, shortly before the male deposits a spermatophore. According to Sever et al. (1997), this structure contributes to female stimulation and stabilizes the female precisely over the spot where the spermatophore will be dropped and can be picked up suc-

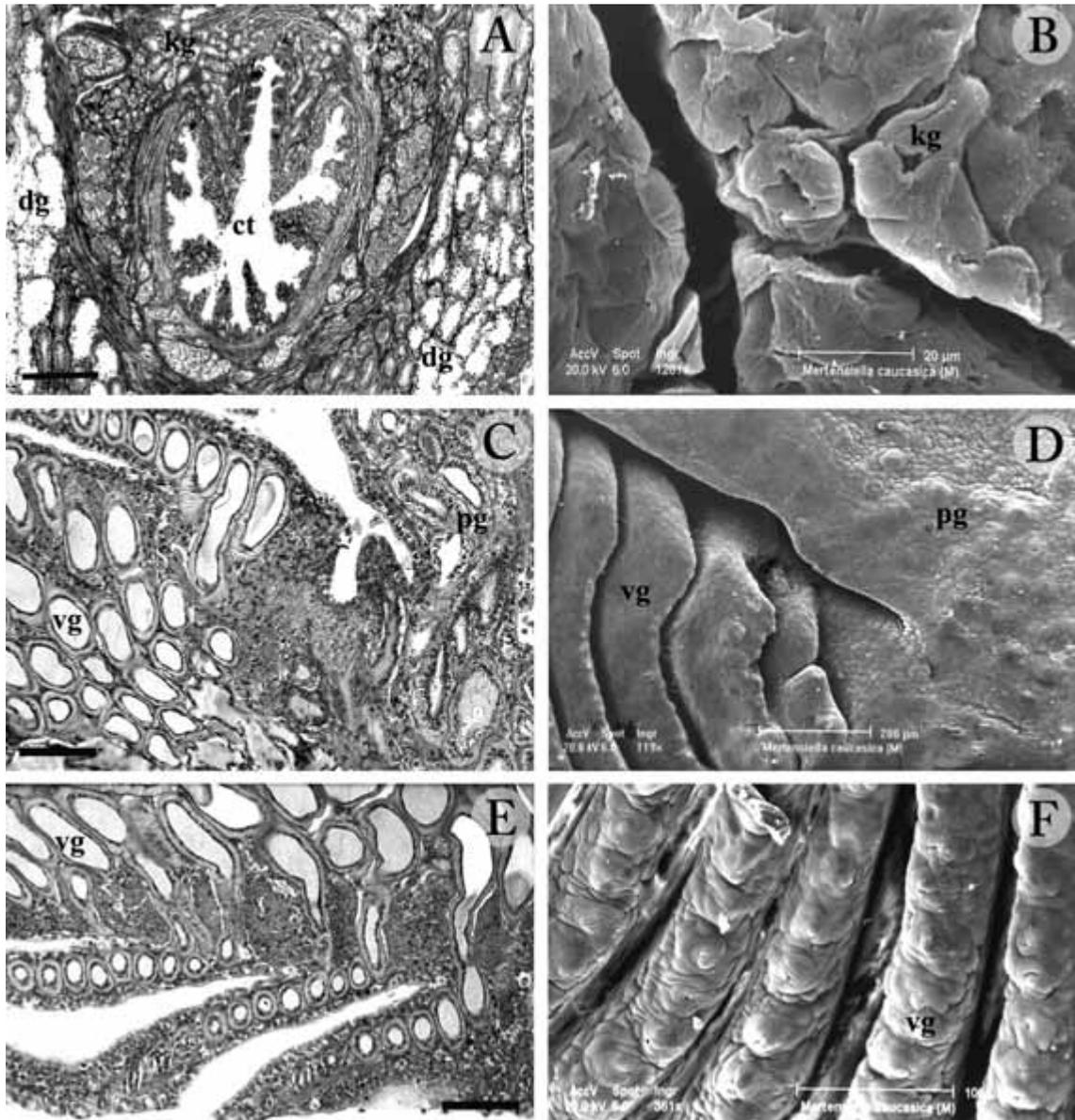


Fig. 2. Patterns of male cloacal glands in *M. luschani* (A) and *M. caucasica* (B – F). LM observations (A, C, E) allow to recognize, under SEM (B, D, F), the corresponding cloacal regions and the different secretory pores. **A:** Transverse section of the cloacal tube (ct) surrounded by Kingsbury (kg) and dorsal glands (dg); **B:** Kingsbury's gland outlets in form of stumpy papillae; **C – D:** Secretory tubules and pores of pelvic (pg) and ventral glands (vg); **E – F:** Ventral gland outlets aligned along typical cloacal folds. Scale bars in A, C, E are 120 µm.

cessfully. Nonetheless, the presence of a tubercle rudiment we observed in females of *M. luschani* (as also noticed by Basoglu and Atatur (1975) and Basoglu and Baran (1976) suggests a wider outlook on the original function of this anatomical trait. Owing to its localization just at the tail base, the dorsal tubercle marks a very critical region in case of attack by a predator. In this connection, the original function of this structure, also provided by a rich supply of noxious cutaneous glands (see Sever et al., 1997), may cor-

respond to a mechanical-chemical deterrent device, possibly occurring in the common ancestors (of both sexes) of *Mertensiella*. The specialisation of the tubercle as a sexually dimorphic trait may be hypothesised as a further step.

On the whole, male cloacal glands of *Mertensiella* correspond to those reported in most salamandrids (Sever, 1992; Brizzi et al., 1996a, 1996b). Nonetheless, the present study confirms slight interspecific differences, mainly related to tubule number and position of the secretory

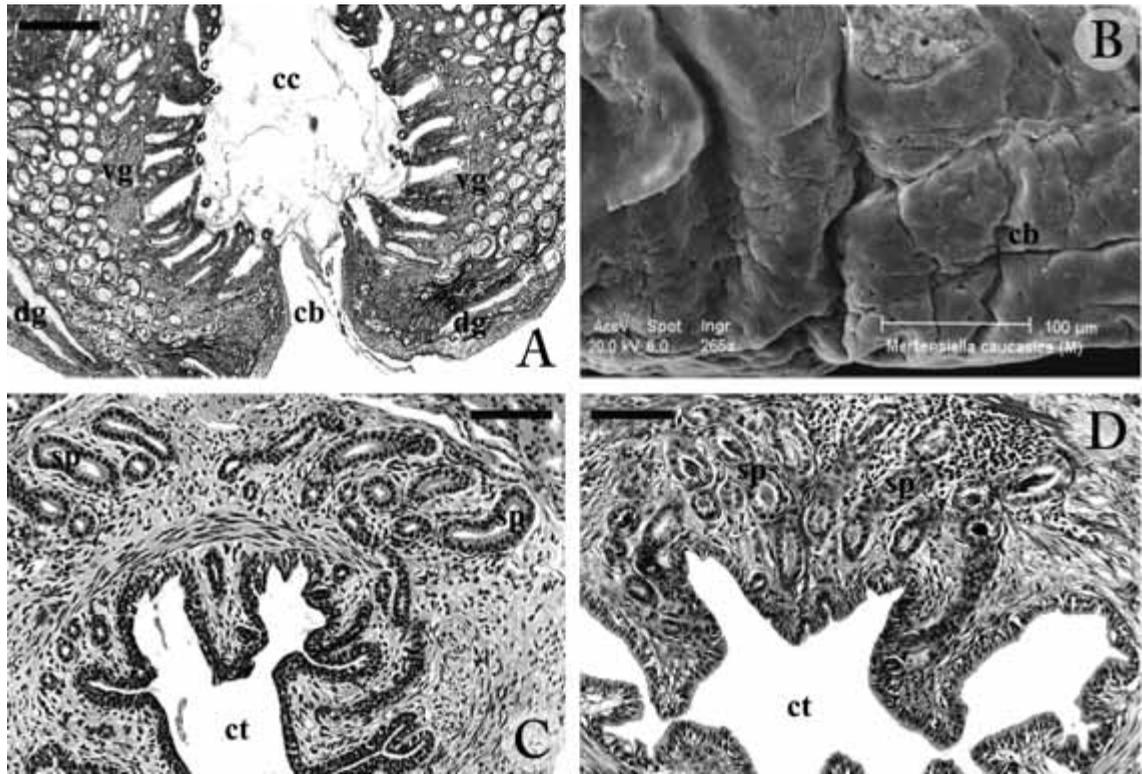


Fig. 3. A – B: Male cloaca chamber (cc) in *M. luschani* (A) and in *M. caucasica* (B). At this level the cloaca opens externally and the cloacal border (cb) appears to be keratinised. Note in (A) ventral (vg) and dorsal glands (dg) around the cloaca; C – D: Spermathecae (sp) around the cloacal tube (ct) of female *M. caucasica* (C) and *M. luschani* (D). Scale bars are 250 µm (A) and 120 µm (C – D).

pores (as previously reported by Brizzi et al., 1996a). More similar appear the spermathecal features occurring in the females of both *Mertensiella* species, possibly in relation to their general role in sperm collection. After all, these data, particularly those related to tail tubercle and male cloacal glands, indicate that some distinct character states may have functional importance also in species with a tight phylogenetic relationship, as *M. caucasica* and *M. luschani*.

REFERENCES

- Arnold S. J. (1987), "The comparative ethology of courtship in salamandrid salamanders, I. *Salamandra* and *Chioglossa*," *Ethology*, **74**, 133 – 145.
- Basoglu M. and Atatur M. (1975), A new population of the Lycian salamander, *Mertensiella luschani* (Steindachner) in the Antalya region of southwestern Anatolia," *Ege Univ. Fen. Fak. Mec. Ser.*, **B40**, 89 – 93.
- Basoglu M. and Baran T. (1976), The subspecific status of the population of *Mertensiella luschani* (Steindachner) in the Antalya region of southwestern Anatolia," *Ege Univ. Fen. Fak. Ilmi Raporlar Ser.*, **253**, 3 – 13.
- Brizzi R., Calloni C., Delfino G., and Lotti S. (1996a), "The male cloaca of *Salamandra lanzai* with notes on the dorsal glands in the genera *Salamandra* and *Mertensiella* in relation to their courtship habits," *Herpetologica*, **52**, 505 – 515.
- Brizzi R., Delfino G., and Jantra S. (1996b), "Comparative anatomy and evolution of the male dorsal glands in the Salamandridae (Amphibia, Urodela)," *Acta Biol. Benrodis*, **8**, 61 – 77.
- Griffiths R. A. (1996), *Newts and Salamanders of Europe*, T. & A. D. Poyser Natural History, Cambridge.
- Özeti N. (1979), "Reproductive biology of the salamander *Mertensiella luschani antalyana*," *Herpetologica*, **35**, 193 – 197.
- Schultschik G. (1994a), "Zur Fortpflanzungsbiologie von *Mertensiella caucasica* (Waga, 1876) (Urodela: Salamandridae)," *Abh. Ber. Naturk. Magdeburg*, **17**, 163 – 175.
- Schultschik G. (1994b), "*Mertensiella luschani*: Haltung, Nachzucht und Freilandbeobachtungen," *Salamandra*, **30**, 161 – 173.
- Sever D. M. (1992), "Comparative anatomy and phylogeny of the cloacae of salamanders (Amphibia: Caudata). IV. Salamandridae," *Anat. Rec.*, **232**, 229 – 244.
- Sever D. M., Sparreboom M., and Schultschik G. (1997), "The dorsal tail tubercle of *Mertensiella caucasica* and *M. luschani* (Amphibia: Salamandridae)," *J. Morphol.*, **232**, 93 – 105.

AMPA RECEPTORS LOCALIZATION BY IMMUNOHISTOCHEMISTRY IN *Xenopus* TADPOLES

J. Estabel¹ and J.-M. Exbrayat¹

Keywords: *Xenopus laevis* tadpoles, glutamate receptors.

INTRODUCTION

Glutamate (Glu) is an amino acid that occurs mainly in the central nervous system. It acts as a major excitatory neurotransmitter by stimulating or exciting the postsynaptic neurons (Gasic and Hollmann, 1992). At high concentrations, Glu acts as a neurotoxin capable to induce some severe neuronal damages.

Glutamate receptors (GluRs) can be characterized by their sensitivity to specific glutamate analogues (Hollmann and Heinemann, 1994). The structure of GluR agonists and antagonists are similar to that of glutamate, and these molecules bind onto the same receptors. Two class of receptors have been characterized: the ionotropic receptors as the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and the metabotropic receptors coupled to G proteins.

In several studies, the glutamate receptors (GluRs) are studied in nervous system (Chen et al., 2002; Chung and Han, 2003; Lerma, 2003; Llado et al., 1999). Nevertheless they have been also localized in some peripheral tissues. The GluRs in non-neural tissues may be implied in mediating endocrine, cardiorespiratory or hormonal and reproductive functions (Gill and Pulido, 2001). The knowledge of this distribution in *Xenopus laevis* tadpoles is useful for *in vivo* toxicological studies.

MATERIAL AND METHODS

All the animals were handled according to the French legislation concerning the animal care. Adult *Xenopus laevis* toads were obtained from the CNRS breeding facility (UPRESA 6026, Rennes, France). The stages of tadpoles, grown from fertilised eggs using standard methods, were determined according to Niewkoop and Faber normal table (1967). The animals were anaesthetised with MS-222 (Sigma, St Louis, MO), then fixed with cold para-

formaldehyde (4%) and embedded in paraffin. The animals studied were observed at stage 58 and 60, i.e., during the metamorphosis.

Immunohistochemistry: The sections (5 μ m) were incubated with an anti-GluR_{2/3} and an anti-GluR₁ (Chemicon) in order to detect the AMPA receptors. In order to visualize the specific antibody, a secondary antibody was coupled to Alexa 488 (Molecular Probes), giving a green colour with a fluorescent light.

RESULTS

GluR_{2/3} and GluR₁ labelling was cytoplasmic. The localization of the two proteins was similar.

In the spinal cord, the motoneurons were strongly labelled (Figs. 1 and 2). This labelling permitted to validate protocols and was used as positive control. The radial cells (cells lining the cerebral ventricles in amphibians) were stained by use of the anti-GluR_{2/3} antibody only.

In the stomach and the intestine, the apical zone of the epithelial cells was labelled (Figs. 3 and 4). Neither the

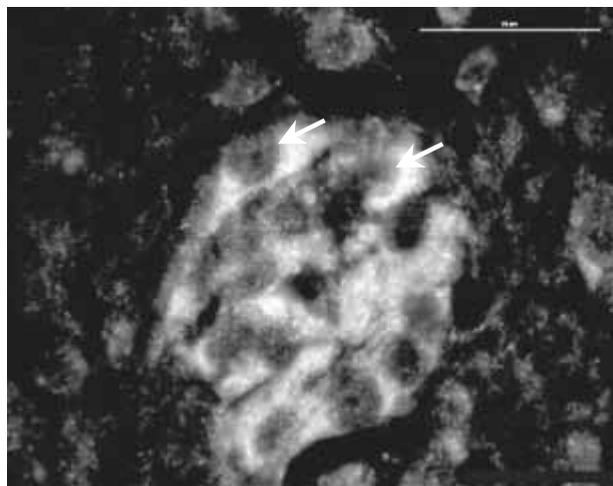


Fig. 1. Labelling of GluR_{2/3} in motoneurons. Scale bar is 50 μ m.

¹ Laboratoire de Biologie générale, Université Catholique de Lyon and Laboratoire de Reproduction et Développement des Vertébrés, Ecole Pratique des Hautes Etudes, 25 rue du Plat, 69288 Lyon Cedex 02 France; E-mail: jestabel@univ-catholyon.fr.

microvilli border of the absorptive cells nor the goblet cells present any labelling.

In the kidney, the cells of proximal tubules were labelled (Fig. 5). The cardiac and skeletal muscular cells were labelled with the two antibodies.

The liver, gills and bone were labelled neither with anti-GluR₁ nor anti-GluR_{2/3}.

CONCLUSIONS

In *Xenopus* tadpoles, the glutamate receptors (GluRs) were mainly studied in nervous system but they are also located in the peripheral tissues. This study showed a large distribution of AMPA GluRs (compound by subunits

GluR₁ to GluR₄) and the same localization for the two proteins. The most stained organs were the digestive tract, the skeletal and cardiac muscles and the kidney. Liver and gills were two negative organs.

In these locations, AMPA receptors may play a physiological role as target-effector sites for excitatory compounds. The knowledge of this distribution in *Xenopus laevis* tadpoles could be useful for *in vivo* toxicological studies.

In *Xenopus*, the localization of AMPA receptors resembles that observed in mammals (Gill and Pulido, 2001; Gill et al., 1998, 2000). These results confirm that these receptors have been conserved throughout the evolution (Estabel et al., 1999; König et al., 2001).

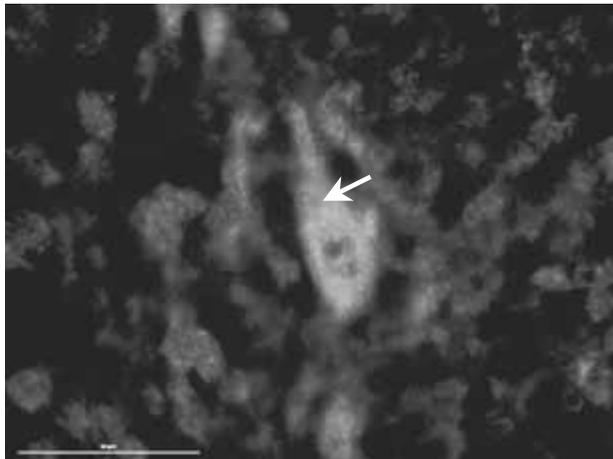


Fig. 2. Labelling of GluR_{2/3} in motoneurons. Scale bar is 50 μ m.

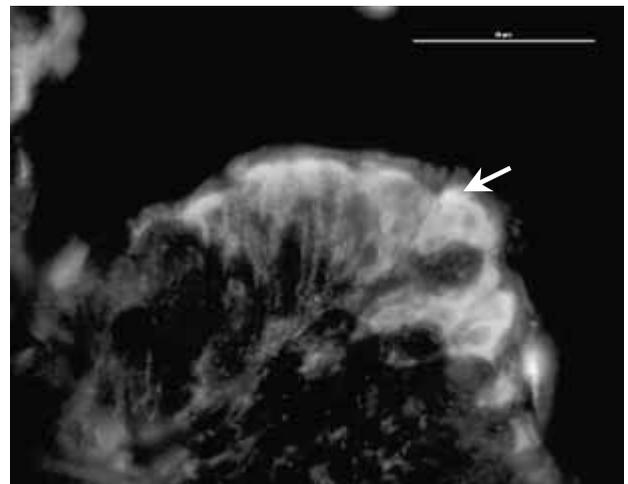


Fig. 4. Labelling of GluR_{2/3} in stomach epithelium. Scale bar is 50 μ m.

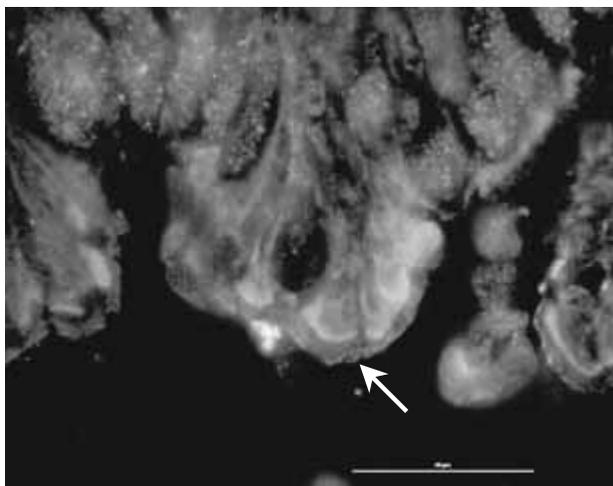


Fig. 3. Labelling of GluR₁ in stomach epithelium. Scale bar is 50 μ m.

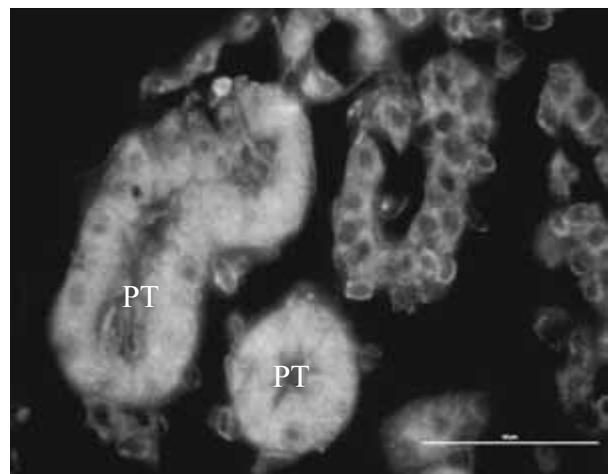


Fig. 5. Labelling of GluR₁ in proximal tubule (PT) epithelium. Scale bar is 50 μ m.

REFERENCES

- Chen Z., Ljunggren H. G., Bogdanovic N., Nennesmo I., Winblad B., and Zhu J.** (2002), "Excitotoxic neurodegeneration induced by intranasal administration of kainic acid in C57BL/6 mice," *Brain Res.*, **931**, 135 – 145.
- Chung S. Y. and Han S. H.** (2003), "Melatonin attenuates kainic acid-induced hippocampal neurodegeneration and oxidative stress through microglial inhibition," *J. Pineal. Res.*, **34**, 95 – 102.
- Estabel J., König N., and Exbrayat J.-M.** (1999), "AMPA/kainate receptors permeable to divalent cations in amphibian central nervous system," *Life Sci.*, **64**, 607 – 16.
- Gasic G. P. and Hollmann M.** (1992), "Molecular neurobiology of glutamate receptor's," *Ann. Rev. Physiol.*, **54**, 507 – 536.
- Gill S. S., Pulido O. M., Mueller R. W., and McGuire P. F.** (1998), "Molecular and immunochemical characterization of the glutamate receptors in the rat heart," *Brain Res. Bull.*, **46**, 429 – 434.
- Gill S. S., Mueller R. W., McGuire P. F., and Pulido O. M.** (2000), "Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity," *Toxicol. Pathol.*, **28**, 227 – 284.
- Gill S. S. and Pulido O. M.** (2001), "Glutamate receptors in peripheral tissues: current knowledge, future research, and implications for toxicology," *Toxicol. Pathol.*, **29**(2), 208 – 223.
- Hollmann M. and Heinemann S.** (1994), "Cloned glutamate receptors," *Ann. Rev. Neurosci.*, **17**, 31 – 108.
- König N., Poluch S., Estabel J., Durand M., Drian M. J., and Exbrayat J.-M.** (2001), "Synaptic and non-synaptic AMPA receptors permeable to calcium," *Jap. J. Pharmacol.*, **86**, 1 – 17.
- Lerma J.** (2003), "Roles and rules of kainate receptors in synaptic transmission," *Nature Rev.*, **4**, 481 – 495.
- Llado J., Caldero J., Ribera J., Tarabal O., Oppenheim R. W., and Esquerda J. E.** (1999), "Opposing effects of excitatory amino acids in chick embryo spinal cord motoneurons. Excitotoxic degeneration or prevention of programmed cell death," *J. Neurosci.*, **19**, 10803 – 10812.
- Nieuwkoop P. D. and Faber J.** (1967), *Normal Table of Xenopus laevis (Daudin)*, North Holland, Amsterdam.

THE DEVELOPMENT, DIFFERENTIATION AND GROWTH OF GONADS IN *Typhlonectes compressicauda* (AMPHIBIA, GYMNOPHIONA)

J.-M. Exbrayat¹ and E. Anjubault¹

Keywords: *Dermophis mexicanus*, *Typhlonectes compressicauda*, Gymnophiona, development, gonads.

INTRODUCTION

Gymnophiona are limbless burrowing tropical animals. Growth of gonads has been studied in *Dermophis mexicanus* (Wake, 1980) and *Typhlonectes compressicauda* (Exbrayat, 1986a, 1986b). Development of gonads has been the subject of a few works (Spengel, 1876; Brauer, 1902; Tonutti, 1931; Seshachar, 1936).

MATERIAL AND METHODS

Specimens of *Typhlonectes compressicauda*, an aquatic viviparous species, were captured in French Guyana. The sexual cycles are linked to the seasons. In the adult male, each testis is constituted with 12 to 20 lobes disposed on a common duct. In females, the ovaries are paired elongated structures throughout which several germinal nests are disposed on a segmented manner.

RESULTS AND DISCUSSION

Development of *Typhlonectes compressicauda*

The development of *Typhlonectes compressicauda* can be described in four main stages. Stage I groups the first stages of development. Stage II is divided into twelve subdivisions in which the embryos possess a more or less abundant yolk mass, and they are surrounded by a mucous envelope. Intra-uterine hatching occurs at stage 25 or 26. Stage III is divided into 6 divisions (26 to 31); the yolk mass is exhausted, the embryos are free in the uterine lumen, where they can move and grasp the wall. Metamorphosis occurs approximately from stage 30 to 32. Stage IV is divided into 3 subdivisions (32 to 34); each animal is an intra-uterine larva resembling a small adult enveloped within two large vesiculous gills (Sammouri et al., 1990).

Development of Gonads

Between the stages 23 and 25, primordial germ cells (PGC), oval shaped and larger than the other endodermal cells, are observed on the dorsal part of the endoderm of the posterior intestine. They group into an endodermic dorsal crest and enlarge and become rounded. Some of them are in mitosis. Then, PGC use a temporary median groove originating from the dorsal mesentery to reach a median genital ridge located under the aorta. At stages 26 and 27, PGC leaves the median genital ridge to join the median region of two lateral genital glands situated on the sides of the aorta, where they become gonidia. At stage 29, the genital glands are paired segmented structures parallel to the kidneys (Fig. 1). Each genital gland presents a cortex and a medulla, with a poorly defined limit. The pea-shaped genital glands enlarge and divide into two parts: an internal side giving the gonad and an external side giving the corresponding fat body. At stage 30, gonidia migrate in a centripetal direction but they are not particularly situated in the cortex or the medulla, i.e., there is no visible differentiation in an ovary or a testis. Some voluminous cells are situated on the periphery of each gonad. Fat bodies enlarge (Fig. 2). At stages 31 and 32, the center of the gonad contains some ovoid cells with a granular nucleus. The cells situated on the periphery of the medulla are larger. Several small germ and follicle cells are found in the cortical region. Follicle cells begin to surround some small gonidia (Figs. 3 and 4). At stage 33, secondary gonidia, originating from the division of primary ones, are observed in some animals. It is still not possible to recognize the sex according to the position of germ cells in the gonad. At stage 34, at birth, the gonads are morphologically differentiated into testis or ovary that can be recognized by histology only. On 28 new-born, we could find 16 males and 12 females.

Growth of Gonads

In new-born, each lobe of a testis is constituted with several lobules. Each lobule contains an islet of primary spermatogonia surrounded with several follicle cells, are grouped together at the end of a very small duct. The cytoplasm of follicle cells can be developed, with a fibrous as-

¹ Laboratoire de Biologie Générale, Université catholique de Lyon and Laboratoire de Reproduction et Développement des Vertébrés, 25 rue du Plat, F-69288 Lyon Cedex 02, France;
E-mail: jmexbrayat@univ-catholyon.fr.

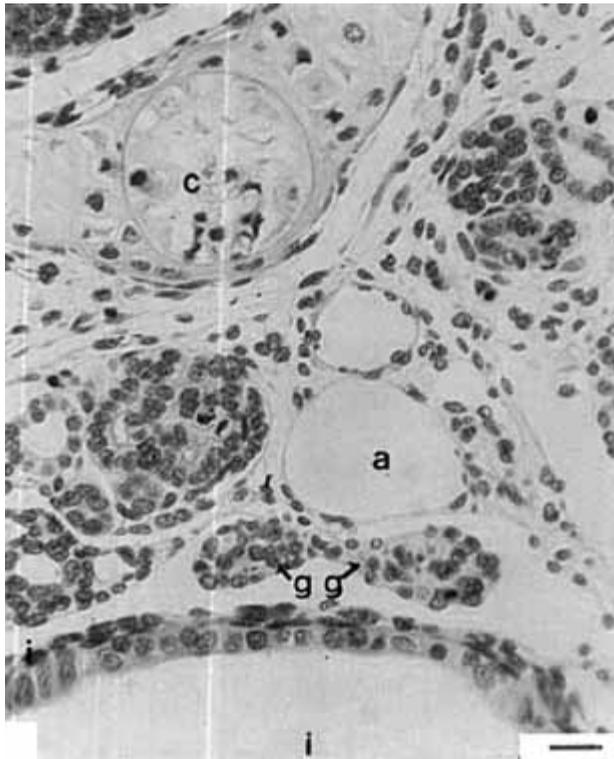


Fig. 1. Stage 29. Lateral genital glands: a, aorta; c, chord; gg, genital gland. Scale bar is 12.5 μ m.

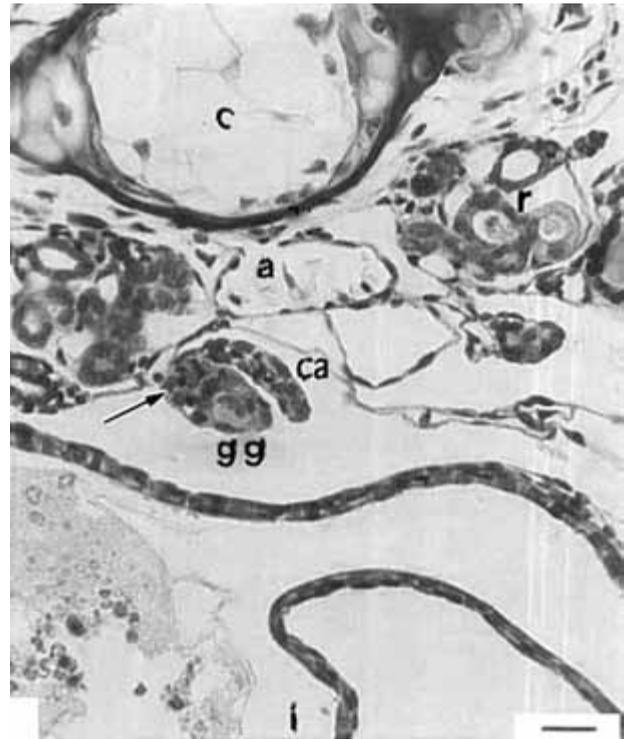


Fig. 2. Stage 30. Gonads and fat bodies: a, aorta; c, chord; ca, fat body; gg, genital gland; r, kidney. Scale bar is 25 μ m.

pect with light microscopy, resembling to the Sertoli cells of the adult. The nucleus of these cells is applied against the wall of lobule. In one year old animal, each follicle cell elongates. The nucleus moves away the spermatogonia, and the basal pole contacts the germ cell. The cytoplasm presents some filamentous structures with fat globules. Primary and secondary spermatogonia are observed. During the second year, the biometric data indicate some variations looking like that of the adult testis. In January and February, some primary and secondary spermatocytes and spermatids are observed. In May – June (18 months old animals), the spermatids and spermatozoa are observed, but they degenerate and are eliminated. A proliferation of spermatogonia and spermatocytes is also observed at this period. In August, the testis resembles that of the adult. In three years old animals, a normal sexual cycle with evacuation of spermatozoa is observed: the sexual maturation is reached.

In new-born female, the ovaries resemble a pair of small strikes containing several oogonia and oocytes with or without any follicle cells. When the ovaries develop, the oocytes (150 to 300 μ m in diameter) are surrounded with follicle cells; In the most developed ovaries, the oogonia

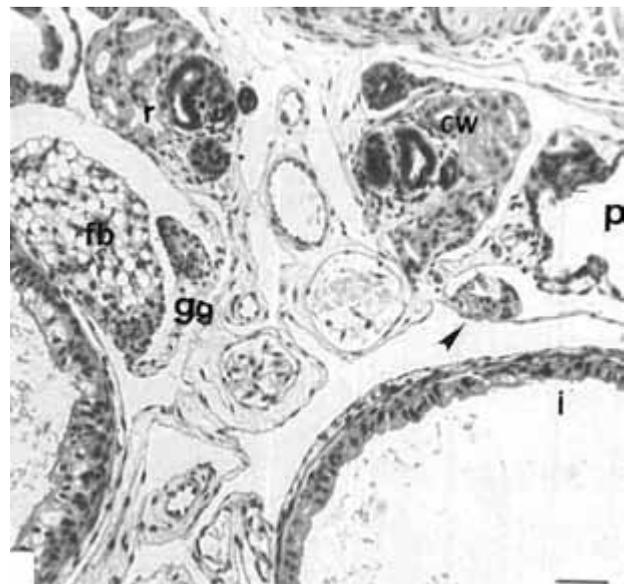


Fig. 3. Stage 32. Gonads: cw, Wolffian duct; fb, fat body; gg, genital gland; p, lung. Scale bar is 25 μ m.

are grouped within germinal nests, between them several follicles are observed. In 20 months old females, some larger follicles (600 to 750 μ m) with a tiny vitellin mem-

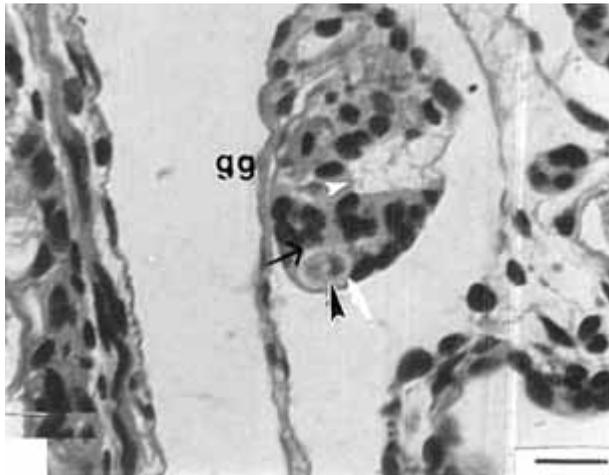


Fig. 4. Stage 32. Details of gonads: gg, genital gland; black arrow, medulla; white arrow, cortex. Scale bar is 50 μ m.

brane are observed. In 25 months old animals (2 years, August), the first vitellogenic oocytes and atretic follicles are observed. The animals become adult in January (February of the third year 24 to 30 months old animals). The smallest pregnant female are 24 to 30 months old according to their size. These results show that the sexual maturation of females is also reached in 2 to 3 years old animals.

CONCLUSIONS

To summarize the development of gonads in *Typhlonectes compressicauda*, we can say that PGC migrate from an endodermic dorsal ridge to an impaired gonadic crest then a paired primordial genital gland. The origin of gem cells is endodermic, like in Anura (Bounoure et al., 1954; Smith, 1965, 1966; Blackler, 1970; Gipouloux, 1970a, 1970b; Lamotte and Xavier, 1972). In contrary, in Urodela, the PGC are of mesodermal origin (Humphrey, 1928; Nieuwkoop, 1947; Blackler, 1958; Houillon, 1967; Capuron, 1972; Satarurja and Nieuwkoop, 1974; Wakahara, 1996). In the Anura and Urodela (except for the neotenic species), the sexual differentiation is observed just before or after the metamorphosis (Lofts, 1974). In *Typhlonectes compressicauda*, the morphological differentiation is delayed to the birth, e.g., two months after the metamorphosis. The sexual maturation of *Dermophis mexicanus*, another Gymnophiona, has also been observed in two to three year old animals (Wake 1980).

During the first year after the birth, the physiology of the animal is mainly oriented towards the growth. The testes and ovaries enlarge, the germ cells begin to organize

themselves in a functional structure. During the second year of the life, several variations are reminiscent of the sexual cycle of adult in testes as in ovaries. For both sexes, the maturation occurs during the third year.

REFERENCES

- Blackler A. W.** (1958), "Contribution to the study of germ cells in the Anura," *J. Embryol. Exp. Morphol.*, **6**, 491 – 503.
- Blackler A. W.** (1970), "The integrity of the reproductive cell line in the amphibia," *Curr. Topics Dev. Biol.*, **5**, 71 – 88.
- Bounoure L., Aubry R., and Huck M. L.** (1954), "Nouvelles recherches expérimentales sur les origines de la lignée reproductrice chez la grenouille rousse," *J. Embryol. Exp. Morphol.*, **2**, 245 – 263.
- Brauer A.** (1902), "Beitrag zur kenntniss der Entwicklung und Anatomie der Gymnophionen. III. Die Entwicklung der Excretionsorgane," *Zool. Jahrb. Anat.*, **16**, 1 – 176.
- Capuron A.** (1972), "Mise en évidence de gonocytes dans divers territoires isolés au stade du bourgeon caudal et cultivés *in vitro* chez *Pleurodeles waltlii* Michah (Amphibien Urodèle)," *C.R. Acad. Sci. Paris*, **274**, 277 – 279.
- Exbrayat J.-M.** (1986a), *Quelques aspects de la biologie de la reproduction chez Typhlonectes compressicaudus (Duméril et Bibron, 1841). Amphibien Apode. Doct. Sci. Nat.*, Univ. Paris VI.
- Exbrayat J.-M.** (1986b), "Le testicule de *Typhlonectes compressicaudus*; structure, ultrastructure, croissance et cycle de reproduction," *Mém. Soc. Zool. Fr.*, **43**, 121 – 132.
- Gipouloux J. D.** (1970a), "Recherches expérimentales sur l'origine, la migration des cellules germinales et l'édification de crêtes génitales chez les Amphibiens Anoures," *Bull. Biol. Fr. Belg.*, **104**, 21 – 93.
- Gipouloux J. D.** (1970b), "Précisions sur le stade auquel débute la migration des cellules germinales chez le Crapaud commun (*Bufo bufo*)," *C.R. Acad. Sci. Paris*, **270**, 533 – 535.
- Houillon C.** (1967), *Embryologie. Coll. Méthodes*, Hermann, Paris.
- Humphrey R. R.** (1928), "Sex differentiation in gonads developed on transplants of intermediate mesoderm of *Ambystoma*," *Biol. Bull. Mar. Bull. Woods Hoole*, **55**, 367 – 399.
- Lamotte M. and Xavier F.** (1972), "Recherches sur le développement embryonnaire de *Nectophrynoides occidentalis* Angel, Amphibien Anoure vivipare. I. Les principaux traits morphologiques et biométriques du développement," *Ann. Embryol. Morphol.*, **5**, 315 – 340.
- Lofts B.** (1974), "Reproduction," in: Lofts B. (ed.), *Physiology of the Amphibia. Vol. II*, Acad. Press, London – New York, pp. 53 – 106.
- Nieuwkoop P. D.** (1947), "Experimental investigations on the origin and determination of the germ cells and on the development of the lateral plates and germ ridges in Urodeles," *Arch. Neerl. Zool.*, **8**, 1 – 205.
- Sammouri R., Renous S., Exbrayat J.-M., and Lescure J.** (1990), "Développement embryonnaire de *Typhlonectes compressicaudus* (Amphibia: Gymnophiona)," *Ann. Sci. Nat. Zool. Paris. 13ème Sér.*, **11**, 135 – 163.

- Satasurja L. A. and Nieuwkoop D.** (1974), "The induction of the primordial germ cells in the Urodeles," *Wilhelm Roux's Arch.*, **175**, 199 – 220.
- Seshachar B. R.** (1936), "The spermatogenesis of *Ichthyophis glutinosus* (Linn.) I. The spermatogonia and their division," *Z. Zellforsch. Mikr. Anat.*, **24**, 662 – 706.
- Smith J. W.** (1965), "Transplantation of the nuclei of primordial germ cells into enucleated eggs of *Rana pipiens*," *Proc. Natl. Acad. Sci. USA*, **54**, 101 – 107.
- Smith J. W.** (1966), "The role of a 'germinal plasma,' in the formation of primordial germ cells in *Rana pipiens*," *Dev. Biol.*, **14**, 330 – 347.
- Spengel J. W.** (1876), "Das Urogenitalsystem der Amphibien. I Theil. Der Anatomische Bau des Urogenitalsystem," *Arb. Zool. Inst. Wurzburg*, **3**, 51 – 114.
- Tonutti E.** (1931), "Beitrag zur Kenntnis der Gymnophionen. XV. Das Genital-system," *Morphol. Jahrb.*, **68**, 151 – 292.
- Wakahara M.** (1996), "Heterochrony a neotenic salamanders: possible clues for understanding the annual development and evolution," *Zool. Sci.*, **13**(6), 765 – 766.
- Wake M. H.** (1980), "Reproduction, growth and population structure of the central American Caecilian *Dermophis mexicanus*," *Herpetologica*, **36**, 244 – 256.

DIVERSITY IN THE TIMING OF DIGIT OSSIFICATION WITHIN THE LIMB DEVELOPMENTAL BAUPLAN IN CAUDATA

J. R. Hinchliffe,¹ E. I. Vorobyeva,² and D. N. Mednikov²

At the early prechondrogenic stage, within an overall common developmental bauplan, the larval urodele limb provides examples of variation in pattern some of which may be classified as caenogenetic or heterochronic, in some cases clearly adaptive. Comparison of ossification pattern in larval limb development has been relatively neglected. The sequence of ossification along the fore and hindlimb digits was examined in *Salamandrella keyserlingii*, *Onychodactylus fisheri*, *Triturus vulgaris*, and *Triturus vittatus*. In *Onychodactylus* and *Triturus vittatus* in almost all digits the ossification sequence is proximo-distal as in the chondrogenic sequence. In *Salamandrella* the normal sequence is also proximo-distal, but in the digits of a number of variants the sequence is distal-proximal. In *Triturus vulgaris*, the sequence is distal-proximal. These differences appear unrelated to phyletic relations or to ecological niche, whether this is rheophilic (*Onychodactylus*) or limnophilic. The variety of normal sequences together with the frequency of individual variation suggests that the ossification sequence may not be tightly controlled. But the varied patterns of chondrogenesis and digit ossification illustrate the dynamic and varied character of the developmental bauplan of the caudate limb.

Keywords: *Salamandrella keyserlingii*, *Onychodactylus fisheri*, *Triturus vulgaris*, *Triturus vittatus*, larval limb development.

INTRODUCTION

For many years there has been active discussion of a possible developmental basis of tetrapod limb homology, that overall similarity of structure which underlies all tetrapod limbs regardless of their specialization. This has been sought particularly in terms of early prechondrogenic patterning of the limb skeleton, considered to reflect shared developmental processes. More recently homology has also been considered to be based in shared patterns of regulatory gene (especially Hox genes) expression (review, Hinchliffe, 2002).

Caudates (urodeles) have provided controversial evidence for this discussion. In particular their antero-posterior sequence of digit development, strikingly different from that of other tetrapod taxa, has been taken as an indication of a fundamental difference in their skeletal bauplan, important enough to justify a theory of their independent origin as tetrapods. However, we have argued in a number of articles that there is in fact a single tetrapod bauplan and that caudates have some unique features (Hinchliffe, 2002; Hinchliffe and Vorobyeva, 1999). Their differences with for example the anurans are minor if com-

parison is made using basal caudates such as the hynobiids. Using immunofluorescence as a means of revealing more clearly the prechondrogenic pattern, basal caudates demonstrate similarities with anurans (together with other tetrapods) in proximo-distal skeletal development at the carpus/tarsus level and in the presence of a digital arch. Their principal difference is in the anterior to posterior sequence of digit development though even here in direct developing caudates there is evidence of a sequence more like that of other tetrapods (Shubin and Wake, 1995).

Viewed as a whole, caudates present evidence of heterochrony and variation within the overall tetrapod skeletal developmental bauplan (Wake and Hanken, 1996). Some of these features are related to their larval limb adaptations: unlike all other tetrapods, caudates use the limb for locomotion during its development. This may account for the general feature of anterior-posterior digit formation, a possible adaptation to the early use of the limb in contacting the substrate in many caudate species. Possibly the general tetrapod “make posterior digits first” rule has been over-ridden in caudates as an adaptive heterochronic modification (Vorobyeva, 1999).

Within caudates, hynobiids show clearly a range of heterochronic and caenogenetic features of their limb development. The limnophilic *Salamandrella keyserlingii* has a larval limb with a temporary web between digits 1

¹ University of Wales, Institute of Biological Sciences, Aberystwyth, SY23 1EY, UK; E-mail: jkh@aber.ac.uk.

² Institute of Ecology and Evolution RAN, 33 Leninsky pr., 117071 Moscow, Russia; E-mail: sevin@orc.ru.

and 2 and which is used for first “planing” (the floating phase of this pelagic larva) and then movement over the substrate. Two rheophilic species have been analyzed. Here a priority is maintaining a position in running water. *Ranodon sibiricus* has early claw development and a small interdigital web. *Onychodactylus fisheri* also has early claw development and a long skin fold along external side of the limb (Vorobyeva and Hinchliffe, 1998). Both have a specialized organ linking the ventral tendon to the digit tip, probably increasing the leverage and therefore the grip which the tendon can exert.

In caudates at the intraspecific level there is a remarkable degree of individual variation during the skeletal patterning of the limb. A study of the variants in the *Salamandrella* larval limb skeleton (Borkhvardt, 1994) and in *Ranodon* (Vorobyeva et al., 1997) demonstrate a great variety of loss or fusions of elements or alternatively of the appearance of new or atavistic elements. In *Salamandrella* only about 30% of individuals have the “standard” pattern. Alberch and Gale (1985) have reported in a variety of caudate species that the different patterns follow certain predictable rules and are a result in some cases of heterochrony. Alberch links this to experimental work on Axolotl limbs where the variants in the cartilaginous skeleton were a result of differences in limb bud size and availability of limb bud mesenchyme for skeletal formation, for example for the last forming digits. He relates this to similar patterns found as the standard morphology in particular species and believes that similar heterochronic events are responsible.

In conclusion we emphasize that within an overall common bauplan, caudates demonstrate the importance of heterochrony, caenogenesis and variation at the early chondrogenic phase of limb development in generating the variety of definitive morphologies which are displayed at both at the intra- and inter-specific level. We focus next on the more neglected subject of the diversity in caudate digits of the sequence of ossification.

RESULTS

Despite the substantial literature which is concerned with the chondrogenic patterns of caudate larval limbs, apart from experimental studies by Keller (1946) on metamorphosis in the Axolotl, much less attention has been given to digit ossification patterns (Wake and Hanken, 1996). Generally digits chondrify in a proximo-distal direction. Do digits ossify in the same direction?

Histological study of the ossification process shows that the skeletal elements must first reach a mature stage of

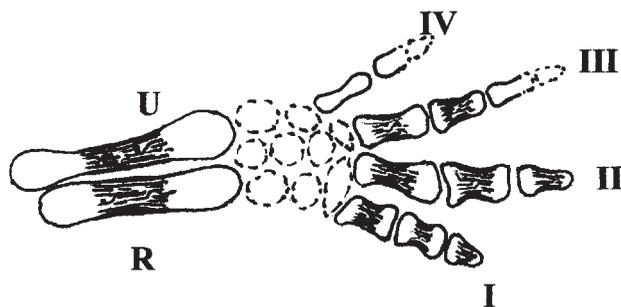


Fig. 1. Schematic drawing of the forelimb skeleton in *Salamandrella keyserlingii* stage 41 larva showing ossified areas (black). R, radius; U, ulna; I – IV, digit no.

chondrogenesis with the hypertrophy of the central chondrocytes of the cartilage element or “model.” First a cylinder of bone forms round this model, then the central hypertrophic cartilage is eroded and replaced by bone and finally the cartilage is restricted to the two ends of the model. This replacement process may be very slow in caudates, sometimes never taking place in the carpus and tarsus elements. However the timing of ossification is subject to the constraint that the cartilage model must be formed first (ossification cannot take place independently from chondrogenesis) and it must be undergoing central hypertrophy. One might therefore expect that the sequence of ossification of the digit elements reflects the initial sequence of formation of the cartilage models. However, this is not the case. It may be that the process of digit cartilage hypertrophy does not reflect this initial sequence: the timing of the two processes may be dissociated.

We report here the ossification sequences in the digits of two hynobiid species, *Salamandrella keyserlingii* and *Onychodactylus fisheri* and also in two salamandrid species: *Triturus vulgaris* and *Triturus vittatus*. Using the Alizarin Red method to stain bone, our findings show that the ossification patterns are varied and range from species where the sequence is proximal to distal in all the digits, through those where it is different in the different digits, to those where it is distal to proximal. However, all the species show an anterior to posterior gradient (digit 2 ossifies first followed by the sequence 3, 4, 5) which is the same as the digit chondrification sequence along that axis.

The proximal to distal sequence appears to be the norm for tetrapods other than caudates (e.g., anurans, mammals [mouse], and birds [chick]) and it is found in the axolotl (Keller, 1946) and *Onychodactylus fisheri* (Vorobyeva and Hinchliffe, 1998), though in both species the evidence is not complete. The complete analysis made of

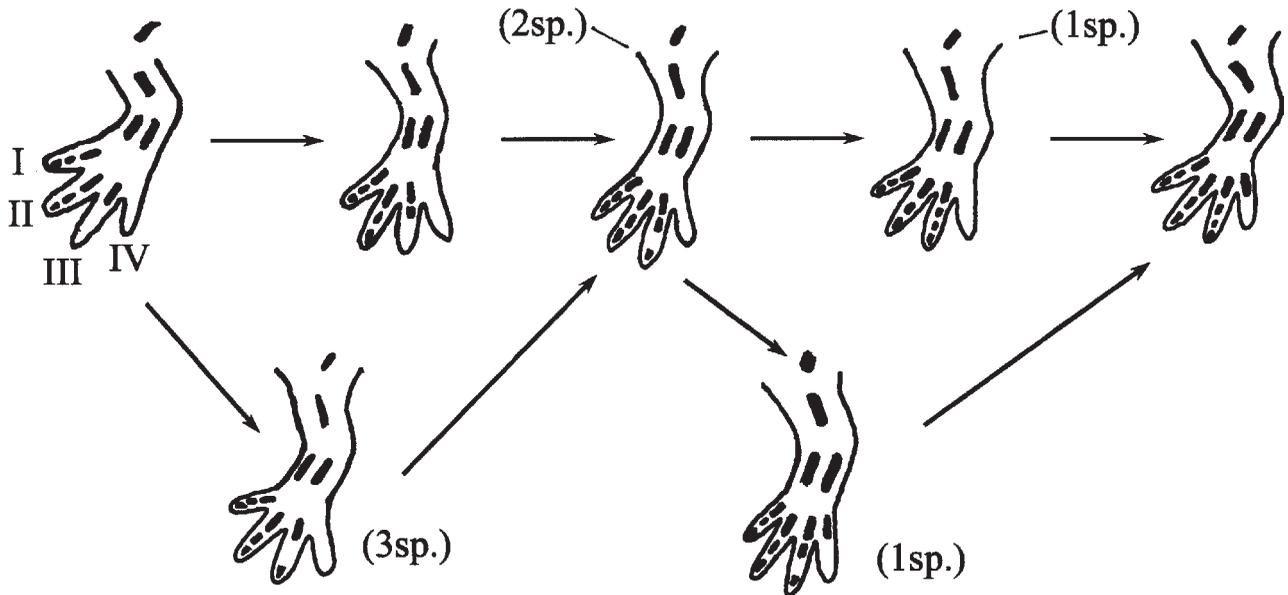


Fig. 2. Schematic drawing of ossification sequence in forelimbs of *Salamandrella* larvae of stages 41 – 43. I – IV, digit no. Specimens showing variation are shown (e.g., 2sp. = 2 specimens) in addition to normal individuals.

the hind limb of *Triturus vittatus* shows a proximal to distal sequence in all digits.

Examples of limbs which show a different ossification sequence in different digits are provided by *Triturus vittatus* and *Salamandrella keyserlingii*. Both species are limnophilic (Kuzmin, 1999) but belong to either to basal (Hynobiidae) or to advanced (Salamandridae) families. In the forelimb of *T. vittatus* digits 2 and 4 the sequence is proximo-distal, while in digits 1 and 3 it is distal-proximal. In *S. keyserlingii* both the fore and hind limb digits are generally proximo-distal (Figs. 1 and 2), but distal-proximal in digit 1 in both fore and hind limbs and also in variants of fore limb digits 3 and 4 (Figs. 1 – 4).

In *Triturus vulgaris* (which is also limnophilic and phylogenetically close to *T. vittatus*) in both fore and hind limbs all digits demonstrate a distal to proximal sequence (Figs. 3 and 4; see Vorobyeva and Mednikov, 2002).

This species also shows some other diversification of ossification. The relative timing of ossification of digits relative to the zeugopod is unusual. The ossification process in the hindlimb starts from the femur (as is typical for caudates as a whole) but at stage 50 (a minimum 14 days after hatching) when hindlimb is still three fingered (all the digit elements of the forelimb have now ossified, except for carpal elements and digit IV) the next element to ossify is metatarsal of the digit II, and only subsequently the tibia. Shortly after this, the ossification of terminal phalanx of digit II and fibula begins (stage 51). Usually

zeugopod elements in caudates (as in other tetrapods) ossify earlier than the digit elements.

The degree and tempo of digit ossification varies among caudates at inter- and intraspecies levels. Among the species studied, *Ranodon* and *Onychodactylus* (rheophilic hynobiids) show wholly ossified phalanges at metamorphosis. In *Salamandrella* the tempo of phalanx ossification is slower and has individual variation. But in general in all caudates, the degree of ossification increases during ontogeny including postmetamorphic and adult animals.

DISCUSSION

Generalizing for caudates as a whole about the chondrification and ossification of their limb skeleton, the initial sequence for both processes is proximal to distal for the stylopod (e.g., humerus, femur) and zeugopod (e.g., radius and ulna, etc.) parts of the limb skeleton (with some exceptions as in *T. vulgaris*). Chondrification within the individual digits in all cases is proximal to distal, though there is an overall anterior to posterior sequence of digit formation. The first digit element to ossify is the metacarpal or metatarsal of digit 2 and the sequence for the digits is always anterior to posterior. In the digits it is the phalanges which show variation (both inter- and intra-species) in the sequence of ossification which is sometimes proximo-distal and sometimes distal-proximal for the limb while in

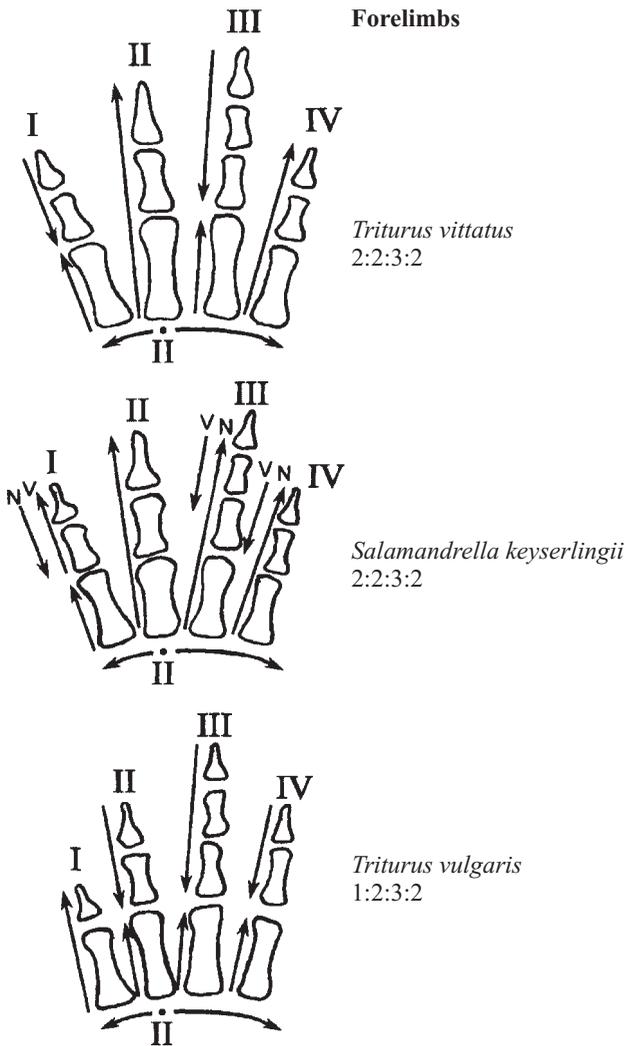


Fig. 3. Ossification sequence of phalanges and metacarpals of digits I–IV of the forelimbs of *Triturus vittatus*, *Salamandrella keyserlingii*, and *Triturus vulgaris*. Variant pattern (V) is shown for *Salamandrella* (N, normal). Ossification begins in digit II in all three species.

some species different digits have different ossification sequences. Tarsus and carpal elements do not ossify in the larvae and in the adult ossification of these elements is slow or even absent.

The timing of the beginning of ossification is related to the time at which the larvae begin to use the limb digits to move on the substrate. This is early in *Triturus* species at about 2–5 days post hatching. This time is later in *Salamandrella* at about the second week since initially on the bottom the larvae use the interdigit 1–2 web as a temporary “finger” until its regression.

The ossification sequence of the digits whether proximo-distal or the reverse does not appear to have special

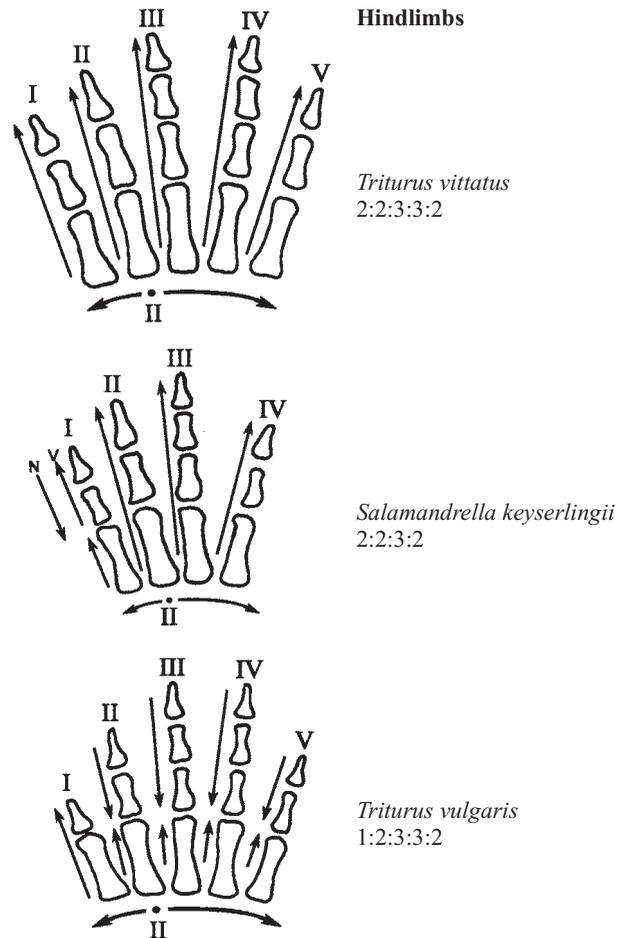


Fig. 4. Ossification sequence of phalanges and metatarsals of digits I–V of the hindlimbs of *Triturus vittatus*, *Salamandrella keyserlingii* and *Triturus vulgaris*. Variant pattern (V) is shown for *Salamandrella* (N, normal). Ossification begins in digit II in all three species. Sequence is proximo-distal in both *Triturus vittatus* and normally for *Salamandrella*.

importance as an adaptational feature in the larvae, for example as between limnophilic and rheophilic species. Once ossification of a digit begins it is completed fairly rapidly. Within the individual digit the pattern changes quickly as the next element begins ossification and this together with the frequency of deviation from the normal pattern for the species suggests that the pattern may not be too tightly controlled. Probably we should see the trajectory of the digit ossification process as directed towards the completion of definitive bone morphology by the end of metamorphosis, enabling the newts to move on the land. Ossification timing for individual digit elements may be less important from a larval adaptive viewpoint. But the varied patterns of digit ossification in caudates — like the varied patterns of chondrogenesis — illustrate the dy-

namic and varied nature of the developmental bauplan of the caudate limb.

Acknowledgments. Our work is supported by RFFR (grant No. 02-04-49292) and by scientific school (grant NSH 1808.2003.4). The Royal Society kindly awarded a travel grant to JRH to enable his participation in this conference.

REFERENCES

- Alberch P. and Gale E. A.** (1985), "A developmental analysis of an evolutionary trend: digital reduction in amphibians," *Evolution*, **39**, 8 – 23.
- Borkhvardt V. G.** (1994), "Limb development in larvae of Siberian newt, *Salamandrella keyserlingii* (Amphibia, Hynobiidae)," *Zool. Zh.*, **73**, 53 – 67 [in Russian].
- Hinchliffe J. R.** (2002), "Developmental basis of limb evolution," *Int. J. Dev. Biol.*, **46**, 835 – 845.
- Hinchliffe J. R. and Vorobyeva E. I.** (1999), "Developmental basis of limb homology in urodeles: heterochronic evidence from the primitive hynobiid family," in: Bock W. (ed.), *Homology*, John Wiley, pp. 95 – 109.
- Keller R.** (1946), *Rev. Suisse Zool.*, **53**, 389.
- Kuzmin S. L.** (1999), *The Amphibians of the Former Soviet Union*, Pensoft, Sofia – Moscow.
- Shubin N. H. and Wake D. B.** (1995), "Implications of direct development for the tetrapod limb bauplan," *Am. Zool.*, **31**, 8A.
- Vorobyeva E. I.** (1999), "The problem of polydactyly in Amphibians," *Russ. J. Herpetol.*, **6**(2), 95 – 103.
- Vorobyeva E. I., Hinchliffe J. R., and Ol'shevskaya O. P.** (1997), "Specific features of development of the paired limbs in *Ranodon sibiricus* Kessler (Hynobiidae, Caudata)," *Ontogenez*, **28**, 188 – 197 [in Russian].
- Vorobyeva E. I. and Hinchliffe J. R.** (1998), "Phylogenetic variability and larval adaptations in the developing limbs of hynobiids," *Adv. Amphibian Res. Former Soviet Union*, **3**, 1 – 9.
- Vorobyeva E. I. and Mednikov D. N.** (2002), "Some specific features in the development and ossification of the limbs skeletal elements of the *Triturus vulgaris* (Caudata)," *Dokl. RAN*, **387**, 565 – 568 [in Russian].
- Wake D. B. and Hanken J.** (1996), "Direct development in the lungless salamanders: what are the consequences for developmental biology, evolution and phylogenesis?," *Int. J. Dev. Biol.*, **40**, 859 – 869.

A COMPARATIVE STUDY OF THE FORM AND EVOLUTIONARY IMPLICATIONS OF THE INTERDIGITAL MEMBRANE OF LARVAL HYNوبيID SALAMANDERS

K. Iizuka,¹ S. K. Sessions,² S. Yasugi,³ T. Nakazato,⁴ and Y. Takeuchi⁵

A comparative study explored limb development among Japanese hynوبيid salamanders including both those with pond-type larvae (*Hynوبيus lichenatus*, *Hynوبيus nigrescens*, *Hynوبيus tokyoensis*) and those with stream-type larvae (*Hynوبيus kimurae*, *Onychodactylus japonicus*). Within the genus *Hynوبيus*, all species with the pond-type larva have early limb development characterized by the transient formation of an interdigital membrane (IM). The IM is completely absent in *O. japonicus*, and present as a vestigial IM in species of *Hynوبيus* that have stream-type larvae. Our observations, along with available information about other hynوبيid and non hynوبيid salamanders, indicate that 1) the IM is probably characteristic of cryptobranchoid salamanders, 2) the IM has an adaptive value in pond habitats but not in stream habitats, and 3) the IM has been reduced or lost two or three times independently within the Cryptobranchioidea. This pattern also suggests that within the genus *Hynوبيus*, species with stream-type larvae are derived relative to pond-type species.

Keywords: limb development, salamander larva, interdigital membrane, pond-type, stream-type, *Cryptobranchus*, *Hynوبيus*, *Onychodactylus*.

INTRODUCTION

Some hynوبيid salamanders have an interdigital membrane (IM), which is a fin-like structure that forms between digits 1 and 2 during early limb development and then disappears as limb development proceeds. The presence of an IM was first described for *Salamandrella keyserlingii* (Schmalhausen, 1910; Vorobyeva and Hinchliffe, 1996) and later for several species of *Hynوبيus* (Sawano, 1947; Iwasawa and Yamashita, 1991). Such a structure has not been found in any other salamander group, although Holmgren (1933) reports a vestigial IM in the North American cryptobranchid salamander *Cryptobranchus alleganiensis*. Holmgren (1933) believed the IM tells us something about the organization and origin of the pattern of development of the vertebrate limb, and others have suggested that the IM has adaptive value for pond-type larvae (Wake and Shubin, 1998). The IM structure is absent or vestigial in stream-type larvae. Here we present the results of a comparative study of limb development in Japanese species of hynوبيid salamanders. Our results suggest that the IM is probably a primitive feature of cryptobranchoid salamanders and that it has been reduced or lost at least twice independently in the cryptobranchoid lineage.

Within the genus *Hynوبيus* the IM has been reduced to a vestigial condition in species that have stream-type larvae, and this suggests that the IM only has adaptive value in quiet water habitats. Our results combined with previous observations suggest that stream-type larvae are derived relative to pond-type larvae, at least in the genus *Hynوبيus*.

MATERIAL AND METHODS

Egg clutches of *Hynوبيus lichenatus* and *Hynوبيus nigrescens* were collected in Shiobara-machi (Tochigi Prefecture, Japan) in the spring of 2001, and egg clutches of *Hynوبيus tokyoensis* were collected in Sano-shi (Tochigi Prefecture) in the spring of 2002. Eggs of *Hynوبيus kimurae* were collected in Hikami-gun (Hyogo Prefecture) in the winter of 2001. Adult males and females of *Onychodactylus japonicus* were obtained in Minamiaizu-gun (Fukushima Prefecture) in the early summer of 2001, and eggs of this species were obtained from the captive adults by means of an injection of gonadotropin hormone. The fertilized embryos were raised under low temperature in laboratory conditions. At selected times the larvae were fixed in buffered 10% formalin and photographed by means of a Leica microscope and digital photography. Developmental stages of the larvae of the three species of pond-type *Hynوبيus* were identified according to the defined stages for *H. lichenatus* (Sawano, 1947) and *H. nigrescens* (Iwasawa and Yamashita, 1991). The developmental stages of the larvae of the stream-type hynوبيids

¹ Yotsuya Jr. High School, Shinjuku-ku, Tokyo 1600004, Japan; Postal address of senior author: Koji Iizuka, 5-10-8 Sendagi Bunkyo-ku Tokyo, 1130022 Japan;

E-mail: iizuka1@cat.zero.ad.jp, hynوبيid.salamander@ezweb.ne.jp.

² Hartwick College, Oneonta, NY 13820, USA.

³ Tokyo Metropolitan University, Hchioghji-shi, Tokyo 1920397, Japan.

⁴ Tamabe Jr. High School, Akishima-shi, Tokyo 1960002, Japan.

⁵ Ohkubokita Jr. High School, Akashi-shi, Hyogo 6740051, Japan.

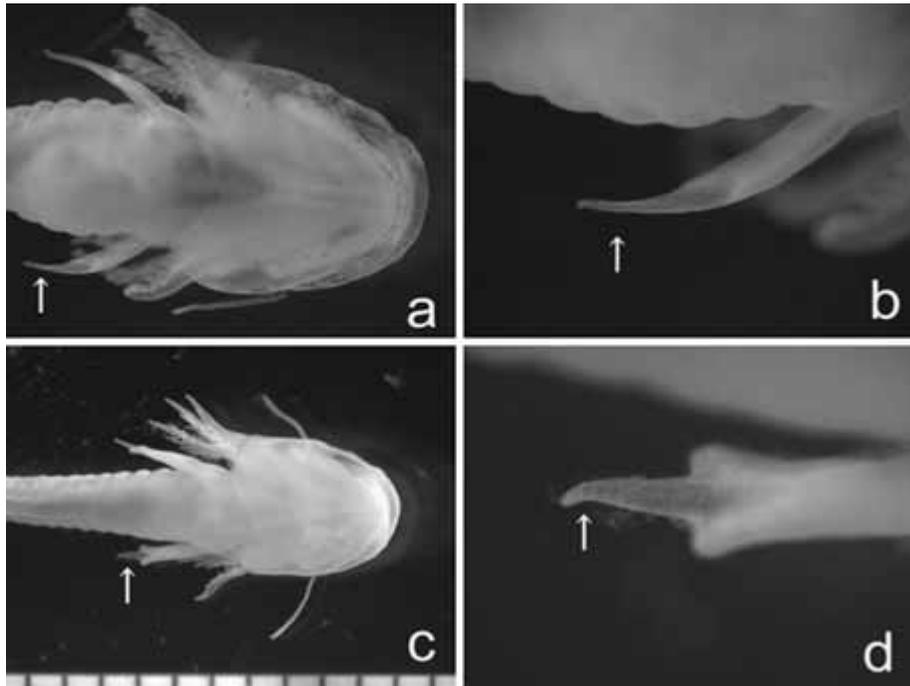


Fig. 1. Limb development in the pond-type larva of *Hynobius lichenatus*. *a, b*: Developmental stage 48. The interdigital membrane (IM) of the forelimbs is fully formed at this stage and is located between digits 1 and 2 (arrows). *c, d*: Forelimbs of *H. lichenatus* at stage 50. The IM is beginning to regress, and development of the first two digits is symmetrical around the central IM (arrows). The larvae have a pair of well-developed balancers that are visible in (*a*) and (*c*). Scale bar is 1 mm (*c*).

were identified according to the defined stages for *H. kimurae* (Akita, 2001) and of *O. japonicus* (Iwasawa and Kera, 1980).

RESULTS

Of the specimens examined in this study, the three species of *Hynobius* with pond-type larvae, *H. lichenatus*, *H. nigrescens*, and *H. tokyoensis*, all had interdigital membranes (Figs. 1 – 3 and Table 1). These pond-type larvae were also characterized by well-developed balancers (Fig. 1*a, c*). The early forelimb buds have a spear-like morphology that, by the two-digit stage, resolves itself as a symmetrical structure consisting of a pointed IM, with digits 1 and 2 developing on either side (Fig. 1*a – d*). As the forelimb develops, the IM regresses and is nearly gone by the 3-digit stage (Fig. 2*a, b*). As in most other salamanders with aquatic larvae, the development of the hind limbs is delayed relative to the forelimbs, but in these species the hind limbs also develop a distinct IM that will regress as the limb develops (Fig. 2*c, d*). The pattern of forelimb and hind limb development appears to be identi-

cal in all three species (Figs. 1 – 3) as well as in *Hynobius nebulosus* (Sessions et al., unpublished; Table 1).

We also examined the limb development of two hynobiid species that have stream-type larvae. Relative to the embryos of the pond-type *Hynobius* species, the embryos of *Hynobius kimurae* are large, yolky, sparsely pigmented, and develop more slowly (Fig. 4*a*), and there is less of a delay between the development of the forelimbs and the hind limbs. The limb buds are less pointed than those of the pond-type larvae (Figs. 4*b – d* and 5*a*) and a full IM never develops. Nevertheless, there is an apparent vestigial IM in the two-digit stage (Fig. 5*b* and Table 1) and then it is completely absorbed by the three-digit stage (Fig. 5*c, d*). The Taiwanese stream-type hynobiid, *Hynobius formosanus*, also possesses a vestigial IM (Kakegawa et al., 1989), so a vestigial IM seems to be characteristic of stream-type *Hynobius* (Table 1). Relative to pond-type *Hynobius*, *Onychodactylus japonicus* has large, yolky, slow-developing embryos with less developmental delay between the forelimb and hind limb (Fig. 6*a*). The forelimbs of *O. japonicus* are almost completely developed by the time of hatching, but the hind limbs have just begun developing digits and are comparable to the hind limbs of pond-type *Hynobius* when the IM is fully formed

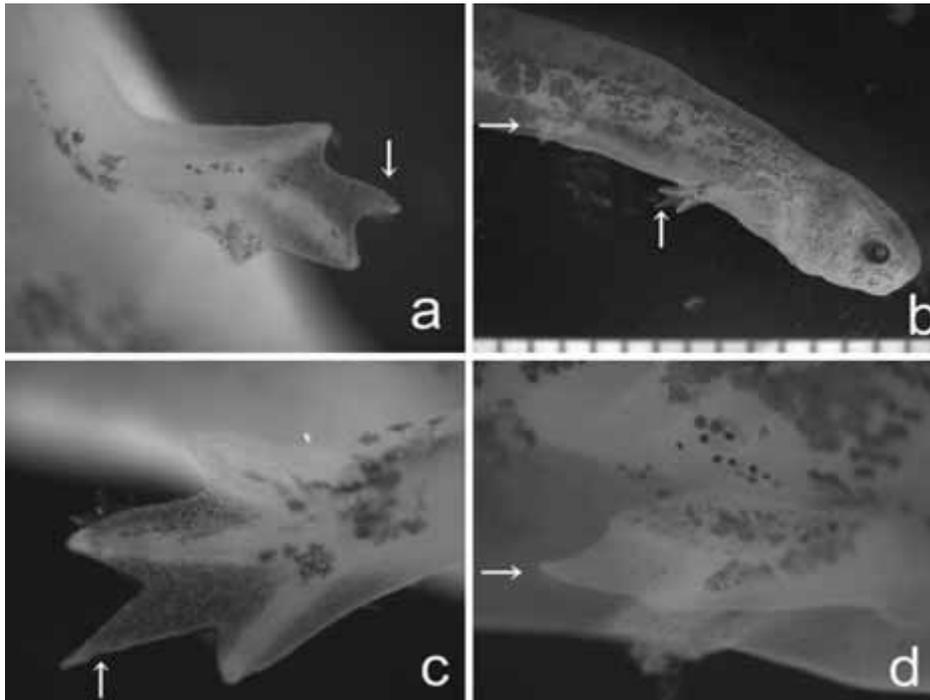


Fig. 2. Limb development in the pond-type larva of *Hynobius lichenatus* at stage 51 (forelimbs at the 3-digit stage). *a, c*: Forelimbs of *H. lichenatus* with three fingers and the IM (arrows) that has undergone further regression. *b, d*: As in most salamander larvae, the hind limbs developing later than the forelimbs, and accordingly the development and regression of the IM is occurring later in the hind limbs (arrows). Scale bar is 1 mm (*b*).

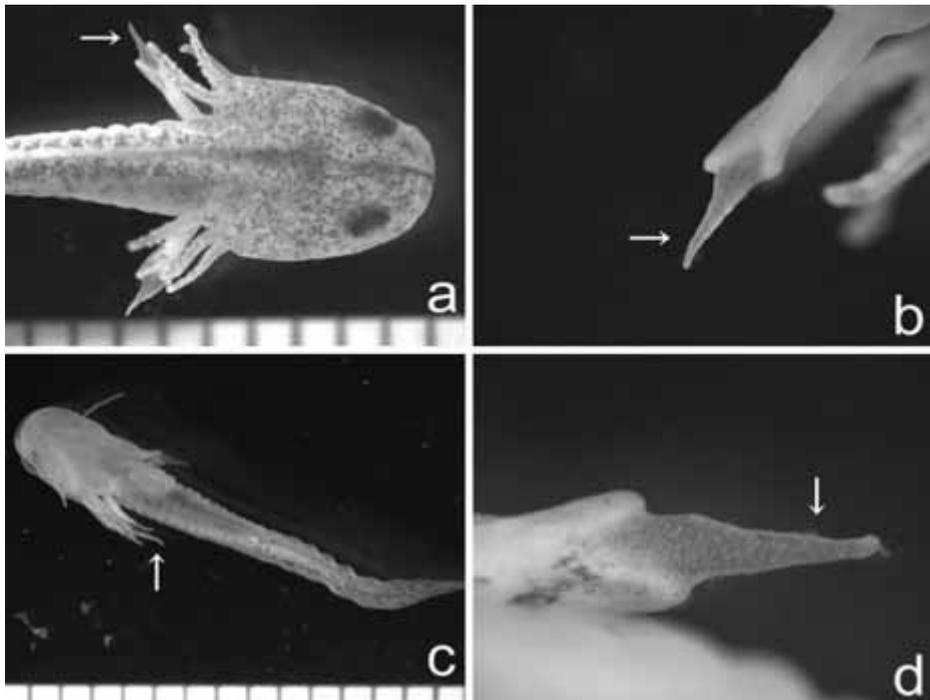


Fig. 3. Limb characteristics in pond-type larva of *Hynobius nigrescens* (*a, b*) and *Hynobius tokyoensis* (*c, d*) showing the IM (arrows). Both are at approximately stage 50. Scale bar is 1 mm (*a, c*).

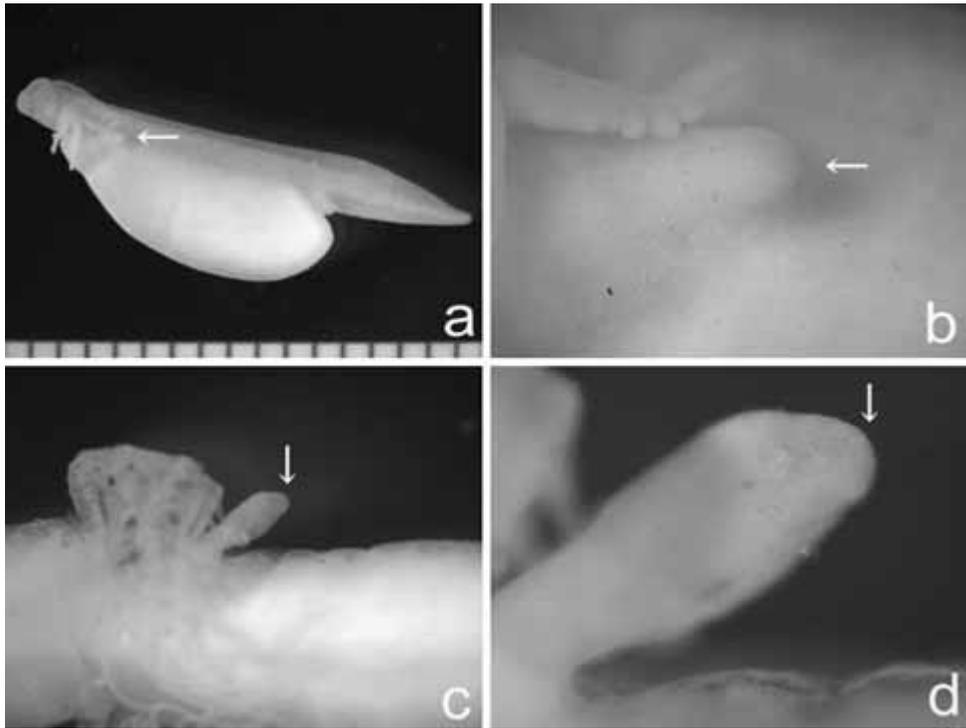


Fig. 4. Limb development in the stream-type larva of *Hynobius kimurae* at stage 42 (*a, b*) and at stage 45 (*c, d*). The limbs (arrows) are poorly developed at these early stages. Scale bar is 1 mm (*a*).

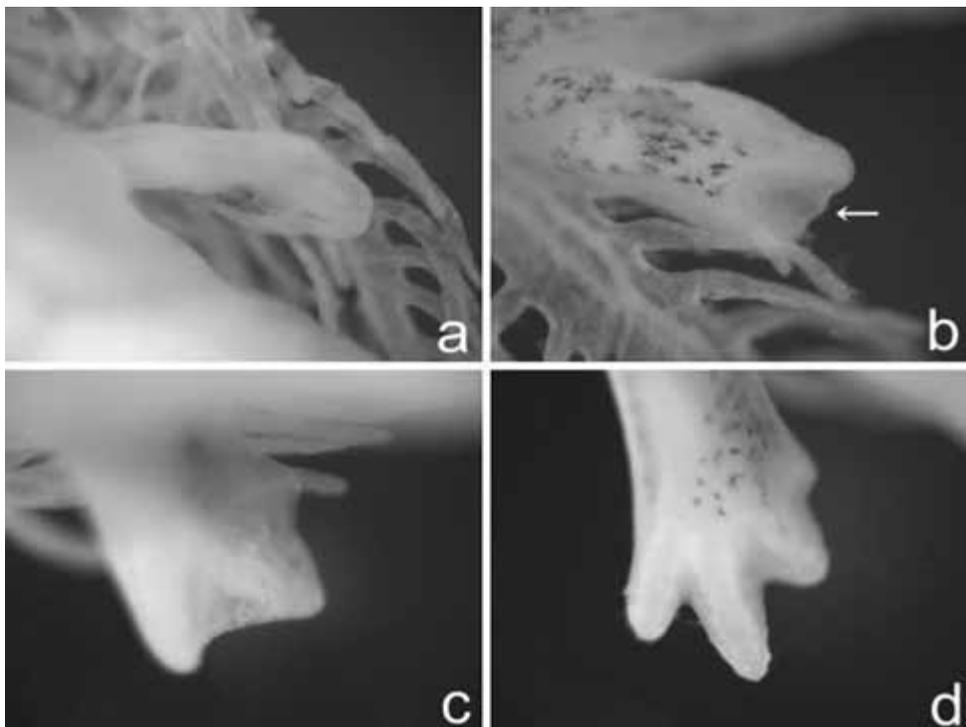


Fig. 5. Limb development in the stream-type larva of *Hynobius kimurae* at stage 46 (*a*), stage 47 (*b*), stage 48 (*c*), and stage 50 (*d*). A vestigial interdigital membrane (arrow) can be seen between digits 1 and 2 at stage 47 (*b*), but has disappeared by the 3-digit stage (*c*).

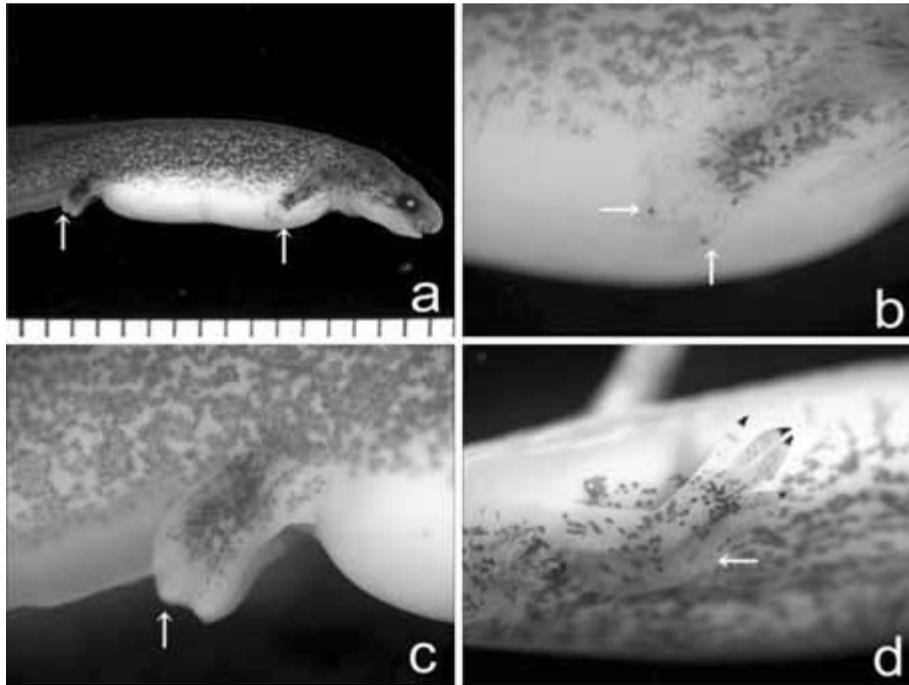


Fig. 6. Limb characteristics of a larva in the stream-type hynobiid *Onychodactylus japonicus* immediately after hatching at stage 56 (*a – c*) and at stage 61 (*d*). The hind limbs (*c*) have no trace of an interdigital membrane (arrow), although a thin fin-like membrane (arrow) was observed along the posterior margins both in forelimbs and hind limbs (*d*). The forelimbs are equipped with black cornified claws (arrows) (*b, d*). Scale bar is 1 mm (*a*).

(Fig. 6*b – d*). However, there is no sign of an IM of any kind in *O. japonicus* (Fig. 6*c* and Table 1). The embryonic limbs of *O. japonicus* larvae also have several special features that appear to be adaptations for stream habitats, including well-developed claws and a thin, fin-like membrane that extends the entire length of the posterior margin of both forelimbs and hind limbs (Fig. 6*d*).

DISCUSSION

The Suborder Cryptobranchoidea includes two families of primitive salamanders, the Cryptobranchidae and the Hynobiidae (Larson and Dimmick, 1993). Phylogenetic relationships among these salamanders, especially within the diverse family Hynobiidae, are poorly understood. Middle Jurassic fossils suggest that cryptobranchid salamanders may be the most ancient member of the “crown-group” (living and recent fossil) urodeles (Gao and Shubin, 2003). Closely related to the Cryptobranchidae are the hynobiid salamanders, an ancient lineage that displays primitive characteristics in their osteology, reproductive biology, and cytogenetics (Morescalchi et al., 1979). This makes the suborder Cryptobranchoidea a particularly interesting group for the study of the evolution of

the vertebrate limb. One of the earliest studies of limb development in a cryptobranchoid salamander was by Schmalhausen (1910), who described the morphology of limb development in the hynobiid salamander *Salamandrella keyserlingii*, which is a species with a pond-type larva and a well-developed IM (Table 1). The presence of an IM in *S. keyserlingii* gives the developing limb a resemblance to the biserial archipterygium of the Australian lungfish *Ceratodus*, a fact noted by Holmgren (1933) who used it to identify homologies in the central axes of vertebrate limbs and fins.

The present study of the IM of hynobiid salamanders has been directed toward understanding the evolutionary significance and possible function of this structure. There are several questions. Is the IM an essential aspect of limb formation, or does it represent a larval adaptation to particular habitats? Also, can the presence or absence of the IM shed any light on phylogenetic relationships among hynobiid salamanders, especially within the genus *Hynobius*?

If the IM is an essential component of limb formation then we would expect to see an IM in both forelimbs and hind limbs, which we do. However, most salamanders, including other genera of hynobiids, do not have anything resembling an IM, so apparently it is not an essential component of limb formation in salamanders. A well-devel-

oped IM is only seen in those species of *Hynobius* that have a pond-type larva, and it is reduced in species of *Hynobius* with stream-type larvae. This correlation suggests that the IM may have adaptive value for larvae inhabiting quiet waters and it has become vestigial in those species that have become secondarily adapted to flowing waters.

One interpretation of the evolutionary significance of the IM of *Hynobius* species is that the stream-type species evolved from a pond-type ancestor, or ancestors, and the adaptation involved the reduction of the IM. This view is supported by the fact that an IM is also found in *Salamandrella keyserlingii*, a species with pond-type larvae and

TABLE 1. A List of the Salamanders of Japan and Taiwan Plus *Cryptobranchus* of North America

Species	Chromosome No.	Larval habitat	Occurrence of IM	
Cryptobranchidae				
<i>Andrias</i> <i>A. japonicus</i> ^{*1}	60	stream	? (+vIM)	
<i>Cryptobranchus</i> <i>C. alleganiensis</i> ^{*2}	60	stream	+vIM	
Hynobiidae				
<i>Salamandrella</i> <i>S. keyserlingii</i> ^{*3}	62	pond	+IM	
<i>Hynobius</i>	<i>H. retardatus</i>	40	pond	
	<i>H. lichenatus</i>	56	pond	+IM
	<i>H. tokyoensis</i>	56	pond	+IM
	<i>H. hidamontanus</i>	56	pond	
	<i>H. takedai</i>	56	pond	
	<i>H. tenius</i>	56	pond	
	<i>H. abei</i>	56	pond	
	<i>H. nigrescens</i>	56	pond	+IM
	<i>H. nebulosus</i> ^{*3}	56	pond	+IM
	<i>H. dummi</i>	56	pond	
	<i>H. tsuensis</i>	56	pond (stream)	
	<i>H. okiensis</i>	56	stream (pond)	
	<i>H. kimurae</i>	58	stream	+vIM
	<i>H. naevius</i>	58	stream	
	<i>H. stejnegeri</i>	58	stream	
	<i>H. boulengeri</i>	58	stream	
	<i>H. arisanensis</i>	58	stream	
	<i>H. formosanus</i> ^{*4}	58	stream	+vIM
	<i>H. sonani</i> ^{*4}	58	stream	+vIM
<i>Onychodactylus</i> <i>O. japonicus</i>	78	stream	—	
Salamandridae				
<i>Cynops</i>	<i>C. pyrrhogaster</i>	24	pond	
	<i>C. ensicauda</i>	24	pond	
<i>Echynotriton</i> <i>E. andersoni</i>	24	pond		

Table compares the diploid chromosome number, larval habitat, and occurrence of an interdigital membrane (IM) or vestigial interdigital membrane (vIM) for these salamanders. ^{*1} Iwama (1968): we could not recognize certain vIM at stage 44; ^{*2} Holmgren (1933); ^{*3} Sessions et al. unpublished data; ^{*4} Kakegawa et al. (1989).

which can be used as an outgroup to the genus *Hynobius*. However, the situation is complicated by the fact that an IM is completely absent in other hynobiids outside of the genus *Hynobius*, including *Onychodactylus*, *Batrachupeirus*, and *Ranodon*, which all have larvae with pronounced adaptations to stream habitats (Vorobyeva et al., 1997; Hinchliffe et al., 2003). Perhaps the IM is a derived feature (synapomorphy) linking *Salamandrella* and *Hynobius* as sister taxa. Little is known about limb development in cryptobranchids, but Holmgren (1933) illustrates a limb bud of *Cryptobranchus alleganiensis* showing what he identified as a vestigial IM (Table 1). If that identification is correct, then we would have to conclude either that an IM is a primitive feature of the Cryptobranchioidea and that it has become reduced or lost independently in several different lineages (Fig. 7a), or that the non-*Hynobius* genera with stream-type larvae are all outgroups to a central lineage consisting of cryptobranchids, *Salamandrella* and *Hynobius* (Fig. 7b). The resolution of these questions will depend further analyses of the phylogenetic relationships within the suborder Cryptobranchioidea.

Acknowledgments. For field assistance, valuable information, and constructive reviews we are grateful to our many colleagues, including N. B. Ananjeva, I. Aoyagi, J. S. Applegarth, N. V. Baleeva, O. Boroznova, E. G. Brede, M. Hasumi, T. Hayashi, J. R. Hinchliffe, H. Hoshi, H. Hoshiba, M. Kakegawa, M. Kuro-o, T. Kusano, K. Noguchi, H. Ohta, N. A. Poyarkov, T. Seto, T. Ueda, T. Utsunomiya, E. I. Vorobyeva, T. Yamada, D.

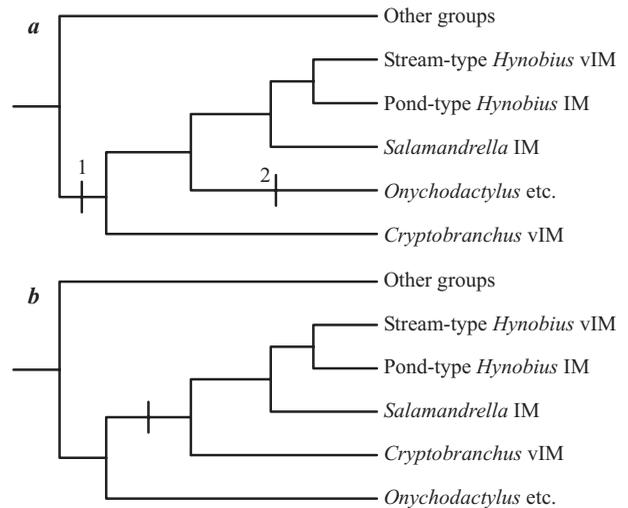


Fig. 7. Alternative phylogenies for the evolution of the interdigital membrane (IM) in hynobiid salamanders. *a*: The IM evolved in an ancestral cryptobranchoid (1) and was later lost (2) in *Onychodactylus* and in other groups that have strong secondary adaptations to stream habitats. The IM becomes vestigial (vIM) in cryptobranchids and stream-type *Hynobius*. *b*: The IM evolved in an ancestor to a lineage including only cryptobranchids, *Salamandrella*, and *Hynobius*.

B. Wake, and J. Wycherley, the students of Science Club in Komagome Jr. High School and the members of the Developmental Program Laboratory in Tokyo Metropolitan University. This project was supported by a Grant-in-aid from the Foundation of The Japan Science Society (F03-222 in 2003 to Koji Iizuka).

REFERENCES

- Akita Y.** (2001), “Normal stages of development of *Hynobius kimurae*,” *Amphibian Hist.*, No. 7, pp. 15 – 26 [in Japanese].
- Gao K.-Q. and Shubin N. H.** (2003), “Earliest known crown-group salamanders,” *Nature*, **422**(6930), 424 – 428.
- Hinchliffe J. R., Vorobyeva E. I., and Mednikov D. N.** (2003), “Diversity of the limb developmental bauplan in Caudata,” in: *Abstrs. of the 12th Ord. Gen. Meeting, St. Petersburg, August 12 – 16, 2003*, Soc. Eur. Herpetol., p. 71.
- Holmgren N.** (1933), “On the origin of the tetrapod limb,” *Acta Zool.*, **14**, 185 – 295.
- Iwama H.** (1968), *Normal Stages of Development of Japanese Giant Salamander, Megalobatrachus japonicus*. Private Publication, Nagoya [in Japanese].
- Iwasawa H. and Kera Y.** (1980), “Normal stages of development of the Japanese lungless salamander, *Onychodactylus japonicus* (Houttuyn),” *Jap. J. Herpetol.* (now *Curr. Herpetol.*), **8**(3), 73 – 89 [in Japanese].
- Iwasawa H. and Yamashita K.** (1991), “Normal stages of development of a hynobiid salamander, *Hynobius nigrescens* Stejneger,” *Jap. J. Herpetol.* (now *Curr. Herpetol.*), **14**(2), 39 – 62 [in Japanese].
- Kakegawa M., Iizuka K., and Kuzumi S.** (1989), “Morphology of egg sacs and larvae just after hatching in *Hynobius sounani* and *H. formosanus* from Taiwan, with analysis of skeletal muscle protein compositions,” in: Matsui M., Hikida T., and Goris R. C. (eds.), *Current Herpetology in East Asia. Proc. of the Second Japan – China Herpetol. Symp., Kyoto, July 1988*, Herpetol. Soc. of Japan (Kyoto University), pp. 147 – 155.
- Larson A. and Dimmick W. W.** (1993), “Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters,” *Herpetol. Monogr.*, No. 7, 77 – 93.
- Morescalchi A., Odierna A., and Olmo E.** (1979), “Karyology of the primitive salamanders, family Hynobiidae,” *Experientia*, **35**, 1434 – 1436.
- Sawano J.** (1947), *Normal Stages of Development of a Hynobiid Salamander, Hynobius lichenatus*. Private Publication, Gurusu Library, Sapporo [in Japanese].
- Schmalhausen J. J.** (1910), “Die entwicklung des extremitäten-skelettes von *Salamandrella keyserlingii*,” *Anat. Anz.*, **37**, 431 – 446.
- Vorobyeva E. I. and Hinchliffe J. R.** (1996), “Developmental pattern and morphology of *Salamandrella keyserlingii* limbs (Amphibia, Hynobiidae) including some evolutionary aspects,” *Russ. J. Herpetol.*, **3**(1), 68 – 81.
- Vorobyeva E. I., Ostrovskaya O. P., and Hinchliffe J. R.** (1997), “Specific features of development of the paired limbs in *Ranodon sibiricus* Kessler (Hynobiidae, Caudata),” *Ontogenez*, **28**(3), 188 – 197 [in Russian].
- Wake D. B. and Shubin N.** (1998), “Limb development in the pacific giant salamanders *Dicamptodon* (Amphibia, Caudata, Dicamptodontidae),” *Can. J. Zool.*, **76**, 2058 – 2066.

THE DISTAL LIMB PART'S VARIABILITY IN AMPHIBIA AND REPTILIA

Yu. I. Kruzhkova¹

This paper deals with acro- and basipodial variability in Tetrapoda on the whole and in Amphibia and Reptilia in particular. The variability analysis by “method of spectra” (Kovalenko, 1996) was applied. The characters, chosen for the analysis, were shown to combine with each other in an unfree way. The regularities of their combination were found. The comparative analysis of variability trends of mentioned structures in Amphibia and Reptilia allowed to reveal the qualitative and quantitative criteria of their real acro- and basipodial variability diapasons, discriminating these groups from each other. Besides distinctions, reptilians and amphibians were shown to have common regularities, differing from birds and mammalians.

Keywords: acropodium, basipodium, variability, Amphibia, Reptilia.

It is well known that the most variable segments in tetrapod extremity are basi- and acropodium. This is not surprising because of the great number of elements they contain and tendency towards fusion and new formation within them. Most of researches into the evolution of pentadactyl limb add up to the consideration of just these parts alterations (Shmalgauzen, 1915; Severtsov, 1950; Alberch and Gale, 1985; Shubin et al., 1995; Lee, 1997; etc.).

The aim of the present investigation is to find out if there is any correlation between variation of acropodium and basipodium in tetrapod limbs and to compare variation ranges of these structures in Amphibia and Reptilia.

MATERIAL AND METHODS

The material we possess includes data on extant and extinct forms. The total number of analyzed orders, families and species is presented in Table 1. The descriptions were carried out on the osteological collections of the Department of Vertebrate Zoology, St. Petersburg State University, and Zoological Institute of the Russian Academy of Science.

All material was analyzed by the “method of spectra”, proposed by Kovalenko (Kovalenko, 1996).

DISCUSSION

The first step according to the “method of spectra” is determination of the features’ variability ranges. At this stage of research the author dwelled on the variation of

quantitative characters. It was found out that the number of elements in basipodium varies from 0 to 15, whereas the number of rays in acropodium, from 0 to 11. Combining the states of both features’ with each other and arranging their combinations in lattice form according to the acropodial feature in columns and according to the basipodial one in lines, we obtain the theoretical spectrum (St) of limb variability (Fig. 1). Hence, the sum of all possible combinations amounts to 192.

Then the cells corresponding to the variants found in Tetrapoda should be marked in this lattice. The marked cells form the pattern of the real spectrum (Sr). The number of met combinations is 60. This accounts for only 31% of the theoretically calculated sum. Such low percentage could be explained by two reasons:

1. our material is too scanty and does not include all the variants occurring in the group;
2. the characters, chosen for the analysis are combined with each other in unfree way; thus, not all theoretically possible combinations can be realized in ontogenesis.

If the attributes do vary independently, their real combinations have to fill the lattice uniformly. The scantiness of material would result in the randomly situated empty cells and the obtained pattern of the real spectrum should be mosaic. Nevertheless in fact (Fig. 1) one can see the realized variants grouped in two opposed sectors: upper left

TABLE 1. Material

Group	Extant		Extinct		Total number of species
	orders	families	orders	families	
Amphibia	2	17	6	10	70
Reptilia	4	38	15	19	160
Aves	40	139	5	6	154
Mammalia	15	33	1	3	153

¹ Department of Vertebrate Zoology St. Petersburg State University, Universitetskaya nab., 7/9, 199034 St. Petersburg, Russia;
E-mail: Yulya_Kruzhkova@mail.ru.

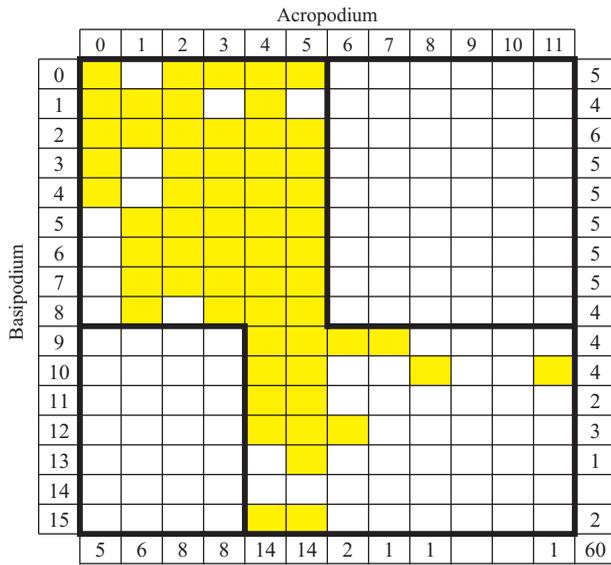


Fig. 1. The spectrum of real variants of limb structure (Sr) of Tetrapoda, inscribed in spectrum of theoretical variants (St) of Tetrapoda. The figures, situated above the lattice and on its left, are the codes of columns and lines, which indicate the number of acropodial rays and basipodial elements, respectively. The figures under the lattice and on its right indicate the number of variants of basipodial and acropodial structure, respectively, which were met at the certain state of second attribute in the investigated group. □, The variant of limb structure, which was not met in the investigated group; ■, the variant of limb structure, which was met in the investigated group.

and lower right. Such ordered arrangement supports our assumption about correlation between acro- and basipodial variability. However, there is no direct relation between the numbers of elements within these limb parts since at a certain state of one feature the other one, could have a rather wide variation range. Interestingly, that only two acropodial states, pentadactyl and close to it tetradactyl, allow practically all basipodial patterns to be realized. The fourth and fifth columns in Sr are the fullest and their upper and lower parts contain marked cells, while, to the left and to the right of them one can see restrictions on the large groups of combinations (empty cells in Fig. 1).

As it has been mentioned before, the common spectrum of Tetrapoda contains data on limb structure in Amphibia, Reptilia, Aves, and Mammalia. In other words, it was composed of the limb variability diapasons of these groups. In Fig. 2 one can see the positional relationship of these diapasons within the theoretical spectrum. It is noteworthy, that being overlapped in lattice center, either of the four spectra, stretches in its own direction. Such a picture suggests the different regularities of limb variation in the above groups. In order to test this suggestion let us compare the real spectra of Amphibia and Reptilia (Fig. 3).

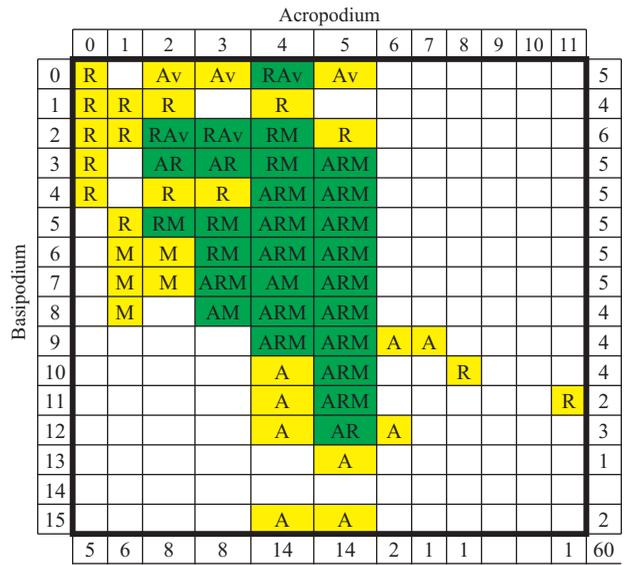


Fig. 2. The positional relationship of Sr of Amphibia, Reptilia, Aves and Mammalia in St of Tetrapoda. The letters in cells indicate the classes of Tetrapoda, in which the given variant of limb structure was found: A, Amphibia; Av, Aves; R, Reptilia; M, Mammalia. ■, The variant of limb structure, which was met in several classes of Tetrapoda; ■, the variant of limb structure, which was met in only one class of Tetrapoda. For other designations see Fig. 1.

These spectra differ from each other by several characteristics. The first of them is the sum of realized combinations or the spectrum fullness. In amphibians we found 27 variants (Fig. 3a), while in reptilians, 41 (Fig. 3b). thus, the limb diversity in Reptilia is half as much again, as in the former group.

The second distinction is the pattern formed by real variants in the theoretical lattice. In amphibians (Fig. 3a) the most part of marked cells are situated within the fourth and fifth columns (10 and 12, respectively). Thus, in penta- and tetradactyl limb the range of basipodial variability is very wide, whereas at other acropodial states, basipodium did not vary at all. The pattern of reptilian spectrum looks quite different (Fig. 3b). The fourth and fifth columns as well, as in amphibians are the fullest, but there is a rather wide diapason of basipodium variability also at many other acropodium states, all columns of lattice left half contain several marked cells. Combinations from this part of the spectrum correspond to different variants of reduction in both acro- and basipodium. Their diversity is much higher in reptilians, than in amphibians. Decreasing of elements number was observed in both limb pairs in either class, but in reptilians the hind extremities tend to greater reduction, than the fore ones. In amphibians the situation changes to vice versa. As a rule the number of

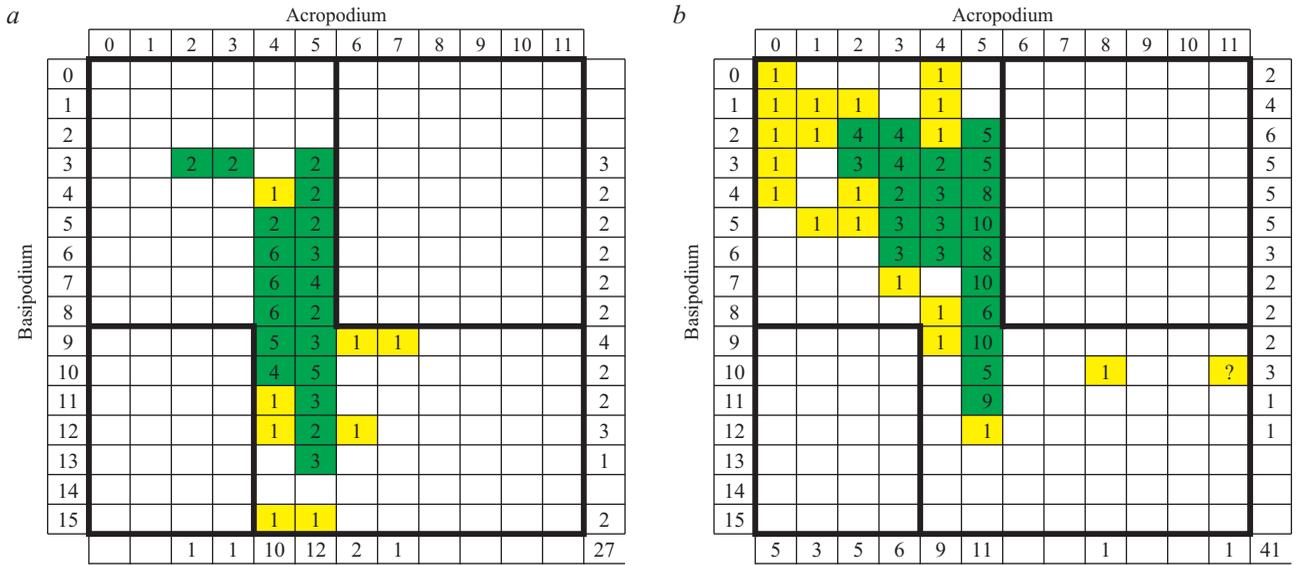


Fig. 3. Sr of Amphibia (a) and Reptilia (b) in St of Tetrapoda. The figures within the filled cells indicate the number of families in which the given combination of mentioned characters was met. ?, the exact number of families in which this variant was found is unknown. ■, The variant of limb structure, which was met in several families of the corresponding class; ■, the variant of limb structure, which was met in only one family of the corresponding class. For other designations see Fig. 1.

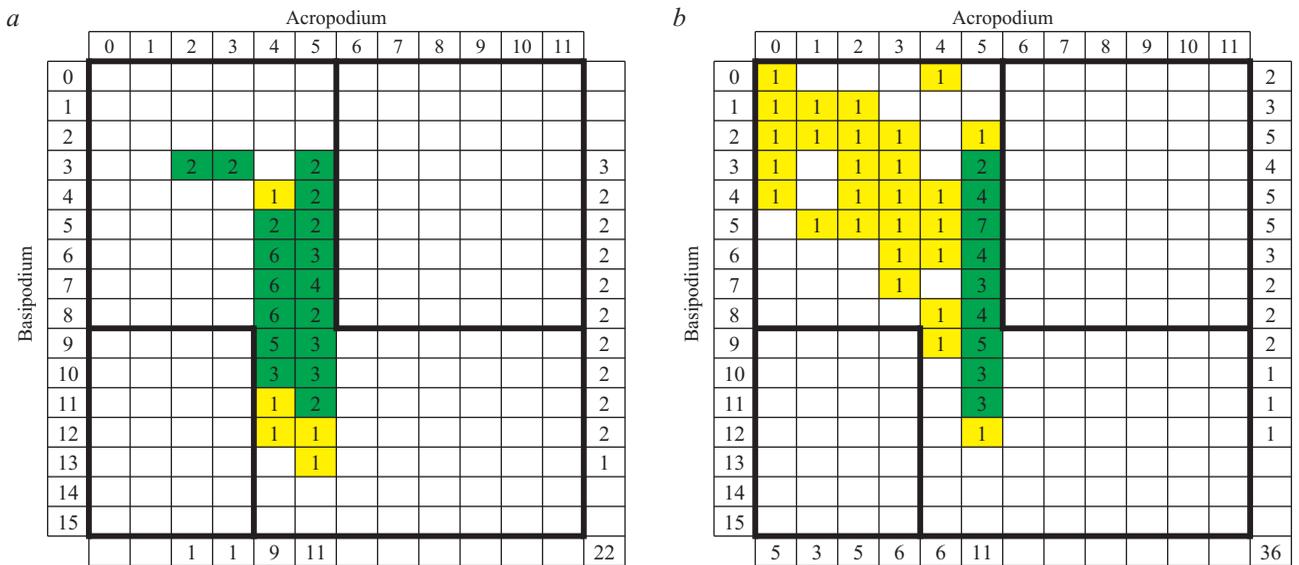


Fig. 4. Sr of extant Amphibia (a) and Reptilia (b) in St of Tetrapoda. For other designations see Fig. 3.

elements in the fore limbs of the latter class representatives is less, than in their hind extremities.

The percentage ratios between low- and high-frequency combinations are also different in these classes. In Amphibia variants, which were met in only one family (monotypic variants) account merely to 29% of their Sr, while in reptilians, to 45%. The difference between these

figures will be much larger, if we retain in spectra the data on only extant forms (Fig. 4). The percentage of monotypic variants in amphibians will account to 23%, in reptilians, to 76%. The presence of such low-frequency combinations is due to inter- and intraspecies variability in some families of these classes and characteristic features of Sr patterns of these families. For example, in Hynobiidae and

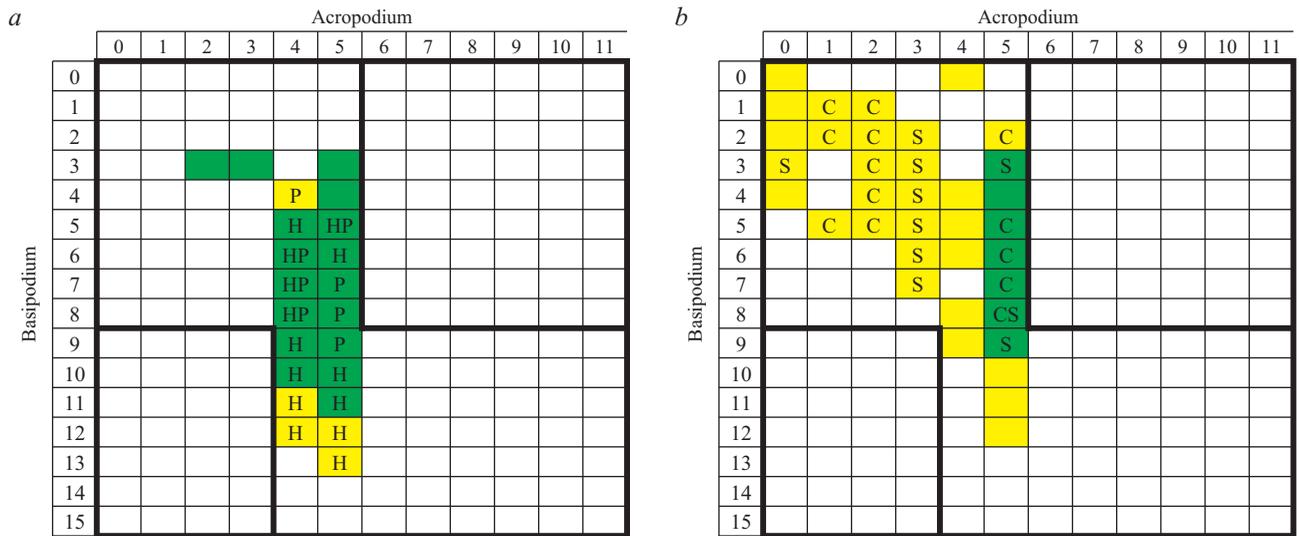


Fig. 5. Limb variability diapasons of different families against the background of Sr of Amphibia (a) and Reptilia (b). The letters in cells indicate the families, in which the given variant of limb structure was found: C, Cordilidae; H, Hynobiidae; P, Plethodontidae; S, Scincidae. For other designations see Fig. 3.

Plethodontidae fourteen and eight variants of limb structure were described, respectively, (Hanken and Dinsmore, 1986; Dwyer and Hanken, 1990; Borkhvardt and Ivashintsova, 1993, Vorobyeva and Hinchliffe, 1998). It can be seen in Fig. 5a, that spectra of these families have more or less similar patterns and coincide with each other and with the spectra of the rest of the families throughout a significant area. Hence, they contribute only 27% of the variants (five combinations) in common Sr of extant amphibians (Fig. 4a). In Reptilia we see another kind of picture. They also demonstrate a very high inter- and intraspecies variation of limb structure; for example, in Cordilidae and Scincidae 13 and 10 variants were described, respectively, (Essex, 1927; Stokely, 1947; Severtsov, 1950). However, in comparison with amphibians, limb variability ranges of these reptilian families demonstrate different patterns (Fig. 5b) and overlap with each other and with those of the rest of families to a much lower degree. Thus, in spite of the fact that in Scincidae and Cordilidae we described an approximate number of variants, as in Hynobiidae and Plethodontidae, the former two groups contribute to the common reptilian Sr much more combinations, than the latter two groups to the amphibian one. Their contribution comes to 45% of common Sr of extant Reptilia (sixteen combinations). All these facts being considered, we can conclude, that amphibian families demonstrate similar tendencies of the limb variability, whereas in Reptilia one can see the interfamily diversification of the tendencies.

In spite of all listed differences between limb variability diapasons in Amphibia and Reptilia, there are several

characteristics common to them, which allow to distinguish the above classes from all other Tetrapoda. Only in these groups the increasing number of acropodial rays was described (Fig. 1): up to eight in Acanthostega (Coates and Clark, 1990) and up to eleven in Ichthyosauria (Cherpanov and Ivanov, 2001). In warm-blooded animals, even in secondary aquatic ones, the maximum digits number is five (Fig. 2). The presence of interspecies variability of distal limb parts also is a distinctive feature of lower Tetrapoda. In birds and mammalians, as a rule, a certain variant of fore and hind limb structure corresponds to a certain order (in birds even to a group of orders).

Acknowledgments. The author is very thankful to all the members of morphological group of the Department of Vertebrate Zoology, St. Petersburg State University, for discussion of results, presented in this paper, and is especially grateful to E. E. Kovalenko for the very valuable advises during preparation of the manuscript.

REFERENCES

Alberch P. (1983), "Morphological variation in the neotropical salamander genus *Bolitoglossa*," *Evolution*, **37**(5), 906 – 919.
 Alberch P. and Gale E. (1985), "A developmental analysis of an evolutionary trend: Digital reduction in amphibians," *Evolution*, **39**, 8 – 23.
 Auffenberg W. (1966), "The carpus of land tortoises (Testudini-nae)," *Bull. Florida State Mus.*, **10**(5), 159 – 191
 Bateson W. M. A. (1894), *Materials for the Study of Variations*, Macmillan, London – New York.

- Borkhvardt V. G. and Ivashintsova E. B.** (1993), "Some regularities in the variation of the paw skeleton of Siberian salamander, *Salamandrella keyserlingii* (Amphibia, Hynobiidae)," *Zool. Zh.*, **72**(6), 66 – 73 [in Russian].
- Carroll R. L.** (1992), *Vertebrate Paleontology and Evolution*, Mir, Moscow [Russian translation].
- Case E. C.** (1904), "On the For Foot of Dimetrodon," *J. Geol.*, **12**(4), 312 – 315.
- Cherepanov G. O. and Ivanov A. O.** (2001), Fossil Higher Vertebrates, Izd. SPbGU, St. Petersburg [in Russian].
- Coates M. I. and Clarck J. A.** (1990), "Polydactyly in the earliest known tetrapod limbs," *Nature*, **347**(6288), 66 – 69.
- Dwyer Ch. M. and Hanken J.** (1990), "Limb skeletal variation in the Jamez Mountains salamander, *Plethodon neomexicanus*," *Can. J. Zool.*, **68**, 1281 – 1287.
- Essex R.** (1927), "Studies in reptilian degeneration," *Proc. Zool. Soc. London*, **2**, 879 – 925.
- Fabrezi M. and Alberch P.** (1996), "The carpal elements of Anurans," *Herpetologica*, **52**(2), 188 – 204
- Gans C.** (1975), "Tetrapod limbleness: evolution and functional corollaries," *Am. Zool.*, **15**, 455 – 467.
- Gertsen I. G.** (1939), "Materials on comparative anatomy of ankle joint," *Arkh. Anat. Histol. Émbriol.*, **20**(2), 320 – 330 [in Russian].
- Gollmann G.** (1991), "Osteological variation in *Geocrinia laevis*, *Geocrinia vectoriana* and their hybrid populations (Amphibia, Anura, Myobatrachinae)," *Z. Zool. Syst. Evol.-Forsch.*, **29**(4), 289 – 303.
- Grandison A. G. C.** (1981), "Morphology and phylogenetic position of the West African *Didynamipus sjoestedti* Andersson 1903, (Anura, Bufonidae)," *Ital. J. Zool.*, **11**, 187 – 215.
- Hanken J.** (1985), "Morphological novelty in the limb skeleton accompanies miniaturization in salamanders," *Science*, **229**, 871 – 874.
- Hanken J. and Dinsmore Ch. E.** (1986), "Geographic variation in the limb skeleton of the red-backed salamander, *Plethodon cinereus*," *J. Herpetol.*, **20**(1), 97 – 101.
- Hinchliffe J. and Jonson D. R.** (1980), *The Development of the Vertebrate Limb*, Oxford.
- Jungersen H. F.** (2001), "Remarks on the structure of the hand in *Pipa* and *Xenopus*," *Ann. Mag. Nat. Hist.*, **8**, 193 – 206.
- Kingsley J. S.** (1925), *The Vertebrate Skeleton from the Developmental Standpoint*, London.
- Klimov A. F.** (1927), *Extremities of Farming Animals*, Gosizdat, Moscow [in Russian].
- Kovalenko E. E.** (1996), "Analysis of anuran sacrum variability. 1. A method of analyzing anuran sacrum variability," *Zool. Zh.*, **75**(1), 52 – 66 [in Russian].
- Lebedev O. A. and Coates M. I.** (1995), "The postcranial skeleton of the Devonian tetrapod *Tulerpeton curtum* Lebedev," *Zool. J. Linn. Soc.*, **114**, 307 – 348.
- Lee M. S. Y.** (1997), "The evolution of the reptilian hindfoot and the homology of the hooked fifth metatarsal," *J. Evol. Biol.*, **10**, 253 – 263.
- Lewis O.** (1964), "The homologies of the mammalian tarsal bones," *J. Anat. London*, **98**(2), 195 – 208.
- Manzyi S. F.** (1961), "Mammalian wrist from the point of view of their fore limbs evolution and function," in: *Proc. of the VI All-Union Congr. Anat., Histol., and Embryol. Vol. 1*, pp. 224 – 225 [in Russian].
- Romankowowa A.** (1961), "The sesamoid bones of the autopodia of bats," *Acta Theriol.*, **5**(10), 125 – 140
- Ruiz-Carranza P. M. and Hernander-Camacho J. I.** (1976), "Osornophryne, genero nuevo de Anfibios Bufonidos de Colombia y Ecuador," *Caldasia*, **11**(54), 93 – 148.
- Savage J. M. and Kluge A. G.** (1961), "Rediscovery of the strange Costa Rica toad, *Crepedius epioticus* Cope," *Rev. Biol. Tropical*, **9**(1), 39 – 51.
- Senaris J. C., Ayarzagueno J., and Gorzula S.** (1994), "Las sapas de la Familia Bufonidae (Amphibia: Anura) de las tierras altas de la Guayana Venezolana: Descripción de un nuevo genero y tres especies," *Publ. Ciones Ass. Amigos Donana*, **3**, 1 – 37.
- Severtsov A. N.** (1950), *Coll. Works. Vol. 2. The Origin and Evolution of Limbs*, Moscow – Leningrad [in Russian].
- Shmalgauzen I. I.** (1915), *Development of Amphibian Limbs and Their Significance for the Problem of Origins of Land Vertebrate Limbs*, Moscow [in Russian].
- Shubin N., Wake D., and Crawford A.** (1995), "Morphological variation in the limbs of *Taricha granulosa* (Caudata: Salamandridae): evolutionary and phylogenetic implications," *Evolution*, **49**(5), 874 – 884.
- Stokely P. S.** (1947), "Liblensness and correlated changes in the girdles of a comparative morphological series of Lisards," *Am. Midl. Natur.*, **38**(3), 725 – 754.
- Stovall J. W., Price L. I., and Romer A. Sh.** (1966), "The postcranial skeleton of the giant Permian pelycosaur *Cotylorhynchus romeri*," *Bull. Mus. Comp. Zool.*, **135**(1), 1 – 30.
- Terlyayev V. A.** (1960), "On the nature of pteroid in the pterosaurs' wing," *Zool. Zh.*, **39**(2), 278 – 281 [in Russian].
- Vokken G. G.** (1946), "Ontogenesis of bony skeleton of fore limb (paddle) of Black Sea dolphin (*Delphinus delphis*)," *Zool. Zh.*, **25**(6), 551 – 564 [in Russian].
- Vorobyeva E. I.** (1999), "The problem of polydactyly in Amphibians," *Russ. J. Herpetol.*, **6**(2), 95 – 103.
- Vorobyeva E. I. and Hinchliffe R. J.** (1998), "Phylogenetic variability and larval adaptations in the developing limbs of the Hynobiidae," *Adv. Amphibian Res. Former Soviet Union*, **3**, 21 – 34.

THE SKIN SENSE ORGANS OF LIZARDS OF *Teratoscincus* GENUS (SQUAMATA: SAURIA: GEKKONIDAE)

Natalia G. Nikitina¹ and Natalia B. Ananjeva¹

Keywords: Sauria, Gekkota, Iguania, Gekkonidae family, *Teratoscincus*, integument, microstructure, skin organs.

INTRODUCTION

Structure, distribution and number of the skin sense organs is the source of important data in taxonomy and phylogeny. They are epidermal structures with a dermal papilla entering from below. Numerous studies demonstrate great diversity in types of sense organs in Iguania and Gekkota (see reviews in Ananjeva et al., 1991; Matveyeva and Ananjeva, 1995). Presence of sense organs in the skin is a typical feature of the squamate integument. Their structure reflects the complex vertical differentiation of the epidermis which is typical of Squamata. Among the agamids, iguanids and gekkonids there are two basic types of receptors: first type has a hair-like process seta, or bristle, which the second type lacks.

The list of species examined for the microanatomy of skin organs was greatly increased during the last decades but it is still not complete; it concerns a list of examined geckos too (Bauer and Russell, 1988, 1989; Duisebayeva, 1995; Matveyeva and Ananjeva, 1995). Receptors with bristle are known only in Gekkota and Iguania among lizards and aquatic snakes of the family of Acrochordidae. The bristled receptors of the geckos are different from those of the agamids and iguanids in some respects and at first by smaller number of modified germinate cells above the dermal papilla, by thinner bristles and by absence of lamellar body around the regenerated bristles of the gecko receptors (Duisebayeva, 1995). It could be regarded as additional evidence for possible independent origin of the bristled receptors in these lizard groups.

Organs with “hairs” are typical of the geckos. However even based on published data alone, a number of differences can be found in the detailed structure of the sense organs of geckos. A single receptor can be supplied with one or several bristles (up to 5). Different types of microstructure of bristles and receptors were noted in *Phyllurus* (Hiller, 1971), *Tarentola* (Joger, 1984), and other genera. Organs with “hairs” are typical of the geckos of the subfamily Gekkoninae.

The plate-tailed geckos of the genus *Teratoscincus* Strauch, 1863 includes 6 species with distribution range covering deserts of Middle and Central Asia, including southern Mongolia, northern and north-western China, as well as Iran and Afghanistan, Pakistan, and east of United Arab Emirates (Anderson, 1999; Ananjeva et al., 2004). These lizards of medium size with a large wide and high head and big eyes are characterized by delicate skin, especially in *T. scincus* (Anderson, 1999), which could be partially lost during captures of these lizards. This genus was regarded by some herpetologists as belonging to its own subfamily, Teratoscincinae (Kluge, 1987; Grismer, 1988). However the most recent study of phylogeny of gekkotan lizards using C-mos nuclear DNA sequence data shows no evidence for the support of the Teratoscincinae (Han et al., 2005).

Scale organs were examined earlier only in *T. scincus* (Matveyeva and Ananjeva, 1995). We here provide an effort to study skin organs of the most species of genus *Teratoscincus* whose skin sensory organs have not been studied previously.

MATERIAL AND METHODS

The cutaneous receptors of five following species of genus *Teratoscincus* were examined: *T. scincus* (32) and *T. keyserlingii* (5)², *T. roborowskii* (1), *T. przewalskii* (16), and *T. bedriagai* (3). The number of specimens of each species studied is given in brackets. Parts of skin were taken from specimens in the herpetological collection of the Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia. Fragments with size 5 × 5 mm were taken from dorsal and ventral parts of body and different regions of the head (frontal region, regions of ear and supralabial shield) and examined using a Stereoscan. Material examined by SEM had been previously fixed in ethanol. Skin samples were dehydrated and coated with gold before examination. The magnifications used were 40× to 6000× and the voltage was 30 kV.

¹ Department of Herpetology and Ornithology, Zoological Institute, Academy of Sciences, 199034 St. Petersburg, Russia;
E-mail: agama@NA4755.spb.edu.

² Specific status for this taxon is recommended (Macey et al., 2005, in press).

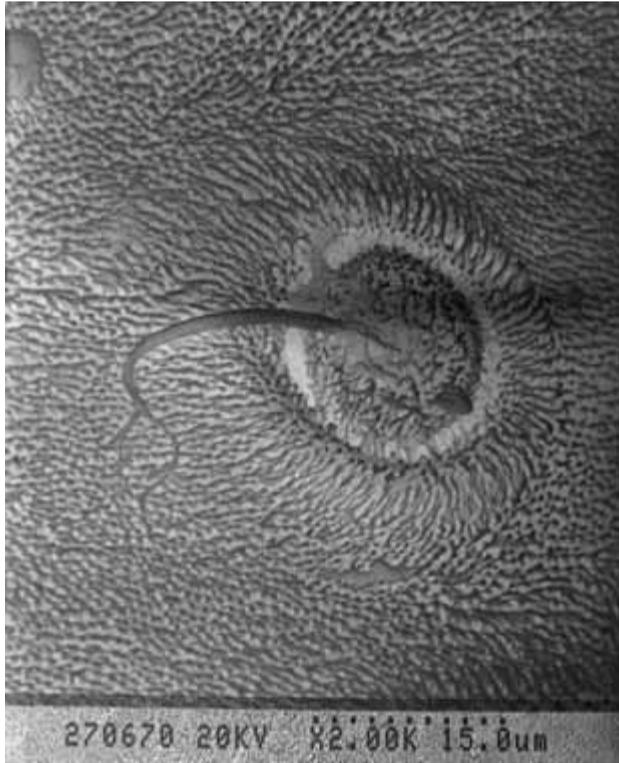


Fig. 1. Receptor and microstructure of dorsal scale of *T. bedriagai*.



Fig. 2. Receptors on ventral scale of *T. scincus*.

RESULTS

Dorsal scales of species studied have rounded-rhomboid shape, homogenous surface and slightly wavy edge. *T. przewalskii* has plicated microstructure of scales in their peripheral regions. Skin receptors of plate-tailed geckos are situated along the edge of scale in its free distal part (10 – 15 receptors per scale in *T. scincus* and *T. przewalskii* and up to 7 receptors per scale in *T. roborowskii* and *T. bedriagai*). Very small skin organs look like roundish depressions with ring-shaped protuberance on the bottom. The bristle with bent top sets out from the center of this protuberance (Fig. 1).

Ventral scales of *T. roborowskii* and *T. bedriagai* have flat surface that look fleecy under 1000 times magnification. Other species have plicated microstructure of these scales (under the same magnification). Edges of scales are flat or slightly wavy (if microstructure is plicate). Receptors are small, distributed along the very edge of scale closer to its free distal part. There are 5 – 6 skin organs per scale (in *T. roborowskii* and *T. bedriagai*) and up to 15 in other species. They look like irregular depressions with protuberance on the bottom (Figs. 2, 3). The bent bristles were noted only in *T. bedriagai*.

Scales of the upper surface of the head are elongated (Fig. 4), of different size. Microstructure with large plications; the lateral surface of plications is fine-grained. We did not record skin organs on the each scale. In the integument of all species studied these round structures (1 – 2 per scale) are situated along the distal edge of the scale. Skin organ looks like round depression with protuberance on the bottom. As a rule we were not able to find the bristle. Sometimes the part of shield with receptor is separated by transversal semicircular fold.

The scales of this region are elongated, irregular or rectangular shape; microstructure with large folds. As a rule two folds are especially notable: longitudinal lateral fold (separating receptor-bearing part of scale) and transverse distal fold; sometimes only single lateral fold is recorded. In the ear region the number of receptors (Fig. 5) is increased up to 3 – 4 per scale; these round relatively large depressions with protuberance on the bottom are recorded in each scale and often have a bristle.

Labial scales have the highest number of skin sense organs. The whole scale surface with fine-grained plications is spotted by numerous small receptors with typical structure. Each receptor looks like depression (Figs. 6, 7) encircled with closed small folds. We have not recorded bristled receptors on labial scales.

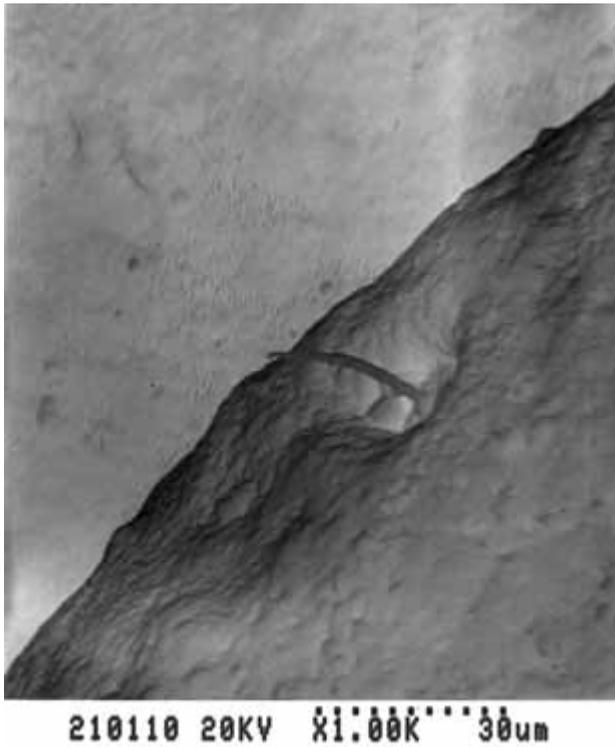


Fig. 3. Receptor on ventral scale of *T. roborowskii*.

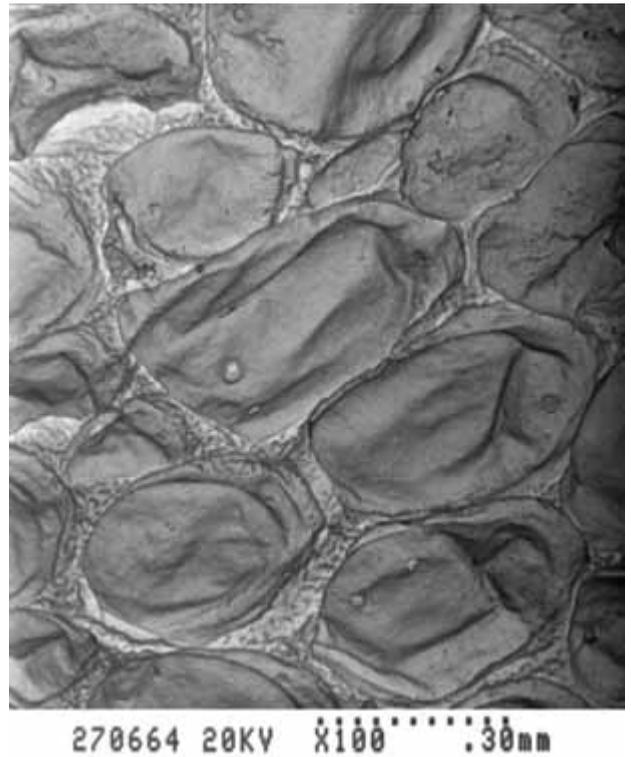


Fig. 4. Head scales of *T. bedriagai*.

DISCUSSION

Scalation of the body of plate-tailed geckos is distinguished from their cephalic scales by large size, rhomboid shape, flatter and more homogenous microstructure (cephalic scales have clear plicate structure). Their arrangement and position of the border between of large cycloid dorsal scales and small scales in the neck or head are important as the main characters using under identification of species of *Teratoscincus* genus (Szczerbak and Golubev, 1986; Macey et al., 1997; Anderson, 1999). Increasing of the body scales to the back of the head in *T. microlepis* and *T. scincus* was noted by Kluge (1967).

All plate-tailed geckos of the genus *Teratoscincus* are able to lose the particles of skin (sometimes large areas) that was described also for other geckos and named “regional integumentary loss” (Bauer and Russell, 1989). This ability is described by Anderson (1999) who considered “the easily torn skin in these and other gecko species” as an adaptation facilitating escape from the grasp of predators.

Bristled receptors are typical in general of the lizards of the Gekkoninae subfamily (Bauer and Russell, 1988). The structure, distribution and number of the skin organs of *Teratoscincus scincus* were described by Matveyeva and Ananjeva (1995). They noted that integument of this



Fig. 5. Receptors on scales in the ear region of *T. roborowskii*.

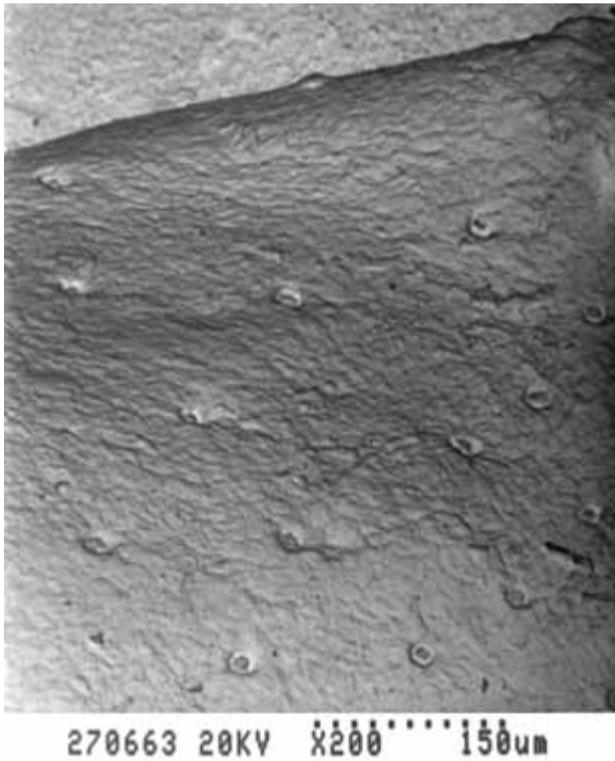


Fig. 6. Receptors on labial scale of *T. bedriagai*.



Fig. 7. Receptor of labial scale of *T. bedriagai*.

species is unique with very high receptor density and in particular with high receptor number of 15 – 20 in the ventral scales. It is much larger in comparison with those of many agamid, iguanid and geckonid species. The greatest number (60 and more) of skin organs arranged in 2 – 3 rows on their free distal border was noted on the dorsal caudal scales. Our data confirm these results. They demonstrate high density of receptors which are located along peripheral area of scale in its free distal border. We have noted 10 – 15 receptors as maximum density in ventral scales of *T. scincus*, *T. keyserlingii*, and *T. przewalskii*, and more low (5 – 6) number in *T. roborowskii* and *T. bedriagai*.

All studied species of *Teratoscincus* are characterized by high density of receptors in labial scales.

This high number of receptors on ventral scales in the genus *Teratoscincus* is unusual for lizards; in most of them the highest density is recorded on cephalic (nasal, labial) and caudal scales (Ananjeva et al., 1991; Matveyeva and Ananjeva, 1995). In cephalic scales (except for labials) is recorded the lesser number of receptors of bigger size than in dorsal and ventral scales. Matveyeva and Ananjeva (1995) assume that the highest number of receptors in the large ventral scales of *Teratoscincus* could be in accordance with the postulate of an inverse relationship be-

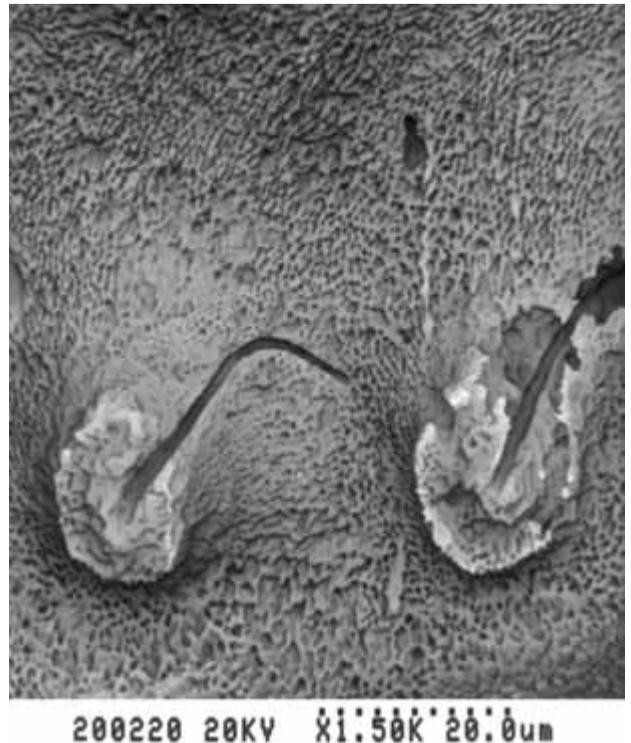


Fig. 8. Receptors and microstructure of scales in the ear region of *T. keyserlingii*.

tween receptor number and degree of contact with the ground. The body of these lizards is held above the substratum, without any snake-like ground-contact during locomotion.

The skin sense organs are localized on the free dorsal border of the dorsal, ventral and caudal scale. Cephalic scales can have skin organs on their periphery or receptors scattered regularly on the shield surface.

The most of receptors bear bent bristle (Figs. 1, 8). It is remarkable that we did not record a bristled receptors on labial scales of all species we studied. This data could be considered as an evidence of existence of two different morphological types of skin sense organs in *Teratoscincus*. However we suppose that non-bristled organs could be just receptors with broken bristles in the labial scales. This fact was already noted for agamid lizards and could be explained by strong functional pressure to this skin regions ((Dujsebajeva and Ananjeva, 2001). Two types of receptors (with bristle and without it) were described in Carphodactylini geckonid lizards the *Nephrurus* genus (Bauer and Russell, 1988). The authors explained that the absence of bristles on the subdigital and scales and scales of the knob-tail is correlated with the lack of epidermal microarchitecture in *Nephrurus* that is considered as perhaps a pedomorphic feature). Bauer and Russell (1988) also suppose that it could be associated with functional demands that expose the sensilla to constant direct substrate contact.

Two species, *T. bedriagai* and *T. roborowskii*, demonstrate some differences in comparison with other species studied, *T. scincus*, *T. keyserlingii*, and *T. przewalskii*. In particular two former species have lesser number of skin organs within body scales (5–7 contrary to 10–15 in three latter species). They are also remarkable by fleecy microstructure of ventral scales. There are no visible difference in density, topography and structure of cephalic shields between all species studied.

Summarizing our data we like to note several peculiarities of distribution, number and structure of skin sense organs of *Teratoscincus*. They are very small and are visible only under magnification not less than 200×. Dujsebajeva (1995) reported for *Teratoscincus scincus*: receptor width 13 µm, receptor height 7 µm, bristle width 1 µm and its length 10 µm. It was shown two different types of receptor's arrangement: peripheral and scattered regularly on the scale (in the latter case sense organs are more numerous); sense organ itself looks like depression with ring or semi-ring-shaped protuberance on the bottom). Most receptors are bristle-bearing; there were no records the bristles in receptors of the labial scales.

Acknowledgment. This study was supported by Russian Fund for Basic Research (grant No. 05-04-48156 to Natalia B. Ananjeva).

REFERENCES

- Ananjeva N. B., Dilmuchamedov M. E., and Matveyeva T. N. (1991), "The skin sense organs of some iguanian lizards," *J. Herpetol.*, **25**(2), 186 – 199.
- Ananjeva N., Orlov N., Khalikov R., Darevsky I., Ryabov S., and Barabanov A. (2004), *Atlas of Reptiles of the North Eurasia: Taxonomic Diversity, Distribution, Conservation Status*, Zoological Institute, Russian Academy of Sciences, St. Petersburg.
- Anderson S. C. (1999), *The Lizards of Iran*, Soc. for the Study of Amphibians and Reptiles, pp. 185 – 190.
- Bauer A. M. and Russell A. P. (1988), "Morphology of geckonid cutaneous sensilla, with comments on function and phylogeny in the Carpodactylini (Reptilia: Gekkonidae)," *Can. J. Zool.*, **66**, 1583 – 1588.
- Bauer A. M., Russell A. P., and Shadwick R. E. (1989), "Mechanical properties and morphological correlates of fragile skin in geckonid lizards," *Exp. Biol.*, **145**, 79 – 102.
- Dujsebajeva T. N. (1995), "The microanatomy of regenerated bristled receptors of two gecko species, *Cyrtopodion fedtschenkoi* and *Sphaerodactylus roosevelti* (Reptilia: Gekkonidae)," *Russ. J. Herpetol.*, **2**(1), 58 – 64.
- Dujsebajeva T. and Ananjeva N. (2001), "Whether Professor Scortecci was right?," *J. Morphol.*, **248**(3), 226.
- Grismer L. L. (1988), "The phylogeny, taxonomy, classification, and biogeography of eublepharid geckos (Reptilia: Squamata)," in: Estes R. D. and Pregill G. K. (eds.), *Phylogenetic Relationships of the Lizards Families*, Stanford Univ. Press, Stanford, pp. 369 – 470.
- Han D. M., Zhou K. Y., and Bauer A. M. (2004), "Phylogenetic relationships among the higher taxonomic categories of gekkotan lizards inferred from C-mos nuclear DNA sequences," *Biol. J. Linn. Soc.*, **83**, 353 – 368.
- Hiller U. (1971), "Form und Funktion der Hautsinnesorgane bei Gekkoniden. I. Light und rasterelektronenmikroskopische Untersuchungen," *Forma Functio*, **4**, 240 – 253.
- Joger U. (1984), "Morphologische und biochemisch-immunologische Untersuchungen zur Systematik und Evolution der Gattung *Tarentola* (Reptilia, Gekkonidae)," *Zool. Jahrb. Anat.*, **112**, 137 – 256.
- Kluge A. G. (1967), "Higher taxonomic categories of geckonid lizards and their evolution," *Bull. Am. Mus. Natur. Hist.*, **135**, 1 – 60.
- Macey R. J., Ananjeva N. B., Wang Y., and Papenfuss T. J. (1997), "A taxonomic reevaluation of the geckonid lizard genus *Teratoscincus* in China," *Russ. J. Herpetol.*, **4**(1), 8 – 17.
- Macey J. R., Fong J. J., Kuehl J. V., Shafiei S., Ananjeva N. B., Papenfuss T. J., and Boore J. L. (2005), "The complete mitochondrial genome of a gecko and the phylogenetic position of the Middle Eastern *Teratoscincus keyserlingii*," *Mol. Phylogen. Evol.* (in press).
- Matveyeva T. N. and Ananjeva N. B. (1995), "The distribution and number of the skin sense organs of agamid, iguanid and geckonid lizards," *J. Zool. Lond.*, **235**, 253 – 268.
- Szczerbak N. N. and Golubev M. L. (1996), *The Gecko Fauna of the USSR and Adjacent Regions*, Soc. for Study of Amphibians and Reptiles, Ithaca.

HEMATOLOGICAL INDEXES OF *Rana ridibunda* IN CLEAN AND CONTAMINATED PONDS

T. Y. Peskova¹ and T. I. Zhukova¹

Keywords: *Rana ridibunda*, Hematological indexes, Pesticides.

INTRODUCTION

Experimental research has been performed of the influence of pesticides on blood of some species of anurans (Gromysz-Kalkowska and Szubartowska, 1986; Szubartowska, 1990; Zhukova and Peskova, 1996). We had determined in laboratory experiments that the blood reaction of *Rana ridibunda* on pesticide pollution depends on dose of toxicant and duration of its influence (Zhukova and Peskova, 1999). In this paper there are some results of our study of hematological indexes of *Rana ridibunda* living in natural ponds with different degree of pesticide pollution.

MATERIAL AND METHODS

The study was conducted in 1998 – 1999 in the Kuban Region (Northern Caucasus). The object is *Rana ridibunda* (200 individuals) from the rice field contaminated by pesticides and a clean pond in arboretum of Kuban State

¹ Kuban State University, Stavropolskaya St., 224/1-49, 350040 Krasnodar, Russia; E-mail: peskova@kubannet.ru.

TABLE 1. The Contents of Residual Chlororganic Compounds (COC) in Water (mg/liter), Ground (mg/kg), and tissues of *Rana ridibunda* (mg/kg)

Object of research	γ -HCCH	DDT	DDD	DDE	Σ COC
Rice field					
Water	N.S.	0.0030	N.S.	0.003	0.0033
Ground	0.0281	0.0343	0.0100	N.S.	0.0724
<i>Rana ridibunda</i>					
muscles	N.S.	0.0027	0.0034	N.S.	0.0061
liver	0.0296	0.0400	0.0229	N.S.	0.0925
Pond in arboretum					
Water	0.0003	0.0006	0.0001	N.S.	0.0010
Ground	0.0030	0.0038	N.S.	N.S.	0.0068
<i>Rana ridibunda</i>					
muscles	0.0003	0.0025	N.S.	N.S.	0.0028
liver	0.0012	0.0048	N.S.	N.S.	0.0060

N.S., not significant.

University. Residual quantities of chlororganic and phosphororganic compounds in water and ground of these ponds as well as in tissues of *Rana ridibunda* are estimated using the method of gasliquid chromatography in the Laboratory of Water Toxicology (KrasSRIFI).

RESULTS AND DISCUSSION

The differences in total chlororganic compounds (COC) in water of rice field and pond of arboretum are connected to quantity of DDT (it is 5 times higher in water of rice field); remaining components (HCCH and metabolites of DDT) are about identical in water of both ponds (Table 1).

The sum of COC in ground of rice field is 10 times higher, than in a pond from arboretum (difference are marked for HCCH, DDT and DDD; only DDE is found in not significant quantities in both ponds). The contamination of ponds promotes accumulation of chlororganic compounds in tissues of *Rana ridibunda*. The maximum concentrations of toxicants are found in a liver. It is equal to the level of concentrations in ground of both ponds and higher, than in water (6 times in arboretum and 28 times in rice field).

The maximum levels of erythrocytes and hemoglobin are shown for frogs in both ponds in summer; in spring and in autumn these indexes significantly declined. The level of leukocytes for frogs from the rice field in summer and

TABLE 2. Hematological Indexes of Adult Males of *Rana ridibunda* from Clean and Contaminated Ponds of Northern Caucasus, in Different Seasons of Researches ($X \pm m$)

Time of research	Pond	Erythrocytes, thousand per mm ³	Hemoglobin, g %	Leukocytes, thousand per mm ³
Spring	clean	290 \pm 10.9	8.1 \pm 0.48	11.0 \pm 2.1
	contaminated	340 \pm 12.0	8.9 \pm 0.77	13.4 \pm 1.9
Summer	clean	520 \pm 13.1	11.5 \pm 0.71	12.1 \pm 2.0
	contaminated	650 \pm 12.2	14.2 \pm 0.82	22.5 \pm 2.1
Autumn	clean	260 \pm 10.5	7.9 \pm 0.52	14.7 \pm 1.9
	contaminated	295 \pm 10.1	8.2 \pm 0.47	19.8 \pm 2.1

TABLE 3. Hematological Indexes of *Rana ridibunda* from Clean and Contaminated Ponds of Northern Caucasus in Summer

Age	Pond	Erythrocytes, thousand per mm ³		Hemoglobin, g %		Reticulocytes, %	
		males	females	males	females	males	females
Adults	clean	520 ± 13.1	550 ± 14.0	11.5 ± 0.71	12.8 ± 0.73	0.3 ± 0.05	0.7 ± 0.06*
	contaminated	650 ± 12.2	695 ± 14.2*	14.2 ± 0.82	16.5 ± 0.91	1.3 ± 0.07	1.6 ± 0.06*
Juveniles	clean		390 ± 11.2		7.5 ± 0.65		1.8 ± 0.08
	contaminated		490 ± 12.0		9.7 ± 0.70		4.1 ± 0.10

* The differences are significant in comparison with the males ($P = 0.05$).

autumn is authentically higher, than this index in spring (Table 2).

We have conducted further analysis of hematological indexes of *Rana ridibunda* in summer taking into account sex and age of animals (Table 3). We had noted sexual dimorphism in number of erythrocytes in the contaminated pond, and also in number of reticulocytes (both in contaminated pond, and in a clean pond); in all cases these indexes are authentically higher for females.

The number of erythrocytes and hemoglobin in juveniles is authentically less than in adults. The quota of reticulocytes in juveniles is more (2.8 – 3.6 times), than in adults, thus the hemopoiesis was more intensive in juveniles.

All hematological indexes of *Rana ridibunda* (both adults and juveniles) in summer are authentically higher (by 21 – 29%) in contaminated pond. The number of reticulocytes significantly increases in contaminated pond 2.1 – 4.3 times. Similar changes were marked earlier (Zhukova and Fic, 1996). However absolute values of these indexes in our research are authentically higher, than it is known from the literature. It is possible to suspect that it is connected with the duration of habitation of amphibians under conditions of pesticide contamination.

The number of leukocytes of *Rana ridibunda* from the rice field is authentically higher (1.9 times), than of amphibians from a pond, that testifies to an increase of protective function of a blood. The number of neutrophils is more in the leukocytic formula of frogs' blood in contaminated ponds.

There is a sexual dimorphism of index of heart in *Rana ridibunda*: in spring (in spawning period) it is higher in females. The index of heart is higher in frogs from

the rice field than from the pond: in adult animals are 7.9 ± 0.66 and $5.0 \pm 0.71\%$, in juveniles are 9.6 ± 0.72 and $6.0 \pm 0.55\%$. The increase of index of heart testifies to higher level of metabolism of amphibians.

The picture of red blood of amphibians permanently inhabiting ponds with pesticide pollution (DDT and its metabolites) is like the one under the short duration of small doses of pesticides (Zhukova and Peskova, 1999). The increase of oxygen capacity of amphibian's blood in the presence of pesticides is long-term adaptation; it is manifestation of effect of directional selection.

REFERENCES

- Gromysz-Kalkowska K. and Szubartowska E. (1986), "Changes in the blood of *Rana temporaria* L. after different doses of trichlorfon," *Folia Biol.*, **34**(1), 21 – 33.
- Szubartowska E. (1990), "Changes in the blood of the frog (*Rana temporaria*) after different doses of ekatin," *Ann. UMCS. C*, **45**, 67 – 78.
- Zhukova T. I. and Fic I. V. (1996), "The comparative characteristic of hematological indexes of *Rana ridibunda* living in ponds of different degree of contamination," in: *Aktual'nye Voprosy Ékologii i Okhrany Prirodi Ékosistem Yuzhnykh i Tsentral'nykh Regionov Rossii [Actual Questions of Ecology and Nature Conservation of Ecosystems of Southern and Central Regions of Russia]*, Krasnodar, pp. 160 – 162 [in Russian].
- Zhukova T. I. and Peskova T. Yu. (1996), "The adaptations of amphibian red blood to the influence of pesticides," in: *Sustainable Development: System Analysis in Ecology*, Sevastopol, pp. 155 – 156 [in Russian].
- Zhukova T. I. and Peskova T. Yu. (1999), "The blood reactions of anurans on pesticide pollution," *Ecology*, No. 4, 288 – 292.

THE RELATIONSHIP BETWEEN BODY LENGTH AND FEMUR BONE THICKNESS IN *Lacerta agilis boemica* AND *L. strigata*. IMPLICATIONS FOR GROWTH INFERENCES FROM SKELETOCHRONOLOGICAL DATA

E. S. Roitberg¹ and E. M. Smirina²

Keywords: body size, femur bone thickness, geographic variation, *Lacerta agilis boemica*, *Lacerta strigata*, lizards, skeletochronology, sexual size differences.

INTRODUCTION

Mark-recapture and experimental studies — the main tool to obtain data on growth and longevity of animals — are very time-consuming. A promising alternative tool to get such data for reptiles and amphibians is investigation of growth layers in their bone tissue. This method provides not only an accurate age determination, but also a quantitative estimation of the pattern of bone growth (Smirina, 1974; Castanet et al., 1977; Castanet and Smirina, 1990; Hemelaar, 1988; Castanet and Baez, 1991; etc.). Due to a generally high correlation between the bone thickness and body size, inferences about body growth are also possible

(Smirina, 1983; Marunouchi et al., 2000 and references therein) provided that the relationship between the body size and the size of growth mark (bone thickness) is known in detail.

MATERIAL AND METHODS

Snout-vent length (*SVL*) and femur bone thickness (*D*, measured as the mean of the minimal and maximal diameter on transverse sections in the middle of the femur diaphysis) were recorded for 320 adults and yearlings of *Lacerta agilis* (subspecies *L. a. boemica*) and a related species *L. strigata* from five lowland and mountain localities in the eastern North Caucasus (Table 1, Fig. 1). The relationship between *SVL* and *D* in homogenous (for species, sex and locality) samples was examined with an allometric

¹ Chair of General & Systematic Zoology, Rostock University, Universitätsplatz 2, D-18055 Rostock, Germany; E-mail: eroit@web.de.

² Koltsov Institute of Developmental Biology Russian Academy of Sciences, Vavilova ul., 26, 119991 Moscow, Russia; E-mail: elsmir@mail.ru.

TABLE 1. Characteristics of the Regression Lines of the Femur Diameter on the Snout-Vent Length in Male and Female Samples of *Lacerta agilis boemica* and *L. strigata* from Eight Populations in the Eastern North Caucasus*

Samples			<i>n</i>	Slope, <i>a</i> **		Y-intercept, <i>ln b</i> **		<i>r</i> ***
Species	Locality	Sex						
<i>agilis</i>	Kostek	M	24	1.069	(0.976 – 1.162)	–5.271	(–6.333) – (–4.210)	0.981
<i>agilis</i>	Kostek	F	30	1.000	(0.851 – 1.149)	–4.539	(–6.222) – (–2.857)	0.933
<i>agilis</i>	Sergokala	M	19	1.158	(0.976 – 1.340)	–6.246	(–8.301) – (–4.191)	0.956
<i>agilis</i>	Sergokala	F	18	1.154	(0.969 – 1.339)	–6.285	(–8.370) – (–4.200)	0.957
<i>agilis</i>	Khuchni	M	28	1.022	(0.862 – 1.183)	–4.711	(–6.527) – (–2.895)	0.932
<i>agilis</i>	Khuchni	F	25	0.995	(0.894 – 1.096)	–4.438	(–5.578) – (–3.298)	0.973
<i>agilis</i>	Termenlik	M	22	0.938	(0.858 – 1.019)	–3.774	(–4.683) – (–2.866)	0.983
<i>agilis</i>	Termenlik	F	29	0.903	(0.825 – 0.982)	–3.439	(–4.315) – (–2.563)	0.977
<i>agilis</i>	Kuli	M	10	0.969	(0.557 – 1.381)	–4.145	(–8.839) – (–0.548)	0.887
<i>agilis</i>	Kuli	F	13	1.131	(1.035 – 1.226)	–6.061	(–7.150) – (–4.972)	0.992
<i>strigata</i>	Kostek	M	26	1.023	(0.905 – 1.141)	–4.744	(–6.081) – (–3.408)	0.965
<i>strigata</i>	Kostek	F	21	1.037	(0.903 – 1.171)	–4.959	(–6.485) – (–3.434)	0.966
<i>strigata</i>	Sergokala	M	14	0.958	(0.819 – 1.097)	–3.993	(–5.574) – (–2.411)	0.974
<i>strigata</i>	Sergokala	F	13	0.642	(0.370 – 0.913)	–0.519	(–3.590) – (–2.552)	0.843
<i>strigata</i>	Khuchni	M	26	1.111	(0.970 – 1.252)	–5.713	(–7.309) – (–4.117)	0.957
<i>strigata</i>	Khuchni	F	24	1.003	(0.884 – 1.121)	–4.558	(–5.899) – (–3.218)	0.966

* Natural log-transformed data, $\ln D = a \ln SVL + \ln b$; ** Estimation and the 95% confidence interval in parenthesis; *** All correlations significant at $P < 0.001$.

formula, $D = aSVL^b$ which is a general form of relative growth of different parts of the body (Gould, 1966; Mina and Klevezal, 1976; Smirina, 1983).

RESULTS

Linear regressions, $\ln D = a \ln SVL + \ln b$ as well as product-moment correlation coefficients, r between $\ln D$ and $\ln SVL$ were calculated for each sample (Table 1). The correlation coefficients amounted 0.84 – 0.98, with 14 of the 16 values exceeding 0.9. High correlation between the SVL and D (0.80 – 0.98) remained even after truncating the data to the SVL range of 76 – 100 mm which was well represented in all study samples.

Slopes (exponent of the allometric equation) varied about unity: in only three of the 16 study samples, 95% confidence intervals were above or below unity (Table 1). This suggests a linearity of the relationship between body length and bone thickness in the two species, at least within the SVL range of ca. 45 – 105 mm.

Heterogeneity of regression slopes in different study samples was not significant (interaction $\ln SVL \times \text{Sex}$: $F_{1,317} = 0.13$, $P = 0.72$; interaction $\ln SVL \times \text{Population}$: $F_{7,317} = 1.53$, $P = 0.15$), so the usual ANCOVA was used for further analysis. The effect of sex, species and locality on the bone thickness when its correlation with SVL is statistically removed was assessed with a three-factor ANCOVA (Table 2). To maintain orthogonality of data, only sympatric populations were included in this analysis. The effects of Sex, Locality and their interaction were highly significant ($P < 0.001$), whereas all effects involving Species were non-significant or only marginally significant ($0.01 < P < 0.05$) (Table 2).

To examine the effect of locality (population) on the femur diameter relative to SVL in more details we conducted a single-factor ANCOVA for each species-sex combination separately. All five populations of *L. agilis* were included in the analyses. In both species the effect of locality was more pronounced in females (*L. agilis*: $F = 12.00$, $df = 4$, $P < 0.001$, coefficient of intraclass correlation $r_1 = 0.335$; *L. strigata*: $F = 9.67$, $df = 2$, $P < 0.001$, $r_1 = 0.325$;) than in males (*L. agilis*: $F = 3.36$, $df = 4$, $P < 0.013$, $r_1 = 0.096$; *L. strigata*: $F = 2.52$, $df = 2$, $P = 0.088$, $r_1 = 0.072$).

To visualize the pattern of sexual, interlocality and interspecific differences for femur diameter relative to SVL , adjusted means of $\ln D$ from an ANCOVA which involved all 16 samples are presented graphically (Fig. 1). In both species, males had consistently higher values than those of females, but the extent of sex differences did vary

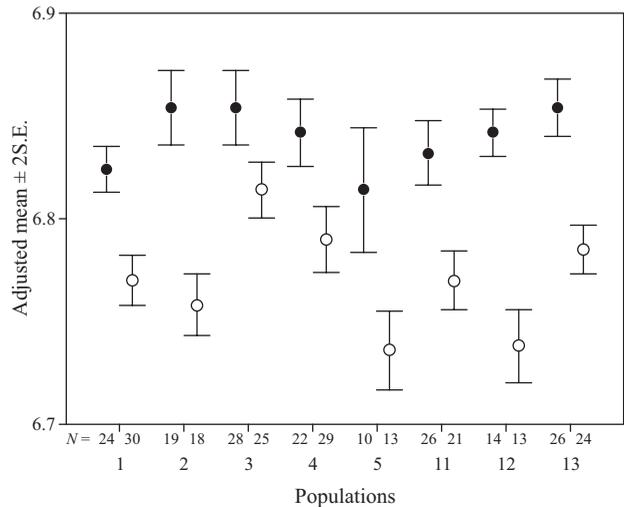


Fig. 1. Adjusted means of ANCOVA with $\ln D$ as the dependent variable, sample as the factor, and $\ln SVL$ as the covariate. Means (solid circles, males; open circles, females) ± 2 standard errors (vertical bars) are indicated. Populations: 1 – 5, *L. agilis*; 11 – 13, *L. strigata*. Localities: 1, 11, Kostek (50 m above sea level); 2, 12, Sergokala (600 m); 3, 13, Khuchni (600 m); 4, Termenlik (960 m); 5, Kuli (1900 m).

among localities (Fig. 1). The latter circumstance was reflected in a highly significant Sex \times Locality interaction of the three-factor ANCOVA considered above. In both species, the interlocality variation in the extent of sex differences is largely determined by the samples of Sergokala exhibiting particularly strong sex differences (Fig. 1). In both sexes of both the species, samples from Khuchni exhibited the highest adjusted mean for $\ln D$ among the five populations of *L. agilis* and the three populations of *L. strigata* (Fig. 1). This has a $(1/5)^2 \times (1/3)^2 \approx 0.0045$ chance of occurring at random which significantly differs from 0.5 (binomial test, $P < 0.001$).

TABLE 2. Analysis of Covariances of the Femur Diameter for Sympatric Populations of *Lacerta agilis boeica* and *L. strigata*

Source	df	F	P
$\ln SVL$	1	2827.965	0.000
Species	1	4.701	0.031
Sex	1	242.841	0.000
Locality	2	21.221	0.000
Species \times Sex	1	4.031	0.046
Species \times Locality	2	2.354	0.097
Sex \times Locality	2	9.053	0.000
Species \times Sex \times Locality	2	0.567	0.568

Notes. The main effects are sex, species, and locality; the covariate is SVL . Morphometric data are log-transformed.

DISCUSSION

Strong and linear interdependence between the *SVL* and femur diameter in the study species enables a quantitative estimation of the pattern of body growth from bone growth using simple back-calculation formulas (Marunouchi et al., 2000; Roitberg and Smirina, in preparation). However, the pattern of this relationship was found to vary not only between sexes but also among populations, especially in females. This provides some problems for use of all regression-based formulas because we should calculate the regression parameters separately for each combination of sex and population whereas the corresponding samples might be insufficiently representative.

Apart from its methodological consequences, the pronounced interlocality variation for femur bone thickness relative to *SVL* revealed in the study species is noteworthy itself. A concordance of this variation in the two species, along with the lack of between-species differences within localities, argues for its exogenous (environmental) determination.

Comparable studies, in which the phalanx of a frog, *Rana temporaria* (Ryser, 1996) and the humerus of a newt, *Notophthalmus viridescens* (Caetano and Leclair, 1996) were used, revealed no or moderate interlocality variation for the relationship between the body length and bone thickness.

Acknowledgment. The work was supported by State Contract 10002-251/P-12/151-150/140503-100.

REFERENCES

- Caetano M. H. and Leclair R. (1996), "Growth and population structure of red-spotted newts (*Notophthalmus viridescens*) in permanent lakes of the Laurentian Shield, Quebec," *Copeia*, **1996**, 866 – 874.
- Castanet J., Meunier F., and Ricqlès A. (1977), "L'enregistrement de la croissance cyclique par le tissu osseux chez les vertébrés poïkilothermes: données comparatives et essai de synthèse," *Bull. Biol. Fr. Belg.*, **111**, 183 – 202.
- Castanet J. and Smirina E. (1990), "Introduction to the skeletochronological method in amphibians and reptiles," *Ann. Sci. Nat. Zool. Paris 13^e Ser.*, **11**, 191 – 196.
- Castanet J. and Baez M. (1991), "Adaptation and evolution in *Gallotia* lizards from the Canary Islands: age, growth, maturity and longevity," *Amphibia-Reptilia*, **12**, 81 – 102.
- Gould S. J. (1966), "Allometry and size in ontogeny and phylogeny," *Biol. Rev.*, **41**, 587 – 640.
- Hemelaar A. M. S. (1988), "Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes," *J. Herpetol.*, **22**, 369 – 388.
- Marunouchi J., Kusano T., and Ueda H. (2000), "Validity of back-calculation methods of body size from phalangeal bones: an assessment using data for *Rana japonica*," *Curr. Herpetol.*, **19**, 81 – 89.
- Mina M. V. and Klevezal G. A. (1976), *Growth of Animals*, Nauka, Moscow [in Russian].
- Roitberg E. S. and Smirina E. M. (in preparation), "Age, body size and growth of *Lacerta agilis boemica* and *L. strigata* in the eastern North Caucasus: a comparative study of two closely related lizard species based on skeletochronology."
- Ryser J. (1996), "Comparative life histories of a low- and high-elevation population of the common frog *Rana temporaria*," *Amphibia-Reptilia*, **17**, 183 – 195.
- Smirina E. M. (1974), "Prospects of age determination by bone layers in Reptilia," *Zool. Zh.*, **53**, 111 – 117.
- Smirina E. M. (1983), "Age determination and retrospective body size evaluation in the live common toads (*Bufo bufo*)," *Zool. Zh.*, **62**, 437 – 444.

THE PATTERNS OF EVOLUTION OF EARLY TRIASSIC HERPETOFAUNA IN EUROPE AND GONDWANA: COMPARISON AND IMPLICATIONS

M. A. Shishkin¹

Keywords: amphibians, reptiles, Early Triassic, faunal evolution.

The Early Triassic (Scythian) history of terrestrial tetrapods is primarily documented by faunal successions of East Europe and South Africa. European succession includes the *Benthosuchus-Wetlugasaurus* (*B-W*) Fauna and succeeding *Parotosuchus* Fauna. These correspond to the regional Vetlugian and Yarenskian Superhorizons and cover the whole range of the Scythian. In South Africa, the coeval faunal units are known from the Karoo basin and termed the *Lystrosaurus* Zone and Lower *Cynognathus* Subzone. These two successions show striking difference in their taxonomic composition, in the patterns of evolutionary change within the earlier units, and, lastly, in the ways of faunal replacement in the transition from the earlier member of each succession to younger one.

Specifically, this implies the following. (1) Both of East European faunas are dominated by temnospondyl amphibians, while those of South Africa by therapsid reptiles. (2) The European *B-W* fauna demonstrates 3 to 4 low-rank transformations through its range, while in its presumed South African equivalent, the *Lystrosaurus* Zone, the biotic changes are difficult to discern. (3) In contrast to the pattern demonstrated by South African succession, in East Europe the evolution from the *B-W* Fauna to *Parotosuchus* Fauna was based mainly on phylogenetic changes or on the replacements within closely related groups.

The latter pattern may be evidenced in many ways. Of temnospondyl genera dominating the European *B-W* Fauna, the capitosaurid *Wetlugasaurus* is very close to the ancestry of succeeding *Parotosuchus*, while the benthosuchid trematosauroids form a morphocline (from *Benthosuchus* to *Angusaurus*) toward the condition of true trematosaurids known from the *Parotosuchus* Fauna. The early Vetlugian aberrant brachyopod *Tupilakosaurus* is replaced by closely related Yarenskian brachyopod *Batrachosuchoides* (with some barren interval between them). Amongst parareptiles, the Vetlugian procolophonids *Tichvinskia* and *Orenburgia* persist into the early Yarenskian and give rise to more advanced taxa. Similarly, of rauisuchid archo-

sauurs, *Tsylmosuchus* ranges across the Vetlugian-Yarenskian boundary and is subsequently replaced by *Vychegdosuchus*. Similar ranging is also suggested for proterosuchid and erythrosuchid archosaurs. All this shows that the Late Scythian (Yarenskian) European herpetofauna was basically autochthonous in origin.

By contrast, the Gondwanan *Lystrosaurus* Zone assemblage is not directly ancestral to the *Cynognathus* Zone community as they are markedly distinct in composition. The guide amphibian groups of these units (lydekkerinids and capitosaurids respectively) are only distantly related. Likewise, the reptilian families of the *Lystrosaurus* Zone do not persist into the succeeding zone, except for the Procolophonidae and possibly some cynodont lineages.

Since late 1980s the attempts were made to account for this discontinuity by suggesting a wide chronological gap between the South African biozones, such that the *Lystrosaurus* Zone was placed in the Early Induan and *Cynognathus* Zone in the Late Olenekian (Anderson and Cruikshank, 1978; Battail, 1988). As the candidates to fill the gap between them there were suggested the fauna of the Arcadia Formation of Australia and/or that of the Middle Sakamena Group of Madagascar. But in fact, none of the Scythian assemblages known “can be shown to be of intermediate position through possession of a transitional taxonomic composition” between the South African biozones (Cosgriff, 1984).

The alternative concept suggests that South African succession is not chronologically disjunct, with the *Lystrosaurus* Zone ranging from the Induan to Early Olenekian (Ochev and Shishkin, 1984, 1989; Shishkin et al., 1995, 1996). This conclusion is primarily based on the fact that in East Europe the appearance of typical, *Procolophon*-like, procolophonids with differentiated teeth falls on the Early Olenekian (late Vetlugian). On the other hand, in South Africa *Procolophon* is most abundant in the upper part of the *Lystrosaurus* Zone which is devoid of the index genus *Lystrosaurus* (Neveling et al., 1999). Of further importance is that the basal beds of the zone contain primitive procolophonid *Owenetta* which is comparable by evo-

¹ Paleontological Institute Russian Academy of Sciences, Profsoyuznaya ul., 123, 117997 Moscow, Russia;
E-mail: shishkin@paleo.ru; schsz@orc.ru.

lutionary level with the Induan *Phaanthosaurus*-related procolophonids of East Europe. All this supports the attribution of the upper part of the *Lystrosaurus* Zone to the Early Olenekian. Additional evidence may be provided by the occurrence in this zone of a rhytidosteid amphibian (*Pneumatostega*). Although the earliest rhytidosteoid record is from the Late Permian, the epibole of the group corresponds to the Early Olenekian as is documented by its record from the Smithian beds of Spitzbergen, Siberia, Australia, and Madagascar.

The upper member of South African Scythian succession, the Lower *Cynognathus* Subzone, is unquestionably Late Olenekian in age (Shishkin et al., 1995), hence a time gap between it and the *Lystrosaurus* Zone seems highly unlikely. Combined with a sharp difference in their taxonomic composition, this fact leads to conclusion that biotic events underlying the rise of the *Cynognathus* assemblage were mostly biogeographic rather than strictly phylogenetic (Shishkin et al., 1996). The amphibian component of the assemblage was suggested to have been derived mainly from the Australo-Tasmanian, and partially the Euramerican, ancestors.

Recently, based on new amphibian finds from the *Lystrosaurus* Zone, some authors (Damiani et al., 2000, 2001) concluded that the zone actually extends to the Late Olenekian, and that some genera are shared by both the *Lystrosaurus* and *Cynognathus* zones, thus pointing to their closer phylogenetic links than it was presumed earlier.

These conclusions are ill-evidenced. Of the finds thought to back them, the first one is the benthosuchid-designed lower jaw, which was originally assigned to trematosaurid Trematosuchus known from the *Cynognathus* assemblage (Neveling et al., 1999), but later re-identified as “Trematosauridae gen. ind.” Its dating by Damiani et al. (2000) is based on rather odd belief that all the trematosaurids are Late Olenekian in age. In reality, the bulk of them belong to the Smithian (*Arctoceras blomstrandii* Zone of Spitzbergen and *Flemingies flemingianus* Zone of Madagascar). In addition, the pattern of postglenoid area (PGA) of the mandible discussed is much more primitive than in trematosaurids because it demonstrates neither the presence of dorsal concavity nor the clear-cut demarcation between the dorsal and lingual PGA sides. With respect to the latter character and limited backward extent of the prearticular, the observed condition is even more primitive than in the late Vetlugian European trematosauroids (Thoosuchinae). All this shows unequivocally that the South African find is not younger in age than the Early Olenekian.

Another alleged evidence of close faunal links between two South African biozones is said to be provided

by a fragment of the capitosaurid mandible from the *Lystrosaurus* Zone (Damiani et al., 2001). It was attributed to so-called Watsonisuchus, a genus based on the holotype of “*Wetlugasaurus*” *magnus* Watson from basal part of the *Cynognathus* Zone. This attribution was also extended by the cited authors to Australian “parotosuchians,” which have been distinguished by Schoch and Milner (2000) as *Rewanobatrachus*. Both the find discussed and *Rewanobatrachus* are the *Wetlugasaurus*-grade capitosaurids showing a primitive design of the PGA (type I by Maryańska and Shishkin, 1996). The latter displays (a) posteriorly tapered dorsal surface; (b) parallel dorsal ridges (crista surangularis and c. medialis) separated only by a groove; (c) weakly developed or absent lingual ridges, with the area above them belonging to lingual surface of the PGA; and (d) shallow postglenoid wall.

However, the discussed South African find is clearly distinct from “*Watsonisuchus*” *magnus* which is actually an advanced, *Parotosuchus*-grade form. Although in its holotype the PGA is incompletely preserved, it allows one to see all principal characters of the *Parotosuchus* PGA pattern (Maryańska and Shishkin, 1996; type III): broad dorsal surface with c. medialis widely diverging from c. surangularis; well developed lingual ridges with the area just above them belonging to the dorsal PGA surface; and high postglenoid wall. In all these respects, the “*Watsonisuchus*” type is indistinguishable from *Kestrosaurus* Houghton, the commonest *Parotosuchus*-grade amphibian of the Lower *Cynognathus* Subzone, and most likely is a junior synonym of *Kestrosaurus*.

Hence, no amphibian genera is actually known to be shared by the *Lystrosaurus* and *Cynognathus* zones of South Africa. However, the very fact of the presence of the capitosaurid in the *Lystrosaurus* Zone is notable and should be assessed in the context of the Early Scythian capitosaurid record in Gondwana overall.

In South Africa, the above-discussed capitosaurid find supplements the lydekkerind-dominated amphibian assemblage which includes also the dissorophoid and rhinesuchid relics along with a rhytidosteid and tupilakosaurid. In the *Lystrosaurus* Zone of Antarctica the capitosaurids are equally known only by a single scrap associated with a lydekkerinid, tupilakosaurid (personal observation) and non-diagnosable temnospondyl remains. Published data on the presence there of brachyopids and rhytidosteids cannot be confirmed (personal observation). In the Panchet Beds of India, apparently of Induan age, the amphibians are also dominated by lydekkerinids but show some increase in the role of capitosaurids. The latter include *Pachygonia* and some of the specimens attributed to the lydekkerinid *Indobenthosuchus* and also *Indolyrocephalus*.

Other local amphibians comprise a lonchorhynchine, a tupilakosaurid and primitive rhytidosteoid.

The only purely terrestrial assemblage from the Early Scythian (Induan?) of Gondwana that shows the abundance of primitive capitosaurids is that of the Arcadia Formation in Australia. Here these forms (*Rewanobatrachus*) are associated primarily with rhytidosteoids, a brachyopod, and early chigutisaurid. In Australo-Tasmanian region the presence of primitive capitosaurids is also evident for the Early Smithian assemblages of the Knocklofty Formation and Blina Shale dominated by rhytidosteids and brachyopids. Another record of Smithian capitosaurids in Gondwana refers to the nearshore marine sediments of the Middle Sakamena Group of Madagascar. Here the capitosaurids are rather common and include *Edingerella* and *Deltacephalus* (often erroneously attributed to lydekkerinids). Like in nearshore Smithian deposits of Spitzbergen, an important role in the assemblage belongs to trematosaurids and rhytidosteids.

In all, these data warrant the conclusion that during the Early Scythian the center of the capitosaurid radiation in southern Gondwana fell on the Australo-Tasmanian region. The rest of the territory, except (to some extent) for India and some coastal biotopes, obviously belonged to remote marginal zones of the capitosaurid dispersal. In this light it seems not unlikely that poorly detectable occurrence of capitosaurids in the *Lystrosaurus* Zone of South Africa was only a short-term episode.

To assess the run of further faunal events in southern Gondwana, of utmost importance is the fact that only in Australo-Tasmania the Early Scythian capitosaurids are associated with brachyopids, with both groups being rather common. The crucial point is that it is just the same combination of temnospondyl families that constitutes the main amphibian component of the Late Scythian *Cynognathus* Subzone assemblage of South Africa. In addition, the commonest Australo-Tasmanian brachyopod, *Blinasaurus*, is very close to *Batrachosuchus* from the *Cynognathus* Zone and sometimes even synonymized with the latter (Warren and Marsicano, 1998). On the other hand, in the antedating *Lystrosaurus* Zone these groups are either lacking (as with brachyopids) or exceedingly scarce. Hence, it seems sound to conclude that they came to South Africa from outside as a result of an overall re-patterning of the tetrapod distribution in Gondwana during Early to Late Olenekian transition. This conclusion is strongly corroborated by a well-known fact of sharp discontinuity between the reptilian components of the *Lystrosaurus* and *Cynognathus* Zones.

Acknowledgment. The paper is sponsored by the Russian Foundation for Basic Research (grant No. 04-05-64741) and the

Program of the Russian Academy of Science: “The Origin and Evolution of the Biosphere.”

REFERENCES

- Anderson J. M. and Cruickshank A. R. I.** (1978), “The biostratigraphy of the Permian and the Triassic. Part 5,” *Palaeontol. Afr.*, **21**, 15 – 44.
- Battail B.** (1988), “Biostratigraphie des formations permo-triassiques continentales à vertébrés tétrapodes et biogéographie du Gondwana,” *Ann. Soc. Géol. Nord*, **57**, 37 – 44.
- Cosgriff J. W.** (1984), “The temnospondyl labyrinthodonts of the earliest Triassic,” *J. Vertebr. Paleont.*, **4**(1), 32 – 46.
- Damiani R., Neveling J., Hancox P. J., and Rubidge B. S.** (2000), “First trematosaurid temnospondyl from the *Lystrosaurus* Assemblage Zone of South Africa and its biostratigraphic implications,” *Geol. Mag.*, **137**(6), 659 – 665.
- Damiani R. J., Neveling J., and Hancox P. J.** (2001), “First record of a mastodontosaurid (Temnospondyli, Stereospondyli) from the Early Triassic *Lystrosaurus* Assemblage Zone (Karoo Basin) of South Africa,” *N. Jb. Geol. Paläont. Abh.*, **221**(2), 133 – 144.
- Hancox P. J., Shishkin M. A., Rubidge B. S., and Kitching J. W.** (1995), “A threefold subdivision of the *Cynognathus* Assemblage zone and its palaeogeographical implications,” *S. Afr. J. Sci.*, **91**, 143 – 144.
- Maryańska T. and Shishkin M. A.** (1996), “New cyclotosaurid (Amphibia: Temnospondyli) from the Middle Triassic of Poland and some problems of interrelationships of capitosauroids,” *Prace Muz. Ziemi*, **43**, 53 – 83.
- Neveling J., Rubidge B. S., and Hancox P. J.** (1999), “A lower *Cynognathus* Assemblage Zone fossil from the Katberg Formation,” *S. Afr. J. Sci.*, **95**, 555 – 556.
- Ochev V. G. and Shishkin M. A.** (1984), “Principles of global correlation of the continental Triassic based on tetrapods,” in: *Abstrs. of the 27th Int. Geol. Congr.*, Vol. 1, p. 146.
- Ochev V. G. and Shishkin M. A.** (1989), “On the principles of global correlation of the continental Triassic on the tetrapods,” *Acta Palaeont. Pol.*, **34**(2), 149 – 173.
- Schoch R. R. and Milner A. R.** (2000), *Handbuch der Paläoherpetologie. Teil 3B. Stereospondyli*, Dr. Friedrich Pfeil Verlag, München.
- Shishkin M. A., Rubidge B., and Hancox J.** (1995), “Vertebrate biozonation of the Upper Beaufort Series of South Africa — a new look on correlation of the Triassic biotic events in Euramerica and southern Gondwana,” in: *Proc. of the Sixth Symp. on Mesozoic Terr. Ecosyst. and Biota, Beijing*, Ocean Press, pp. 39 – 41.
- Shishkin M. A., Rubidge B., and Hancox J.** (1996), “Comparison of tetrapod faunal evolution during Early Triassic in Eastern Europe and South Africa,” in: *Abstrs. of the 9th Biennial Conf. S. Afr. Paleontol. Soc.*, Stellenbosch.
- Warren A. A. and Marsicano C.** (1998), “Revision of the Brachyopidae (Temnospondyli) from the Triassic of the Sydney, Carnarvon and Tasmania basins, Australia,” *Alcheringa*, **22**, 329 – 342.

THE URODELE METAMORPHOSIS: REGULATORY MECHANISMS AND EVOLUTION

S. V. Smirnov¹

Keywords: urodeles, metamorphosis, thyroid hormones, evolution.

The primary amphibian life cycle is represented by the aquatic larva and terrestrial adult. To transform from a larva to an adult, amphibians undergo metamorphosis during which larval specializations disappear and adult ones develop. Such pattern of ontogeny was typical for early fossil amphibians and is displayed by many recent amphibians.

Most views of the amphibian metamorphosis are based on studies on anurans. However, to reconstruct the evolution of the amphibian metamorphosis, study of the urodele metamorphosis seems to be much more promising since it most closely resembles that in early tetrapods (Shishkin, 1973; Boy and Sues, 2000; etc.).

In urodeles, larvae differ from adults in many features. Thus, larvae have external gills, labial folds, and dorsal and caudal fins, among others. In the skin, larvae have Leydig cells as skin glands, whereas adults have multicellular glands. In the blood, larvae have larval hemoglobin which differs from adult one in its affinity to oxygen. Larvae lack a nasolacrimal duct which is characteristic for adult salamanders.

This difference between larval and adult morphologies increases at the transition from primitive to advanced salamanders. Thus, typical urodele larvae have such larval specializations as toothfields represented by several rows of teeth situated on the vomer and palato-pterygoid (Fig. 1a). In adult primitive salamanders, hynobiids, the toothfield on the palato-pterygoid is absent (in parallel with the absence of the underlying dentigerous palatine portion of the palato-pterygoid), whereas the toothfield on the vomer is replaced by one tooth row (Fig. 1b). In adult advanced urodeles, salamandrids, this vomerine tooth row is shifted on the caudal outgrowth of the vomer, vomerine bar, extending along the lateral margins of the parasphenoid (Fig. 1c). In plethodontids, the most advanced urodeles, the complete palato-pterygoid is absent, whereas a new toothfield develops along the parasphenoid (Fig. 1d).

In this case, difference between the larval and adult morphology increases due to the development of new

adult specializations. This difference also increases due to the changes in the larval morphology. Evolution of the urodele larva is accompanied by a progressive freeing a larva from features associated with adult terrestrial stage of life cycle. Whereas in primitive salamanders many adult specializations develop as early as in larvae, their development is shifted toward metamorphosis in evolutionary advanced salamanders. Thus, in hynobiids, an adult hemoglobin, multicellular skin glands, nasolacrimal duct, and nasal capsule, all develop as early as in larvae (Medvedeva, 1975; Lebedkina, 1979; Wakahara and Yamaguchi, 1996). In contrast, in advanced urodeles (salamandrids and plethodontids), they appear just before metamorphosis or during metamorphosis (Vorontsova et al., 1952; Medvedeva, 1975; Lebedkina, 1979; Flavin et al., 1982; Rose, 1995a, 1996; etc.).

Both evolutionary tendencies lead to the increasing complication of metamorphosis since (1) the every system undergoes more complicated transformation and (2) more and more developmental events become concentrated within a brief interval of ontogeny. Consequently, the most complicated metamorphosis is displayed by the most advanced salamanders, plethodontids (Rose, 1996). Surprisingly, it is in plethodontids that metamorphosis is the most shortened.

Hormones of the thyroid gland (TH) are traditionally considered as a global cue inducing the urodele metamorphosis. To reveal a role of TH in mediating the urodele development, cranial ontogeny was examined in animals treated with exogenous TH of different concentrations and in animals with inhibited thyroid activity (Smirnov and Vassilieva, 2002, 2003). It was revealed that cranial bones appearing in the early larval stage are TH-independent, those appearing in the midlarval stage display only a slight TH-dependence, and bones appearing just before metamorphosis or at metamorphosis are triggered by TH. Also, it was revealed that if in phylogeny an early-appearing structure becomes shifted to the late larval stage or metamorphosis, it transforms from TH-noninducible to TH-inducible. Thus, in *Salamandrella keyserlingi* (Hynobiidae), the nasal, maxilla, and prefrontal, all appearing in the mid-

¹ Severtzov Institute of Ecology and Evolution Russian Academy of Sciences, Leninsky pr., 33, 119071 Moscow, Russia;
E-mail: evol_morphol_lab@newmail.ru.

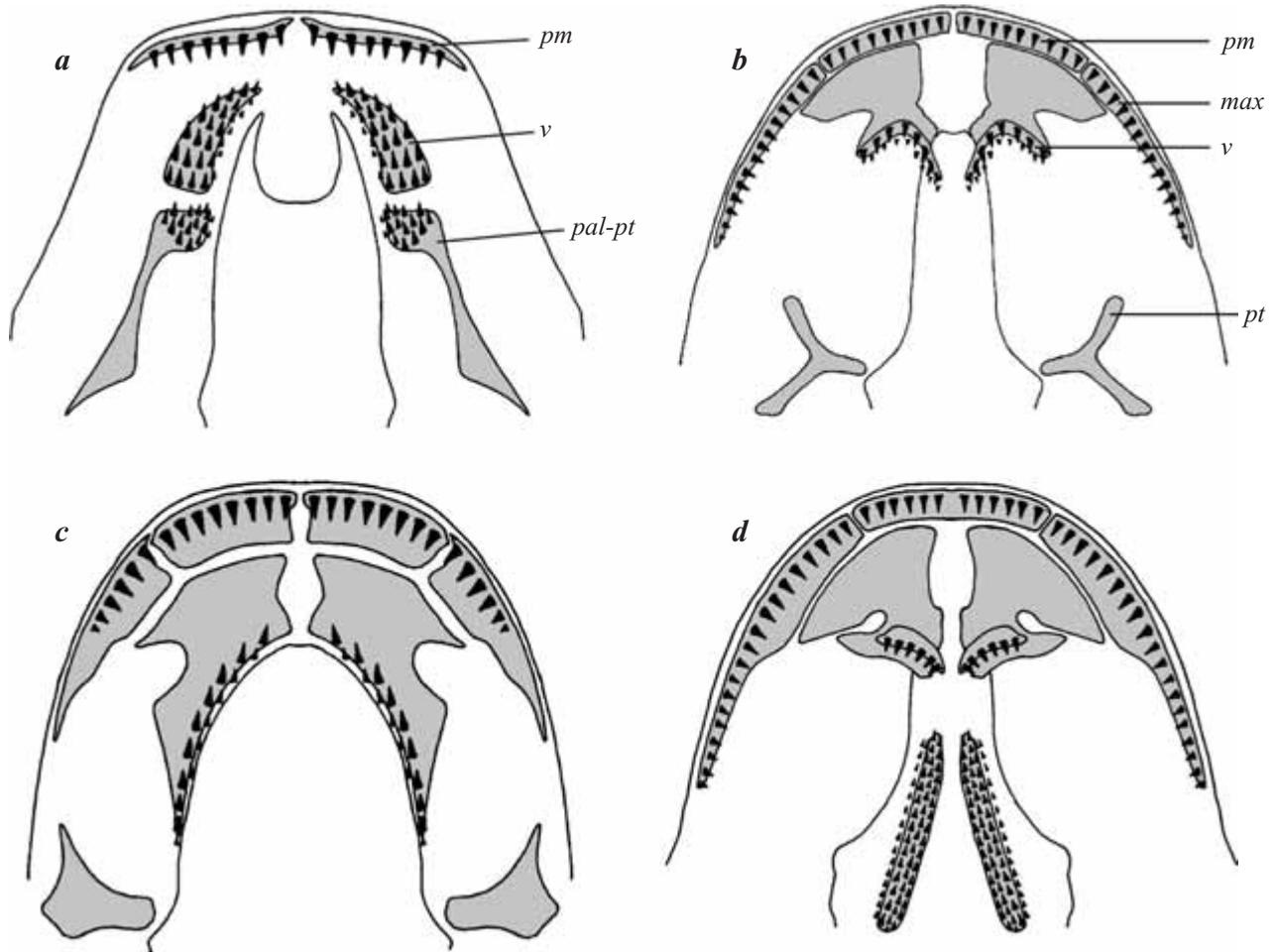


Fig. 1. Ventral view of the urodele skull: *a*, larva; *b*, adult *Salamandrella keyserlingi* (Hynobiidae); *c*, adult *Pleurodeles waltl* (Salamandridae); *d*, adult *Desmognathus brimleyorum* (Plethodontidae). Stippling indicates bones; teeth are in solid black. *max*, Maxilla; *pal-pt*, palato-ptyergoid; *pm*, premaxilla; *pt*, pterygoid; *v*, vomer.

larval stage, develop (though with a delay) even in the absence or deficiency of TH. In contrast, in *Triturus vulgaris* (Salamandridae), these bones appearing just before metamorphosis, fail to develop in the absence of TH. That is, their development is triggered by these hormones. In *Eurycea bislineata* (Plethodontidae), these bones appearing in metamorphosis also are TH-inducible (Rose, 1995b).

Similar tendency may be traced in the development of other systems. For example, in *Hynobius retardatus* (Hynobiidae), replacement of the larval hemoglobin by an adult one begins in the midlarval stage and is TH-independent (Satoh and Wakahara, 1997). In contrast, in *Pleurodeles waltl* (Salamandridae), it begins at the end of larval stage, just before metamorphosis, and is TH-dependent (Flavin et al., 1982).

Consequently, one may conclude that, firstly, urodeles display an evolutionary tendency toward the concentration of more and more ontogenetic events within a metamorphic period. Secondly, developmental events shifted to the metamorphic period, tend to become TH-induced. If summarized, it means that the urodele evolution is accompanied by the progressive increase of the regulatory role of TH as more and more features and systems become TH-dependent. Role of TH is minimal in the ontogeny of primitive salamanders (hynobiids) and maximal in advanced ones (plethodontids).

In some cases, evolution of the thyroid involvement in the urodele ontogeny and metamorphosis may be traced. Thus, in hynobiids, development of such dermal cranial bones as septomaxilla and lacrimal, is under the control of two factors. The nasolacrimal duct acts inductively upon these bones (Medvedeva, 1975), whereas TH promote

their calcification thus playing a minor role in the regulation of their development (Smirnov and Vassilieva, 2002).

Salamandrids lack a separate lacrimal but it is present as a part of the prefrontal (Lebedkina, 1979). In them, TH trigger the development of the latter (Smirnov and Vassilieva, 2003), whereas a nasolacrimal duct plays a minor regulatory role — it determines its shape (Medvedeva, 1975).

In plethodontids, the development of the septomaxilla is induced by TH, whereas a nasolacrimal duct seems not to participate in the regulation of its development (septomaxilla forms even in the absence of this duct) (Rose, 1995b).

This model illustrates the evolutionary switch from non-hormonal control to hormonal one. Developmental events which in primitive salamanders are triggered by inductive tissue interactions become shifted under the hormonal control in advanced salamanders. It seems that it is the increasing involvement of TH in the urodele ontogeny that allows to shorten metamorphosis in advanced salamanders.

In the ontogeny which is regulated mainly via a system of tissue inductive interactions, development unfolds as a network of inductions. That is, each phase of development is induced by the previous one and, in turn, triggers the onset of the next phase of development. In this case, no larval trait can be deleted even if not functional. However, transition from inductive tissue interactions to hormonal factors as regulatory mechanisms allows to eliminate some intermediate larval traits and to shorten both the whole developmental process and metamorphic transition. Moreover, this switch to hormonal control allows to decouple metamorphic events (induced mainly by TH) from larval developmental events (induced mainly by tissue interactions). That is, it provides independence of larval and adult developmental programs, which is considered as a precondition for evolution of direct development (Alberch, 1987; Hanken et al., 1992). Under such circumstances, it is not surprising that among urodeles, the direct development is characteristic only of plethodontids, phylogenetically advanced salamanders with advanced system of TH-mediation.

Acknowledgments. The assistance of Dr. Anna Vassilieva in many aspects of this project is gratefully acknowledged. Kirill Dzerzhinsky assisted in the preparation of the manuscript and illustrations. This study was supported by the Russian Foundation for Fundamental Research (grant No. 04-04-48916a).

REFERENCES

- Alberch P.** (1987), “Evolution of a developmental process: Irreversibility and redundancy in amphibian metamorphosis,” in: Raff R. A. and Raff E. C. (eds.), *Development as an Evolutionary Process*, Alan R. Liss, New York, pp. 23 – 46.
- Boy J. A. and Sues H.-D.** (2000), “Branchiosaurs: larvae, metamorphosis and heterochrony in temnospondyls and seymouriamorphs,” in: Heatwole H. and Carroll R. L. (eds.), *Amphibian Biology*, Vol. 4, Surrey Beatty and Sons, Australia, pp. 1150 – 1197.
- Flavin M., Duprat A. M., and Rosa J.** (1982), “Effect of thyroid hormones on the switch from larval to adult hemoglobin synthesis in the salamander *Pleurodeles waltlii*,” *Cell. Differ.*, **11**, 27 – 33.
- Hanken J., Klymkowsky M. W., Summers C. H., Seufert D. W., and Ingebrigtsen N.** (1992), “Cranial ontogeny in the direct-developing frog, *Eleutherodactylus coqui* (Anura: Lepidodactylidae), analyzed using whole-mount immunohistochemistry,” *J. Morphol.*, **211**, 95 – 118.
- Lebedkina N. S.** (1979), *The Evolution of Amphibian Skull*, Nauka, Moscow [in Russian].
- Medvedeva I. M.** (1975), *The Olfactory Organ of Amphibians and Its Phylogenetic Significance*, Nauka, Leningrad [in Russian].
- Rose C. S.** (1995a), “Skeletal morphogenesis in the urodele skull: I. Postembryonic development in the Hemidactyliini (Amphibia: Plethodontidae),” *J. Morphol.*, **223**, 125 – 148.
- Rose C. S.** (1995b), “Skeletal morphogenesis in the urodele skull: III. Effect of hormone dosage in TH-induced remodeling,” *J. Morphol.*, **223**, 243 – 261.
- Rose C. S.** (1996), “An endocrine-based model for developmental and morphogenetic diversification in metamorphic and paedomorphic urodeles,” *J. Zool.*, **239**, 253 – 284.
- Satoh S. J. and Wakahara M.** (1997), “Hemoglobin transition from larval to adult types in a salamander (*Hynobius retardatus*) depends upon activity of the pituitary gland, but not that of the thyroid gland,” *J. Exp. Zool.*, **278**, 87 – 92.
- Shishkin M. A.** (1973), *The Morphology of the Early Amphibia and Some Problems of the Lower Tetrapod Evolution*, Nauka, Moscow [in Russian].
- Smirnov S. V. and Vassilieva A. B.** (2002), “The bony skull of the Siberian salamander, *Salamandrella keyserlingi* (Amphibia: Urodela: Hynobiidae) and the role of thyroid hormones in its development,” *Dokl. Biol. Sci.*, **385**, 387 – 389.
- Smirnov S. V. and Vassilieva A. B.** (2003), “Skeletal and dental ontogeny in the smooth newt, *Triturus vulgaris* (Urodela: Salamandridae): role of thyroid hormone in its regulation,” *Russ. J. Herpetol.*, **10**(2), 93 – 110.
- Vorontsova M. A., Lyozner L. D., Markelova I. V., and Puchalskaya E. Ch.** (1952), *Newt and Axolotl*, Sovetskaya Nauka, Moscow [in Russian].
- Wakahara M. and Yamaguchi M.** (1996), “Heterochronic expression of several adult phenotypes in normally metamorphosing and metamorphosis-arrested larvae of a salamander *Hynobius retardatus*,” *Zool. Sci.*, **13**, 483 – 488.

ON THE MORPHOFUNCTIONAL PECULIARITIES OF THE JAW APPARATUS OF OPHIOPHAGOUS ELAPID SNAKES AND ON THE SOME STAGES IN EVOLUTION OF ELAPIDAE

A. Yu. Sokolov¹

Keywords: Reptilia, Serpentes, Elapidae, *Calliophis*, *Bungarus*, *Maticora*, jaw apparatus, functional morphology.

INTRODUCTION

Jaw elongation is an important specialization for swallowing prey of large diameter in most alethinophidian snakes (Macrostomata). Thus the inability of many specialized snake-eating species of elapids (species of the genera *Calliophis*, *Maticora*, *Bungarus*, *Micrurus*, and some others) to gape widely (despite a macrostomatan skull) is surprising. The evolution of limited gape may relate to their selection of narrow and elongate prey, but the reasons for the appearance deserve special consideration.

MATERIAL AND METHODS

Prey capture and swallowing were observed in *Calliophis maccllellandi* (one specimen). Fresh heads of three specimens of *Bungarus multicinctus*, two specimens of *Calliophis maccllellandi* and two specimens of *Maticora bivirgata* were studied by layer-by-layer dissection of muscles under low magnification using an MBC-2 binocular microscope. Cranial kinesis was studied. The interaction with prey was modeled and functional analysis was made. These data were compared with similar data obtained from other elapids (*Acanthophis praelongus*, *Naja kaouthia*, and *N. pallida*) feeding on larger diameter prey and with species of other groups, besides Elapidae, that feed on large diameter prey (Sokolov, 2001a, 2001b).

RESULTS AND DISCUSSION

Earlier (Sokolov, 2001a, 2001b) it was shown, that the classical snake-like prey-transport mechanism of alternately engaging the left and right side has a significant fault when the prey is large in diameter. The cause is using protractor muscles which are fastened on the braincase. The efficiency of the mechanism decreases when prey di-

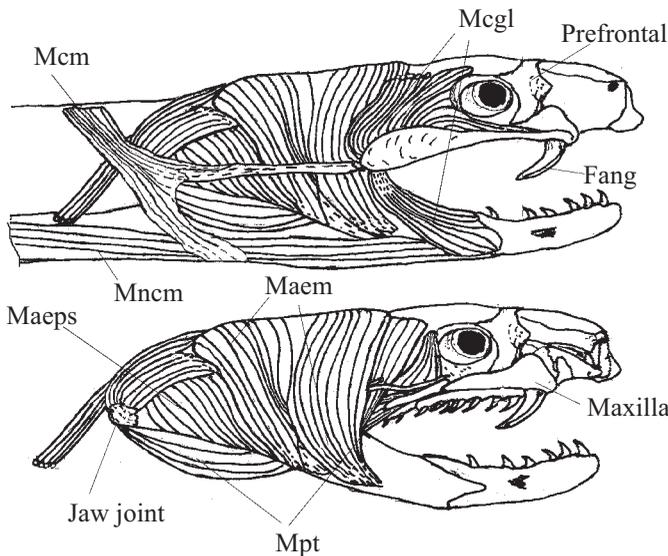
ameter increases. Apparently unilateral transport evolved as a critical specialization of a limbless mud-dwelling snake ancestor to feeding on earthworms or leeches. The swallowing of hard immobile prey alive required advance on the prey by fixed steps. This stage made the snake jaw apparatus plastic (the prey is not crushed [broken] by jaws before entry into the esophagus). After transition to feeding on vertebrates colubroid snakes did not lose the plesiomorphic prey-transport mechanism and only later after transition to feeding on thick vertebrates classical mechanism was supplied by other prey-engaging mechanisms, faultless in swallowing prey of large diameter (other protracting muscles are used in the beginning of step on prey surface). For example advanced Elapidae use the pterygoideus muscle to protract the lower jaw when the teeth of the upper jaw have been fixed on the surface of prey. Specialization for feeding on elongate vertebrates was the first stage of vertebrate-feeding transition and as such, the basal prey-transport mechanism was sufficient. Many specialized snake-eating elapids can use the basal mechanism only (m. pterygoideus has “henophidian” organization and attachment peculiarities). But unlike leeches and earthworms killing or immobilizing little elongate vertebrates is not so hard. The ability to inflict a powerful bite on vital centers of the body, especially on the head or not far from it, is quite important. Some snake-eating snakes, including elapids try to restrain prey using their jaws. During the bite the kinetic upper part of the skull becomes rigid. This is achieved by caudally directed forces applied to the palatamaxillary arches (especially to the maxillae — see Fig. 1) and quadrates and generated by contraction of the palatine retractor muscle (Mrp), m. pterygoideus (Mpt), m. adductor mandibulae externus posterior (Maeps), m. neurocostomandibularis (Mncm), and possibly m. cervicomandibularis (Mcm). These muscles create a “bowstring-like” effect and fix the skull construction in the maximally retracted position (see Cundall and Irish, 1989, for a similar effect in *Casarea*). In this situation the adductor muscles, especially the powerful m. adductor externus medius (Maem), use the jaw apparatus in a crocodile-like man-

¹ Department of Vertebrate Zoology, Faculty of Biology, M. V. Lomonosov Moscow State University, Vorobyevy Gory, 1, k. 12, 119992 Moscow, Russia; E-mail: apophis@hotmail.ru.

ner — the jaw has a long lever arm. In low snake species with flattened skulls vertically oriented muscle fibers are relatively short and limit gape size. Thus in this construction Maem and Maeps apply vertically and caudally oriented forces to the lower jaw. The compressor of the venom gland (Mcgl) is made of most unnecessary as adductor portion — m. adductor externus anterior. The pressing of skull is (forces generated during jaw adduction are) effectively transmitted on to the fang which is situated under or near the prefrontal bone which is fixed in a vertical po-

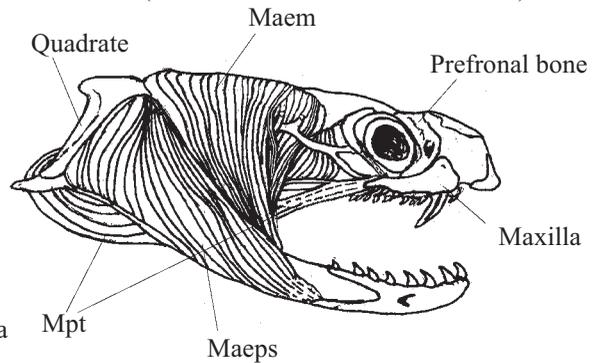
sition. The fang can be used in a mammal-like manner — in perforating some bone structures, the braincase for example. The inflowing of venom through the fang can lead to rapid immobilization of the victim. The analysis of the jaw apparatus of many other elapid species suggested that adaptation occurred during the evolution of family Elapidae. Analysis of the literature (e.g., Haas, 1930; Cundall and Green, 2000) about functional morphology and feeding peculiarities also infer that proteroglyphic sea-snakes experienced modifications of the jaw apparatus similar to

Calliophis maclellandi



Bungarus multicinctus

(some muscles have been extracted)



Scheme of the creation of caudally directed forces during the bite
(model object is the jaw apparatus of *Bungarus multicinctus*)

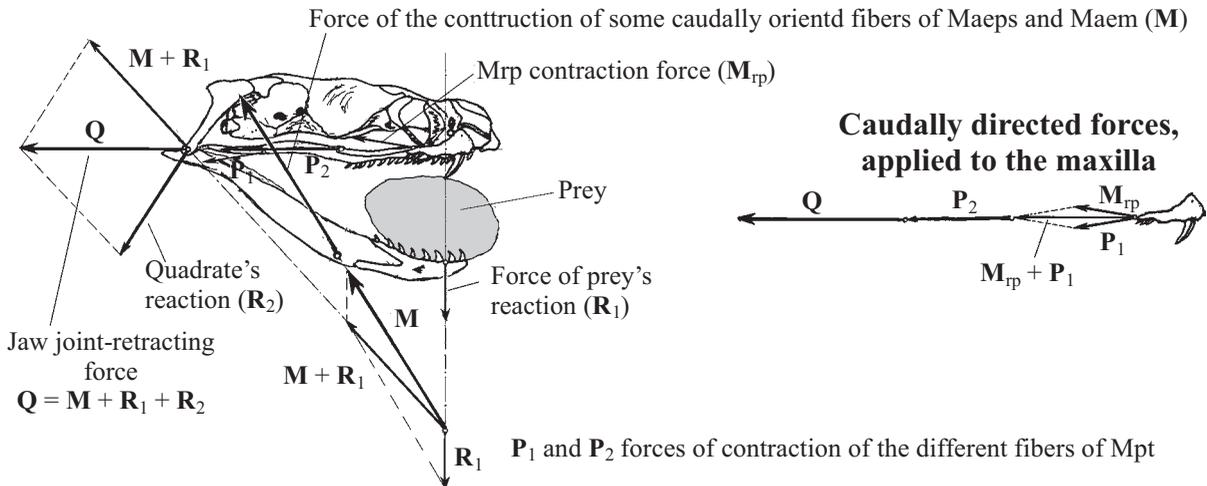


Fig. 1.

those of serpent-eating elapids but associated with eel-eating in hydrophiids and laticaudids. It is surprising that in this aspect proteroglyph snakes appear to have become venomous before so famous “typical ophidian” specialization to “thick prey-swallowing” (in skull of *Calliophis* and *Maticora* with “aniliid-like” proportions the quadrate is short and vertically oriented, postorbital part of braincase and jaws equally elongated, only *Bungarus* has more elongated jaws and elongated slanted quadrate). The snake-eating (or eel-eating in sea-snake case) can be interpreted like preadaptation to appearance of “thick object swallowing” mechanism typical for the advanced proteroglyph snakes which uses pterygoideus muscle. In specialized jaw apparatus of snake-eating elapids the contracted powerful pterygoideus muscle can break quadrate branch of pterygoid bone which is very slender. Thus the disconnection of pterygoid and quadrate with pterygoideum shortening (hyperstreptostylic movability) became very useful. Than the immobilization of the skull front part used for fang’s fixation (it often takes place) results in decreasing of classical prey-engaging mechanism efficiency. Probably besides dolichophagy stage in evolution of the majority of elapid snakes also took place specialization to feeding on small vertebrate victims (several small victims as alternative to one long) which does not require to reconstruct so effective and formidable jaw apparatus. Its result is the look of the majority elapid specimen as active hunters, slender snakes. It is also easy to assume, that the transition from earthworm-eating to vertebrate-eating specialization on some stage could cause not only the snake-eating elapid’s jaw apparatus, able “to switch on regime of rigidity,” but also could cause simpler transformation — the significant decreasing or loss of kinesis by some primitive snakes (a lot of Anilioidea and extinct Dinillysidae) which causes fossil skull preservation to be more probable. They refused from asymmetrical prey-engaging movements of toothed bones to swallow the prey and use outward support. Elongation of the jaws and postorbital

part of the braincase in akinetic or oligokinetic skulls can be accounted for by usefulness of significant lever for adductor muscles. It must be mentioned that the hypothesis that very early snakes used powerful jaws for killing elongate, heavy prey, has already been suggested by some authors (Greene, 1983; Mushinsky, 1987). But my hypothesis about worm-eating specialization of their limbless ancestor [see above and (Cundall and Green, 2000)] and lability of snake jaw apparatus can explain why using powerful jaws did not go beyond the process of prey-killing and why snakes swallow their prey entirely unlike amphisbaenians, which tear off and swallow peaces of large prey.

REFERENCES

- Cundall D. and Irish F. J.** (1989), “The function of the intramaxillary joint in the Round Island boa, *Casarea dussumieri*,” *J. Zool. (Lond.)*, **217**, 189 – 207.
- Cundall D. and Greene H. W.** (2000), “Feeding in snakes,” in: Schwenk K. (ed.), *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates*, Acad. Press, San Diego, pp. 293 – 333.
- Greene H. W.** (1983), “Dietary correlates of the origin and radiation of snakes,” *Am. Zool.*, **23**, 431 – 441.
- Haas G.** (1930), “Über die Schadelmechanik und die Kiefermuskulatur einiger Proteroglypha,” *Zool. Jb. Anat. C*, **52**(2), 347 – 404.
- Mushinsky H. R.** (1987) “Foraging ecology,” in: Siegel R. A., Collins J. T., and Novak S. S. (eds.), *Snakes: Ecology and Evolutionary Biology*, McGraw Hill, pp. 302 – 334.
- Sokolov A. Yu.** (2001a), “On the morphological peculiarities of jaw apparatus of snakes Booidea, Colubroidea, and Acrochordoidea on the basis of the analysis of interaction with super-large prey,” in: *The Problems of Herpetology. Proc. of 1st Meeting of the Nikolsky Herpetol. Soc.*, Pushchino – Moscow [in Russian].
- Sokolov A. Yu.** (2001b), *Morphofunctional Peculiarities of the Jaw Apparatus of Snakes Booidea, Colubroidea, and Acrochordoidea in Connection with Tropical Adaptations. Ph.D. Thesis*, Izd. MGU, Moscow [in Russian].

OBSERVATIONS ON THE GAMASID MITES (PARASITIFORMES, GAMASINA, MACRONYSSIDAE, LAELAPIDAE) PARASITIZING REPTILES (REPTILIA) FROM RUSSIA AND ADJACENT COUNTRIES (EX-USSR)

M. Stanyukovich¹ and L. Iohansen¹

Keywords: Mesostigmata, parasitic gamasid mites, Macronyssidae, *Ophionyssus*, Laelapidae, *Hemilaelaps*, reptiles, Reptilia, ex-USSR.

INTRODUCTION

The cohort Mesostigmata (Acarina: Parasitiformes) unites a large number of families of mites that occupy different ecological habitats and have various life cycles. Among them there occur both freeliving ones and parasites of many groups of vertebrates (mammals, birds and others) and invertebrates.

Mesostigmatic mites parasitizing on reptiles are represented by seven different but often not numerous families, namely Heterozetidae Berlese, 1892 with single species on reptiles *H. oudemansi* Finnegan, 1931; Paramesitidae Tragardh, 1946 with three species from genus *Ophiomegistus* Banks, 1914; Omentolaelapidae Fain, 1961 with single species *Omentolaelaps mehelyae* Fain, 1961.

The gamasid mites (Gamasina) are most numerous among mesostigmatic mites. The following gamasids from four families are parasitizing on (or in) reptiles:

Entonyssidae Ewing, 1923 (specific parasites residing in the respiratory passages of snakes) — 10 species from genus *Entonyssus* Ewing, 1923; *Viperacarus europaeus* Fain, 1960; *Hamertonia bedfordi* (Radford, 1937); *H. psamophis* Till; *Cobranysus schoutedeni* (Radford, 1953); *Entophiophaga colubricola* Fain, 1960 and *E. congolensis* Fain, 1960; *Entophionyssus glasmacheri* (Vitzthum); *Pneumophionyssus aristoterisi* Fonseca, 1940); Laelapidae Berlese, 1892 (nest or permanent ectoparasites) — 1 species from snakes (*Natrix natrix* L.) — *Haemolaelaps natricis* Feider et Solomon, 1960; Ixodorhynchidae Ewing, 1923 (parasites of snakes) — 25 species of genus *Ixodorhynchus* Ewing, 1923 with *I. liponyssoides* Ewing, 1923, *I. johnstoni* Fain, 1961, *I. leptodeirae* Fain, 1962, *I. cubanensis* Fain, 1962; *Ixobioides* Fonseca, 1934 with *I. butantanensis* Fonseca, 1934 and *I. fonsecae* Fain, 1961; *Hemilaelaps* Ewing, 1923 with *H. triangulus* Ewing, 1923 = *H. americanus* Ewing, 1933 = *H. distinctus* Ewing, 1933, *H. javanensis* Fain, 1961, *H. farrieri* (Tibbets, 1954)

= *Ophidolaelaps ponticus* Feider et Solomon, 1959, *H. congolensis* Fain, 1962, *H. causicola* Fain, 1961, *H. dipsodoboae* Fain, 1962, *H. radfordi* (Feider et Solomon, 1959), *H. feideri* Fain, 1962, *H. piger* (Berlese, 1918), *H. imphalensis* (Radford, 1947), *H. novae-guineae* Fain, 1961, *H. ophidius* (Lavoipierre, 1958), *H. schoutedeni* (Fain, 1961), *H. upembae* (Fain, 1961), *H. caheni* Fain, 1961; *Asiatolaelaps* Fain, 1961 with *A. tanneri* (Tibbets, 1954) and *A. evansi* Fain, 1961; *Strantibbetsia* Fain, 1961 with *S. gordoni* (Tibbets, 1967) and *S. brasiliensis* Fain, 1961); Macronyssidae Oudemans, 1936 (nest or permanent ectoparasites, including those of snakes and lizards) — mites of genus *Ophionyssus* Megnin, 1884 (19 species): *Ophionyssus* Megnin, 1884 = *Serpenticola* Ewing, 1923 = *Sauronyssus* Sambon, 1928 = *Neoliponyssus* Ewing, 1929 = *Oudemansiella* Fonseca, 1948 with *O. saurarum* (Oudms.), *O. natricis* (Gervais, 1844) = *O. variabilis* Zenskaya, 1951 = *O. serpentium* (Hirst, 1915) = *O. easti* (Ewing, 1925), *O. eremiadis* Naglov et Naglova, 1960, *O. arnhemlandensis* (Womersley, 1956), *O. luzonensis* Banks, 1914, *O. scincorum* Dowrow, Heath et Kennedy, 1980, *O. ehmani* Domrow, 1985, *O. buloloensis* (Gunther, 1942), *O. clelandi* Womersley, 1958, *O. javanensis* Micherdzinski et Lukoschus, 1987, *O. mabuyae* Till, 1957, *O. lacertinus* (Berlese, 1892), *O. lawrencei* (Till, 1957), *O. myrmecophagus* (Fonseca, 1954), *O. tropidosaurae* (Till, 1957), *O. saurarum* (Oudemans, 1901), *O. gallotocolus* Fain et Bannert, 2000, *O. setosus* Fain et Bannert, 2000, *O. dolatelacensis* Fain et Bannert, 2002, *O. galeotes* Dowrow, Heath et Kennedy, 1980.

RESULTS AND DISCUSSION

The main aim of our present and future investigations is to find out and systematize all information concerning mesostigmatic mites from reptiles of world fauna in general and from ex-USSR reptiles in particular.

¹ Zoological Institute of Russian Academy of Science, Universitetskaya nab., 1, 199034 St. Petersburg, Russia; E-mail: laelaps@zin.ru.

The analysis of the published data as well as that of the collection of Zoological Institute shows that in the territory of the former USSR there are 4 species of gamasids from two families (Laelapidae and Macronyssidae) that parasitize on snakes and lizards. They are: *Ophionyssus saurorum* (Oudemans, 1901) from *Lacerta agilis* L., *L. viridis* Laur., *L. vivipara* Jac., *L. muralis* Laur., *L. strigata* Eich., *L. saxicola* Evers.; *O. natricis* (Gervais, 1844) (= *O. variabilis* Zemskaya, 1951) from *Coluber karelini* Brandt, *Vipera lebetina* (L.), *Echis carinatus* (Schneid.), *Elaphe dione* (Pall.); *O. eremiadis* Naglov et Naglova, 1960 on *Eremias arguta* (Pall.), *E. velox* Pall., *Phrynocephalus guttatus* Gm., *P. mystaceus* Pall. For the first time were found out mites *Hemilaelaps radfordi* (Feider et Solomon, 1959) on the *Natrix natrix* L., 1758 in Ukraine. Formerly these mites had been known on *Coluber jugularis caspius* (Gmelin) and *N. natrix* from Romania.

The mites of *O. natricis* are common in zoos and vivaria where they parasitize on various snakes — *Python molurus* (L.), *Boa constrictor*, *Coluber ravagieri* Men., *Vipera lebetina* (L.), *Echis multisquamatus* Cherlin and others. Occasional findings of gamasids are known for *E. arguta* (Pall.) and *P. mystaceus* Pall.: *Haemolaelaps casalis* (Berl., 1887), *Typhlodromus* sp. etc. The cases when specific parasites of reptiles reside on other vertebrates

(and vice versa) are quite rare. We have found 2 cases like that in the collections of the Zoological Institute: different stages of reptiles mites *O. saurorum* from several nests of voles (*Microtus arvalis*) from Ukraine, and gamasids of the same species (2 protonymphs and 2 males) on a lark from Armenia.

Acknowledgments. The research has been supported partly by the Grant of President of Russian Federation for supporting scientific schools “Scientific School of acad. E. N. Pavlovsky” (No. NSh-1664.2003.4) and the Grant from the Russian Foundation for Fundamental Research (RFFI) (grant No. 03-04-49081).

REFERENCES

- Bregetova N. G.** (1956), *Gamasid Mites (Gamasoidea)*, Moscow – Leningrad [in Russian].
- Naglov V. A. and Naglova G. I.** (1960), “A new species of the genus *Ophionyssus* (Parasitiformes, Gamasoidea),” *Parazit. Sb. Zool. Inst. AN SSSR*, **19**, 164 – 168 [in Russian].
- Zemskaya A. A.** (1951), “Biology and development of the mites from the family Dermanyssidae parasitizing on reptiles in the connection with the problem of appearance of the pasturable parasitism,” *Byull. Mosk. Obshch. Ispyt. Prirody. Otd. Biol.*, **56**(3), 42 – 57 [in Russian].
- Fain A.** (1962), “Les Acariens Mesostigmatiques parasites des serpents,” *Bull. Royal Sci. Nat. Belg.*, **38**(18), 149.

THE DENTAL SYSTEM OF URODELAN AMPHIBIANS AND THE ROLE OF THYROID HORMONES IN ITS METAMORPHIC REMODELING

A. B. Vassilieva¹

Keywords: Urodela, dentition, thyroid hormones, metamorphosis.

INTRODUCTION

Thyroid hormones (TH) are considered to play a major role in the regulation of the metamorphic transformation of the dental system in Urodela. Certain facts support this consideration: for example, it is shown that in naturally neotenic urodeles as in urodeles with artificially blocked TH production the dentition retains the larval features (Chibon, 1972; Gabrion, Chibon, 1973; Clemen, 1988; Greven and Clemen, 1990). Also, the stimulation with exogenous TH affects the development of some dentigerous bones in plethodontid salamanders (Rose, 1995). The present comparative study on the dentitional metamorphosis in different urodeles is intended to clarify the degree of TH-control in this system development and to review if this control changes in the evolution of urodeles.

MATERIAL AND METHODS

We compared the sequence and timing of the dental ontogeny under the different hormonal conditions in several experimental groups of urodeles belonging to the primitive family (Hynobiidae: *Salamandrella keyserlingii*) and the advanced one (Salamandridae: *Triturus vulgaris*, *Pleurodeles waltl*). The hatching larvae were raised in the pure water (control groups), in the solutions of the triiodothyronine of different concentrations or in the solution of the thiourea – a goitrogen that inhibits the thyroid function. The animals were sampled at regular intervals and stained as whole-mounts with Alizarin for calcium deposits. A total of more than 200 specimens of each species was examined.

RESULTS

In hynobiids and salamandrids, the normal development of the dental system follows the similar pattern (Vassilieva and Smirnov, 2001; Smirnov and Vassilieva,

2003). The first teeth to form are nonpedicellate and have a monocuspid crown; as the larvae grow, the polystichous toothfields are established on the vomers, palatine bones and coronoids. In the midlarval stages, the nonpedicellate teeth are replaced by the monocuspid subpedicellate ones, i.e., teeth with a slightly developed weak zone separating the crown from the pedicel. At this stage, the resorption of such toothed bones as the vomers, palatines and coronoids, starts. Further changes are associated with metamorphic remodeling of the dentition. It includes four main events: (1) the splitting of the palatopterygoids and the total resorption of the provisor bones (palatines and coronoids) with their toothfields; (2) the formation of the toothed maxillaries; (3) the remodeling of the vomers and vomerine teeth arrangement; and (4) the replacement of the larval monocuspid subpedicellate teeth by the definitive, pedicellate and bicuspid, ones.

The treatment of the larvae with exogenous TH produces the overall acceleration of metamorphosis that is manifested externally in the accelerated regression of both gills and dorsal fin and the earlier leaving the water medium. The animals reared in the most concentrated TH solution (2×10^{-8} M) fail to complete the metamorphosis; they stop to feed and die soon after the start of gills reduction and first attempts to escape from the water.

The development of the dentition is shown to be affected by the exogenous TH too. Its early stages remain similar in all experimental groups, but the late ones differ greatly from those in the controls.

Thus, in all species the treatment with TH produces the evident acceleration of the main metamorphic events in the dentitional development, such as the onset of provisor bones resorption, splitting of the palatopterygoids, formation of the maxillaries, and (in some cases) the transition from mono- to bicuspidity. The degree of this acceleration depends on the hormone concentration (see Tables 1 and 3) that suggests TH to play an important role in the regulation of the dentitional metamorphosis.

Among urodeles studied here, *S. keyserlingii* is the sole one to complete the dentitional metamorphosis if treated with TH of the so high concentration as

¹ M. V. Lomonosov Moscow State University, Leninskie Gory, 1/12, 119899 Moscow, Russia; E-mail: evol_morphol_lab@newmail.ru.

2×10^{-8} M. In this group, the provisor bones resorption, the palate remodeling, and the teeth types transition all progress more or less synchronously. Nevertheless, there are some abnormalities to occur: though the definitive bicuspid teeth begin to form, the earliest nonpedicellate ones still remain since they have no time to resorb in the so shortened terms. As a result, a mixture of all three teeth types appears on the jaws, that is never seen in the normal development. Furthermore, some stages of dentitional ontogeny fail to be fully realized under these conditions: thus, the polystichous toothfields on the palate bones and coronoids are underdeveloped because the enhanced resorption of these bones counterbalances the fusion of new tooth rows; also, the adult vomers remain underdeveloped.

In *T. vulgaris*, the stimulation with high TH concentration influences greatly the skeletal elements of the dental system (Table 2), but, surprisingly, the teeth properly do not react to it. The timing of their replacement and their morphology don't differ from those in controls, so, by the end of the accelerated metamorphosis, in this group a "chimerical" dentition is established: the metamorphosed palate bears the early-larval nonpedicellate teeth. Maxillaries form very early, but they remain toothless by the larvae's death; also, the vomers fail to remodel fully (Smirnov and Vassilieva, 2003).

In *P. waltl*, the effect of stimulation by this TH concentration is even more prominent (Table 3). Larvae die very early, so the maxillaries have no time to form and the palatopterygoids don't achieve the complete resorption. As in *T. vulgaris*, the dentition remains fully larval and doesn't differ from that in controls of the same age.

The lower concentration of TH (2×10^{-9} M) produces a less dramatic acceleration. The metamorphosis is slightly accelerated if compared with the controls, but it follows the quite normal pattern, resulting in the full metamorphic remodeling of the dentition. Usually these larvae save the vital capacity after the exit to the land.

The similar result was observed in the larvae if immersed in TH only in the late larval stage. In such groups, the metamorphosis was accelerated proportionally to the TH concentration, but the overall developmental pattern remained quite normal.

TABLE 1. Timing of the Dentitional Metamorphosis in *S. keyserlingii* Given as Number of Days After the Hatching

	2×10^{-8} M	2×10^{-9} M	Control group
Maxillaries formation	12 days	14 days	20 days
Resorption onset	13 days	18 days	20 days
Splitting of the palatopterygoid	15 days	29 days	49 days
Bicuspid buds formation	23 days	40 days	55 days

Alternatively, the animals reared in the thiourea failed to metamorphose and retained larval external morphology and the aquatic mode of life over 20 months of the experiment.

In these groups, the dentition develops as in controls till the late-larval stage, but its further ontogeny differs from the normal pattern.

In particular, in all studied species, the resorption of the larval toothed bones is slightly delayed and less intensive; the palatopterygoids never split even in the animals older than 1, 5 years; the toothfields are retained for the very long time; the vomers are only partially remodeled; the maxillaries formation (in salamandrids only) is delayed or even absent; bicuspid teeth do not form. In other words, in the urodeles reared in the TH-deficiency, the dentition remains mainly larval.

However, some events that can be considered as "metamorphic" occur in these groups. For example, in Salamandridae, though the palatines are retained, their dental laminae become inactive (interestingly, at the same time as in controls). Thus, new tooth germs stop to arise and these bones become completely edentate with time. In the old larvae because of both the slower growth of new tooth rows and the progressive resorption of the oldest ones, the toothfields on the vomers and coronoids reduce to only 1 – 2 rows.

CONCLUSIONS

The great effect of the TH excess or deficiency on some events in the dentitional ontogeny of urodeles suggests the development of this system to be highly dependent on them.

However, as the early stages of the dentitional development always remain unchanged, the dental system

TABLE 2. Timing of the Dentitional Metamorphosis in *T. vulgaris* Given as Number of Days After the Hatching

	2×10^{-8} M	Control group
Maxillaries formation	12 days	31 days
Resorption onset	9 days	15 days
Splitting of the palatopterygoid	12 days	42 days
Bicuspid buds formation	—	42 days

TABLE 3. Timing of the Dentitional Metamorphosis in *P. waltl* Given as Number of Days After the Hatching (preliminary results)

	2×10^{-8} M	2×10^{-9} M	Control group
Maxillaries formation	—	16 days	39 days
Resorption onset	8 days	14 days	19 days

seems to acquire the responsiveness to TH only after a certain time. In addition, our results show that in some cases (for example, in Salamandridae exposed to the very high levels of TH and in thiourea-treated ones) the teeth development exhibits some kind of independence upon the bone condition. In contrast, when TH-stimulation is more moderate or begins later, the dentigerous bones and teeth both metamorphose normally and in the perfect synchrony. Presumably, these facts indicate the existence of some inductive interactions between teeth and bones that become established somewhere in the midlarval development. Earlier, the occurrence of such interactions was proposed by Clemen (1988).

Apparently, in more primitive salamanders (Hynobiidae), these interactive correlations are harder to disturb by the exogenous chemical stimulation than in more advanced Salamandridae, in which the overall development depends more prominently on the hormonal control rather than on the morphogenetic correlations.

Finally, our results show that the skeletal elements of the dental system are much more responsive to the TH level than the teeth properly. That means that TH control mainly the osteolytic and osteogenic processes rather than the teeth types alternation.

Acknowledgments. I thank Dr. S. V. Smirnov for the critical review of the manuscript and the linguistic help. The study was

supported by the Russian Foundation for Fundamental Research (grants No. 04-04-48916a and 1808.2003).

REFERENCES

- Chibon P.** (1972), "Etude ultrastructurale et autoradiographique des dens chez les amphibiens. Relations entre la morphogénèse dentaire et l'activité thyroïdienne," *Bull. Soc. Zool. Fr.*, **97**, 437 – 448.
- Clemen G.** (1988), "Experimental analysis of the capacity of dental laminae in *Ambystoma mexicanum* Shaw," *Arch. Biol. Brux.*, **99**, 111 – 132.
- Gabriel J. and Chibon P.** (1973), "Morphologie dentaire et disposition des dents chez les tritons neoténiques (*Triturus helveticus* Raz.)," *C. R. Seanc. Soc. Biol.*, **167**, 50 – 54.
- Greven H. and Clemen G.** (1990), "Effect of hypophysectomy on the structure of normal and ectopically transplanted teeth in larval and adult urodeles," *Acta Embryol. Morphol. Exp.*, **11**, 33 – 43.
- Rose C. S.** (1995), "Skeletal morphogenesis in the urodele skull: III. Effect of hormone dosage in TH-induced remodeling," *J. Morphol.*, **223**, 243 – 261.
- Smirnov S. V. and Vassilieva A. B.** (2003), "Skeletal and dental ontogeny in the smooth newt, *Triturus vulgaris* (Urodela: Salamandridae): role of thyroid hormone in its regulation," *Russ. J. Herpetol.*, **10**(2), 93 – 110.
- Vassilieva A. B. and Smirnov S. V.** (2001), "Development and morphology of the dentition in the Asian salamander *Ranodon sibiricus* (Urodela: Hynobiidae)," *Russ. J. Herpetol.*, **8**(2), 105 – 116.

LIST OF PARTICIPANTS



Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Adnagulov, Eduard	Institute for Water and Ecological Problems, Far East Branch, Russian Academy of Sciences	Kim Yu Chen ul., 65, 680000 Khabarovsk, RUSSIA	E-mail: rfe_herps@mail.ru dv-herp@ivep.khv.ru Tel.: +7 (4212) 21-06-11 +7 (4212)22-75-73 Fax: +7 (4212) 32-57-55
Aguiar Goncalves, Maria Helena	Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), Universidade do Porto	Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vila do Conde, PORTUGAL	E-mail: hgoncalves@mail.icav.up.pt Tel.: +351 (252) 66-04-16 Fax: +351 (252) 66-17-80
Alcobendas, Marina	Museo Nacional Ciencias Naturales (CSIC)	José Gutierrez Abascal, 2, E-28006 Madrid, SPAIN	E-mail: marina@mnnc.csic.es Tel.: +34 (91) 411-13-28 Fax: +34 (91) 564-50-78
Ananjeva, Natalia	President of SEH, Head of Department of Herpetology and Ornithology, Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: azemiops@zin.ru 12SEH@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Andrén, Claes	Department of Zoology University of Göteborg	Västergarden, 170, S-446 91 Alvhem, SWEDEN	E-mail: claes.andren@reptilia.se Tel.: +46 (30) 333-6433 Fax: +46 (30) 333-6477
Arakelyan, Marine	Yerevan State University	Alek Manukyana, 1, 375025 Yerevan, ARMENIA	E-mail: marine@nass.am Tel.: +374 (1) 55-65-68 Fax: +374 (1) 28-68-87
Askenderov, Azim	Dagestan State University	Batiraya ul., 8, 367025 Makhachkala, RUSSIA	E-mail: Mazanaev@mail.ru Tel.: +7 (8722) 67-95-46 Fax: +7 (8722) 68-23-26
Auge, Marc	Laboratoire de Paléontologie Museum National d'Histoire Naturelle	8 rue Buffon, F-75005 Paris, FRANCE	E-mail: Axuxgxex@aol.com Tel.: +33 (3) 24-40-26-56
Ayvazian, Naira	Yerevan State University	Alek Manukyana, 1, 375025 Yerevan, ARMENIA	E-mail: taipan@ysu.am Tel.: +374 (1) 27-81-80
Bakharev, Viktor	Grodno State University	Dzirzinskogo vul., 153-12, 230005 Grondo, BELARUS'	E-mail: bakharev@grsu.by
Bakiev, Andrey	Institute of Ecology of the Volga River Basin Russian Academy of Sciences	Komzina ul., 10, 445003 Tol'yatti, RUSSIA	E-mail: ecology@avtograd.ru Tel.: +7 (8482) 48-95-76 Fax: +7 (8482) 48-95-76
Baleeva, Natalia	Department of Vertebrate Zoology St. Petersburg State University	Universitetskaya nab., 7/9, 199034 St. Petersburg, RUSSIA	E-mail: natally_baleeva@mail.ru Tel.: +7 (812) 328-96-89
Barabanov, Andrei	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Baranovskaja, Natalia	Tomsk Polytechnic University	Pugacheva prosp., 1-65, 634015 Tomsk, RUSSIA	E-mail: valery@ant.tomsk.ru Tel.: +7 (3822) 42-40-08 Fax: +7 (3822) 41-89-10
Bashinskiy, Ivan	Department of Biogeography Faculty of Geography Moscow State University	Leninskie Gory, 119992 Moscow, RUSSIA	E-mail: leolga@geogr.msu.su Tel.: +7 (095) 939-47-17 Fax: +7 (095) 932-88-36
Batista, Vasco	Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO) Universidade do Porto	Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vila do Conde, PORTUGAL	E-mail: vascobat@mail.icav.up.pt Fax: +351 (252) 66-17-80
Bauer, Aaron	Department of Biology Villanova University	800, Lancaster Avenue, Villanova, PA 19085-1699, USA	E-mail: aaron.bauer@villanova.edu Tel.: +1 (610) 519-48-57 Fax: +1 (610) 519-78-63
Bauer, Lucas	Il CORBAU	Sassigné, F-58700 Montenoison, FRANCE	E-mail: l.bauer@wanadoo.nl peruviridis@wanadoo.fr Tel.: +33 (3) 86-60-16-60 Fax: +33 (3) 86-60-16-61
Beebee, Trevor	University of Sussex	Falmer, Brighton BN1 9QG, UK	E-mail: t.j.c.beebee@sussex.ac.uk Tel.: +44 (1273) 60-67-55 Fax: +44 (1273) 67-84-33
Bleyzac, Pierre	Laboratoire de Reproduction et Développement des Vertébrés Université Catholique de Lyon	25 rue du Plat, F-69288 Lyon Cedex 02, FRANCE	E-mail: pbleyzac@univ-catholyon.fr Tel.: +33 (4) 72-32-50-32 Fax: +33 (4) 72-32-50-66
Bobrov, Vladimir	Southern Branch Vietnam-Russian Tropical Centre	3, Ba Thang Hai str., Ho Chi Minh City, VIETNAM	E-mail: s_trop@hcmc.netnam.vn Tel.: +84 (8) 834-10-96 Fax: +84 (8) 834-62-70

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Böhme, Wolfgang	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: w.boehme.zfmk@uni-bonn.de Tel.: +49 (228) 91-22-250 Fax: +49 (228) 91-22-212
Bonacci, Antonella	Ecology Department Calabria University	Via P. Bucci-Rende, I-87036 Cosenza, ITALY	E-mail: bonacci71it@yahoo.it Tel.: +39 (0984) 49-29-72 Fax: +39 (0984) 49-29-86
Bondarenko, Dmitry	The Center of the Sanitary and Epidemiological Surveillance Federal Department "Medbioékstrem"	Rodionova ul., 10-43, Khimki, 141400 Moscow oblast', RUSSIA	E-mail: cgsn@rinet.ru Tel.: +7 (095) 190-48-63 Fax: +7 (095) 190-48-63
Borisovskiy, Alexander	Udmurt State University Institute of Applied Ecology	Universitetskaya ul., 1/1, 426034 Izhevsk, RUSSIA	E-mail: bag@uni.udm.ru Tel.: +7 (3412) 75-57-61
Borkin, Leo J.	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: borkin@spsci.spb.ru Tel.: +7 (812) 323-31-40 +7 (812) 328-41-24 Fax: +7 (812) 328-39-41 +7 (812) 328-41-24
Bozhanskiy, Anatolii	Head of Department on Ecology and Wildlife management Russian State Agriculture External University	Fuchika ul., 1, Balashikha-8, 143900 Moscow oblast', RUSSIA	E-mail: mirr@ok.ru marina@rmc.ru Tel.: +7 (095) 521-45-74 Fax: +7 (095) 529-55-86
Braz, Rui		Travessa da Laranjeira n 26 R/C, 1200-239 Lisboa, PORTUGAL	E-mail: rui braz@hotmail.com Tel.: +351 (96) 238-8209
Brede, Edward	University of Sussex	Falmer, Brighton BN1 9QG, UK	E-mail: e.g.brede@sussex.ac.uk Tel.: +44 (1273) 60-67-55 Fax: +44 (1273) 67-84-33
Brizzi, Rossana	Department of Animal Biology and Genetics University of Florence	Via Romana, 17, I-50125 Florence, ITALY	E-mail: brizzi@dbag.unifi.it Tel.: +39 (055) 228-82-96 Fax: +39 (055) 228-82-99
Brovko, Anastasija	International Solomon University	Sholudenko vul., 1-b, UA-01135 Kyiv, UKRAINE	E-mail: olekstyrov@yahoo.com Tel.: +38 (044) 244-56-40 (h)
Buckley, David	Museo Nacional de Ciencias	C/José Gutiérrez Abascal, 2, E-28006 Madrid, SPAIN	E-mail: davidb@mncn.csic.es Tel.: +34 (91) 411-1328 ext. 1124 Fax: +34 (91) 564-5078
Buckley, John	the Hct	77 Janson Road, Shirley, Southampton SO15 5GL, UK	E-mail: CLAIREJOHNB@aol.com Tel.: +44 (120) 239-1319 (w) +44 (238) 782-966 Fax: +44 (120) 239-2785
Bulakhov, Valentin	Dnipropetrovs'k National University Institute of Biology	Naukova vul., 13, UA-49050 Dnipropetrovs'k, UKRAINE	E-mail: vgasso@mail.dsu.dp.ua vgasso@ua.fm Tel.: +38 (0562) 46-92-82 Fax: +38 (0562) 46-55-23
Bulakhova, Nina	Tomsk State University	Lenina prosp., 36, 634050 Tomsk, RUSSIA	E-mail: bulakhova@bio.tsu.ru sigma44@mail2000.ru Tel.: +7 (3822) 42-61-43 +7 (3822) 63-86-04 Fax: +7 (3822) 41-03-53
Carretero, Miguel A.	Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO) Universidade do Porto	Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vila do Conde, PORTUGAL	E-mail: carretero@mail.icav.up.pt Tel.: +351 (252) 66-04-08 Fax: +351 (252) 66-17-80
Castro, Maria José	Vasco da Gama University School	Quinta de S. Jorge, Estrada da Conraria, Coimbra 3040-714 Castelo Viegas, PORTUGAL	E-mail: mjcastro@uvascodagama.org Tel.: +351 (239) 43-76-24 Fax: +351 (239) 43-76-27
Celani, Antonia	Ecology Department Calabria University	Via P. Bucci-Rende, I-87036 Cosenza, ITALY	E-mail: s.tripepi@unical.it Tel.: +39 (0984) 49-29-72 Fax: +39 (0984) 49-29-86
Cherepanov, Gennady	Department of Vertebrate Zoology St. Petersburg State University	Universitetskaya nab., 7/9, 199034 St. Petersburg, RUSSIA	E-mail: cherepanov_go@front.ru Tel.: +7 (812) 351-30-16 +7 (812) 328-96-89
Chernyshov, Kirill	Faculty of Biology Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: kir-ch@mail.ru Tel.: +7 (095) 939-35-02
Chikin, Yuri	Institute of Zoology Uzbek Academy of Sciences	Navoyi str., 1, 700095 Tashkent, UZBEKISTAN	E-mail: chikinwwf@tkt.uz Tel.: +998 (71) 246-07-18
Ciobanu, Doina	Engelhardt Institute of Molecular Biology Russian Academy of Sciences	Vavilova ul., 32, 119991 Moscow, RUSSIA	E-mail: dia@id.ru Tel.: +7 (095) 492-11-18 Fax: +7 (095) 135-14-05

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Corti, Claudia	Department of Animal Biology and Genetics University of Florence	Via Romana, 17, I-50125 Florence, ITALY	E-mail: claudia.corti@unifi.it Tel.: +39 (055) 228-82-96 Fax: +39 (055) 22-25-65
Crnobrnja-Isailovic, Jelka	Institute for Biological Research	29 Novembra 142, 11060 Belgrade, SERBIA AND MONTENEGRO	E-mail: jelka@ibiss.bg.ac.yu olijela@eunet.yu Tel.: +381 (64) 183-07-71 Fax: +381 (11) 76-14-33
Cruz, Maria João	Departamento de Biologia Animal Faculdade de Ciencias Universidade de Lisboa	Bloco C2, 1749-016 Lisboa, PORTUGAL	E-mail: mjoaac@hotmail.com Tel.: +351 (21) 750-0000 ext. 22177 Fax: +351 (21) 750-0028
Danielyan, Felix	Yerevan State University	Alek Manukyana, 1, 375025 Yerevan, ARMENIA	E-mail: Lacerta@ysu.am Tel.: +374 (1) 55-65-68
Danilov, Igor	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: turtle@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Darevsky, Ilja	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Davlyatov, Yakhya	Institute of Zoology Uzbek Academy of Sciences	Navoyi str., 1, 700095 Tashkent, UZBEKISTAN	Tel.: +998 (71) 246-07-18
de Sá, Rafael	Department of Biology University of Richmond	Richmond, VA 23173, USA	E-mail: rdesa@richmond.edu Tel.: +1 (804) 289-85-42 Fax: +1 (804) 289-82-33
Denoël, Mathieu	Laboratory of Fish and Amphibian Ethology Institute of Zoology	22 Quai Van Beneden, B-4020 Liege, BELGIUM	E-mail: Mathieu.Denoel@ulg.ac.be Tel.: +32 (4) 366-50-84 Fax: +32 (4) 366-50-10
Desfilis, Ester	Instituto Cavanilles de Biodiversidad y Biología Evolutiva Universidad de Valencia	apdo. 2085, E-46071 SPAIN	E-mail: ester.desfilis@uv.es Tel.: +34 (96) 354-3659 Fax: +34 (96) 354-3670
Dmitrieva, Elena	Faculty of Biology Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: dmitrieva@aport.ru Tel.: +7 (095) 148-71-92 Fax: +7 (095) 939-43-09
do Amaral, José Pedro S.	Centro de Biologia Ambiental	Campo Grande, C2, 3 Piso, 1749-016 Lisboa, PORTUGAL	E-mail: amaral@fc.ul.pt Tel.: +351 (93) 281-8128
Dolmen, Dag	NTNU University Museum	NO-7491 Trondheim, NORWAY	E-mail: dag.dolmen@vm.ntnu.no Tel.: +47 73592108 Fax: +47 73592295
Dotsenko, Irina	Zoological Museum National Museum of Natural History National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01030 Kyiv, UKRAINE	E-mail: dotsenko_irina@mail.ru Tel.: +38 (044) 234-70-16 +38 (044) 234-16-13
Drobenkov, Sergey	Institute of Zoology National Academy of Sciences of Belarus	Akademicheskaya vul., 27, 220072 Minsk, BELARUS	E-mail: zoobel@biobel.has-net.by Tel.: +375 (17) 230-28-91
Dunayev, Evgeniy	Zoological Museum, Moscow State University	B. Nikitskaya ul., 6, 103009 Moscow, RUSSIA	E-mail: dunayev@online.ru Tel.: +7 (095) 202-67-82
Dunce, Ilze	Riga Zoological Garden	Meza av. 1, LV-1014 Riga, LATVIA	E-mail: ilzedunce@hotmail.com Tel.: +371 (2) 751-79-74 Fax: +371 (2) 754-00-11
Düsen, Serdar	Department of Biology Faculty of Arts and Sciences Akdeniz University	Antalya 07058, TURKEY	E-mail: serdar2290@yahoo.com sdusen@pascal.sci.akdeniz.edu.tr Tel.: +90 (242) 227-89-00 +90 (242) 310-23-72 Fax: +90 (242) 227-89-11
Exbrayat, Jean-Marie	Université Catholique de Lyon	25, rue du Plat, F-69288 Lyon Cedex 02, FRANCE	E-mail: jmexbrayat@univ-catholyon.fr Tel.: +33 (4) 72-32-50-36 Fax: +33 (4) 72-32-50-66
Faizulin, Alexander	Institute of Ecology of the Volga River Basin Russian Academy of Sciences	Komzina ul., 10, 445003 Tol'yatti, RUSSIA	E-mail: ecovolga@avtograd.ru Tel.: +7 (8482) 48-95-76 Fax: +7 (8482) 48-95-76
Ficetola, Francesco	Department of Biology Section Ecology University of Milano	V. Celoria 26, I-20133 Milano, ITALY	E-mail: francesco.fi_cetola@unimi.it Tel.: +39 (2) 50-31-47-29
Fritz, Uwe	Museum fuer Tierkunde Staatliche Naturhistorische Sammlungen	Koenigsbruecker Landstr. 159, D-01109 Dresden, GERMANY	E-mail: fritz@snsd.de Tel.: +49 (351) 89-26-326 Fax: +49 (351) 89-26-327
Frolov, Vladimir	Moscow Zoo	B. Gruzinskaya ul., 1, 123242 Moscow, RUSSIA	E-mail: svkmzpmask@mtu-net.ru Tel.: +7 (095) 254-79-28 Fax: +7 (095) 973-20-56

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Gasser, Herbert	Institut für Zoologie Universität Wien	Althanstrasse 14, A-1090 Vienna, AUSTRIA	E-mail: a9017793@unet.univie.ac.at Tel.: +43 (1) 427-754-496 Fax: +43 (1) 427-79-544
Gasso, Victor	Department of Zoology and Ecology Dnipropetrovsk National University	Naukova vul., 13, UA-49050 Dnipropetrovsk, UKRAINE	E-mail: vgasso@mail.dsu.dp.ua vgasso@ua.fm Tel.: +38 (0562) 46-92-82 Fax: +38 (0562) 46-92-82
Gernigon- Spycha- lowicz, Thérèse	FSB, USTHB	BP 32 El Alia DZ 16111, ALGERIA	E-mail: tgermigon@yahoo.com Tel.: +213 (21) 44-08-39 Fax: +213 (21) 71-62-38
Glaudias, Xavier	Savannah River Ecology Laboratory University of Georgia	Drawer E, Aiken, SC 29802, USA	E-mail: xavier@srel.edu Tel.: +1 (803) 725-04-22 Fax: +1 (803) 725-33-09
Golay, Philippe	Cultural Foundation Elapsoidea	21, ch. Du Moulin Bernex CH-1233, SWITZERLAND	E-mail: gogo@geneva-link.ch Tel.: +41 (22) 777-11-31
Gollmann, Gunter	Institut für Zoologie Universität Wien	Althanstrasse 14, A-1090 Vienna, AUSTRIA	E-mail: guenter.gollmann@univie.ac.at Tel.: +43 (1) 427-754-467 +43 (1) 979-97-73 Fax: +43 (1) 427-79-544
Golynsky, Eugeny	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Gozdzik, Agnes	University of Guelph	50 Stone Road N1G 2W1, CANADA	E-mail: agozdzik@uoguelph.ca Tel.: +1 (519) 824-41-20 ext. 58569 Fax: +1 (519) 767-16-56
Grechko, Vernata	Engelhardt Institute of Molecular Biology Russian Academy of Sciences	Vavilova ul., 32, 119991 Moscow, RUSSIA	E-mail: grechko@eimb.ru Tel.: +7 (095) 157-68-24 Fax: +7 (095) 135-14-05
Grosjean, Stéphane	Reptiles et Amphibiens Département de Systématique et Evolution Muséum National d'Histoire Naturelle	25 rue Cuvier, F-75005 Paris, FRANCE	E-mail: sgrosjea@mnhn.fr Tel.: +33 (1) 40-79-34-87 Fax: +33 (1) 40-79-34-88
Guseva, Anna		Leningradskaya ul., 2, 153003 Ivanovo, RUSSIA	E-mail: irina_hiterman@mail.ru Tel.: +7 (0932) 30-04-63
Hachtel, Monika	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: m_hachtel@yahoo.com Tel.: +49 (228) 249-57-94 +49 (228) 912-22-49 Fax: +49 (1212) 526-714-382
Hallermann, Jakob	Zoological Museum University of Hamburg	Martin-Luther-King-Platz 3, D-20146 Hamburg, GERMANY	E-mail: hallermann@zoologie.uni-hamburg.de Tel.: +49 (40) 428-382-28-43 Fax: +49 (40) 428-383-937
Hammouche, Sadjia	Boumédiène Laboratoire de Recherche des Zones Arides de la Faculté des Sciences Bolgiques Université des Sciences et de la Technologie Houari	BP 32 El-Alia Bab-Ezzouar 16111, ALGERIA	E-mail: sadjiah@yahoo.fr Tel.: +213 (21) 24-79-13 Fax: +213 (21) 24-72-17
Harris, David J.	Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO) Universidade do Porto	Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vila do Conde, PORTUGAL	E-mail: james@mail.icav.up.pt Fax: +351 (252) 66-17-80
Heatwole, Harold	Department of Zoology North Carolina State University	Raleigh, NC 27695-7617, USA	E-mail: harold_heatwole@nsu.edu Tel.: +1 (919) 515-31-22 Fax: +1 (919) 515-31-22
Helfer, Véronique	Laboratory for Conservation Biology Institut d'Ecologie University of Lausanne	Biology Building, CH-1015 Lausanne, SWITZERLAND	E-mail: vhelpfer@ie-zea.unil.ch Tel.: +41 (216) 924-176 Fax: +41 (216) 924-165
Henle, Klaus	Centre for Environmental Research UFZ Leipzig-Halle Department of Conservation Biology and Natural Resources	Permoserstr. 15, D-04318 Leipzig, GERMANY	E-mail: henle@pro.ufz.de Tel.: +49 (341) 235-42-519 Fax: +49 (341) 135-31-91
Hermida, Gladys	Biodiversity and Experimental Biology Department Facultad de Ciencias Exactas y Naturales University of Buenos Aires	Av. Güiraldess/n — Ciudad Universitaria 1428, ARGENTINA	E-mail: hermida@bg.fcen.uba.ar Tel.: +54 (11) 457-63-372 Fax: +54 (11) 457-63-384
Heyer, Ronald	Smithsonian Institution	MRC 162, P.O. Box 7012, Smithsonian Institution, Washington, DC 20013-7012, USA	E-mail: heyerr@si.edu heyerr.ron@nmnh.si.edu Tel.: +1 (202) 357-21-95 Fax: +1 (202) 786-29-79

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Heyer, Miriam	Smithsonian Institution	MRC 162, P.O. Box 7012 Smithsonian Institution, Washington, DC 20013-7012, USA	E-mail: theheyers@erols.com Tel.: +1 (202) 357-21-95
Hinchliffe, John	Institute of Biological Sciences University of Wales	Aberystwyth Cardiganshire SY23 3DA, UK	E-mail: jkh@aber.ac.uk Tel.: +44 01970622327 Fax: +44 01970622350
Hoedl, Walter	Institut für Zoologie Universität Wien	Althanstrasse 14, A-1090 Vienna, AUSTRIA	E-mail: walter.hoedl@univie.ac.at Tel.: +43 (1) 427-754-495 Fax: +43 (1) 427-79-544
Hraoui-Bloquet, Souad	Faculty of Science II Lebanese University	P.O. Box 26110217, Fanar-Matn, LEBANON	E-mail: sbloquet@ul.edu.lb Tel.: +961 (3) 50-84-79 Fax: +961 (1) 68-02-50
Iantorno, Antonio	Ecology Department Calabria University	Via P. Bucci-Rende, I-87036 Cosenza, ITALY	E-mail: s.tripeci@unical.it Tel.: +39 (0984) 49-29-72 Fax: +39 (0984) 49-29-86
Igolkina, Valentina	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61 +7 (812) 271-60-50
Iizuka, Koji	Rokugo Jr. High School	5-10-8 Sendagi Bunkyo-ku, Tokyo 1130-022, JAPAN (h)	E-mail: iizuka1@cat.zero.ad.jp Fax: +81 (3) 36-84-16-56
Iohanssen, Larissa	Collection manager of Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Ishchenko, Vladimir	Institute of Plant and Animal Ecology Ural Branch Russian Academy of Science	8 Marta ul., 202, 620144 Yekaterinburg, RUSSIA	E-mail: vgi@ipae.uran.ru Tel.: +7 (343) 222-85-70 Fax: +7 (343) 229-41-61
Ivanova, Natalija	Museum "Germanys in St. Petersburg" Association for Research work in St. Petersburg	Novatorov ul., 41-2, 198216 St. Petersburg, RUSSIA	E-mail: nataschaiv@aport2000.ru
Janssen, Ingo	Department of Herpetology Zoological Museum Amsterdam	P.O. Box 94766, Amsterdam 1090 GT, THE NETHERLANDS	E-mail: ijanssen@science.uva.nl Tel.: +31 (20) 525-66-24
Jantra, Silke	Department of Animal Biology and Genetics University of Florence	Via Romana, 17, I-50125 Florence, ITALY	E-mail: sjantra@netscape.net Tel.: +39 (055) 228-82-96 Fax: +39 (055) 228-82-99
Jehle, Robert	Department of Animal and Plant Sciences University Sheffield	Western Bank Sheffield S10 2TN, UK	E-mail: R.Jehle@sheffield.ac.uk Tel.: +44 (114) 222-01-06 Fax: +44 (114) 222-00-02
Joger, Ulrich	Hessisches Landesmuseum	Friedensplatz 1, D-64283 Darmstadt, GERMANY	E-mail: Ulrich.Joger@snhm.niedersachsen.de Tel.: +49 (531) 288-89-210 Fax: +49 (6151) 16-57-65
Kaliontzopoulou, Antigoni	Herpetologia Dpt. Biologia animal (Vertebrate) Facultat de biologia Universitat de Barcelona	av. Diagonal 645, E-08028 Barcelona, SPAIN	E-mail: antkal@hotmail.com Tel.: +34 (93) 204-62-85 +34 (636) 68-47-05
Kalyabina-Hauf, Svetlana	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: kalyabina@hotmail.com Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Kamelin, Eugene	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: ekamelin@mail.ru Tel.: +7 (812) 319-47-61
Karmishev, Yuriy	Herpetological Laboratory Research Institute of Water and Landscape Ecosystems of Ukraine	Lenina vul., 20, UA-72315 Melitopol', UKRAINE	E-mail: mpi@comint.net Tel.: +38 (06192) 6-84-96
Khabibullin, Viner	Bashkir State University	Frunze ul., 32, 450076 Ufa, RUSSIA	E-mail: KhabibullinVF@ic.bashedu.ru Tel.: +7 (3472) 23-66-34
Khalikov, Roman	IT-specialist Department of Herpetology and Ornithology Information and Publishing Department Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: digitalherp@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Khromov, Victor	Semipalatinsk State University	KAZAKHSTAN	E-mail: bolat@semsk.kz
Knopp, Theresa	Department of Ecology and Systematics Division of Population Biology University of Helsinki	P.O. Box 65, Viikinkaari 1, Helsinki, FINLAND	E-mail: theresa.knopp@helsinki.fi Tel.: +358 (19) 15-78-05 +358 (40) 704-91-21 Fax: +358 (19) 248-72-17

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Koehler, Joern	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: J.Koehler.ZFMK@uni-bonn.de Tel.: +49 (228) 91-22-299 Fax: +49 (228) 91-22-212
Kolobajeva, Olga	Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: sevin@orc.ru Tel.: +7 (095) 954-32-62 Fax: +7 (095) 954-55-34
Kordikova, Elena	Kapchagay Geological Survey	Shevchenko ul., 162-Zh, 480008 Almaty, KAZAKHSTAN	E-mail: e_l_n_@hotmail.com Tel.: +7 (3272) 28-12-30
Korolyova, Daria	Shevchenko National University of Kyiv	Volodymyrska vul., 64, UA-01033 Kyiv, UKRAINE	E-mail: olekstytov@yahoo.com
Korshunov, Ilya	Tula Regional Exotarium	Oktyabr'skaya ul., 26, 300002 Tula, RUSSIA	E-mail: rept@tula.net Tel.: +7 (0872) 77-53-92 Fax: +7 (0872) 77-53-92
Korsunen, Anna	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	E-mail: korsanna@mail.ru Tel.: +7 (095) 135-97-93 Fax: +7 (095) 135-41-05
Kosushkin, Sergei	Engelhardt Institute of Molecular Biology Russian Academy of Sciences	Vavilova ul., 32, 119991 Moscow, RUSSIA	E-mail: toki@mail.ru Tel.: +7 (095) 932-74-13 Fax: +7 (095) 135-14-05
Kotenko, Tatiana	Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: kotenko@iz.freenet.kiev.ua Tel.: +38 (044) 252-17-17 Fax: +38 (044) 224-15-69
Kotserzhynska, Inna	Schmalhausen Institute of Zoology National Academy of Science of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: kotserzh@hotmail.com
Kovalenko, Elena	Department of Vertebrate Zoology St. Petersburg State University	Universitetskaya nab., 7/9, 199034 St. Petersburg, RUSSIA	E-mail: kovalenko_i@mail.ru Tel.: +7 (812) 587-82-26
Kraaijeveld-Smit, Femmie	University of Sussex	Falmer, Brighton BN1 9QG, UK	E-mail: f.kraaijeveld-smit@sussex.ac.uk Tel.: +44 (1273) 60-67-55 Fax: +44 (1273) 67-84-33
Krechetova, Oksana	Tomsk State University	Lenina prosp., 36, 634050 Tomsk, RUSSIA	E-mail: Bliss@mail2000.ru blissy@rambler.ru Tel.: +7 (3822) 42-61-43 Fax: +7 (3822) 41-03-53
Krivosheev, Vladimir	Faculty of Ecology Ul'yanovsk State University	L. Tolstogo ul., 42, 432970 Ul'yanovsk, RUSSIA	E-mail: krivosheev@list.ru mk@sv.uven.ru p859@simbir.ru Tel.: +7 (8422) 32-06-45 Fax: +7 (8422) 32-08-10
Kruzhkova, Yulya	Department of Vertebrate Zoology St. Petersburg State University	Universitetskaya nab., 7/9, 199034 St. Petersburg, RUSSIA	E-mail: Yulya_Kruzhkova@mail.ru Tel.: +7 (812) 224-40-36
Kudravytseva, Ksenia	Department of Ecology Russian Peoples' Friendship University	Pavlova ul., 8/5, 113093 Moscow, RUSSIA	E-mail: gvpolyanova@mail.ru Tel.: +7 (095) 170-51-61 Fax: +7 (095) 952-89-01
Kudravytsev, Sergei	Moscow Zoo	B. Gruzinskaya ul., 1, 123242 Moscow, RUSSIA	E-mail: svkmzpmk@mtu-net.ru Tel.: +7 (095) 254-79-28 Fax: +7 (095) 973-20-56
Kukushkin, Oleg	Karadag Nature Reserve National Academy of Sciences of Ukraine	Nauki ul., 24, Kurortnoye, Feodosiya, UA-98188 Crimea, UKRAINE	E-mail: ecol_monit@pochtamt.ru karadag@crimea.com Tel.: +38 (06562) 3-83-31 +38 (06562) 3-84-70 Fax: +38 (06562) 3-83-31
Kupriyanova, Larissa	Department of Karyotaxonomy Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: Vladimir@vz8122.spb.edu Tel.: +7 (812) 328-05-11 Fax: +7 (812) 328-29-41
Kuranova, Valentina	Tomsk State University	Lenina prosp., 36, 634050 Tomsk, RUSSIA	E-mail: Kuranova@bio.tsu.ru boiga@mail2000.ru Tel.: +7 (3822) 42-61-43 Fax: +7 (3822) 41-03-53
Kuzmin, Sergius	Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: ipe51@yahoo.com Tel.: +7 (095) 954-32-62 Fax: +7 (095) 954-10-36
Kuznetsov, V	Mordovian State University	Bol'shevnikov ul., 68, 430000 Saransk, RUSSIA	E-mail: biotech@moris.ru
Lada, Georgii	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: esculenta@mail.ru Tel.: +7 (812) 323-31-40
Lambert, Michael	Environmental Initiatives	12 Cheyne Row, London SW7 5HL, UK	E-mail: Lambertmrk@aol.com Tel.: +44 (207) 352-64-83 Fax: +44 (207) 352-64-83

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Lazareva, Olga	Department of Zoology Ivanovo State University	Leninsky prosp., 136, 153002 Ivanovo, RUSSIA	E-mail: herpet.log@mail.ru Tel.: +7 (0932) 37-01-57
Lebedeva, Anna	Tula Regional Exotarium	Oktyabr'skaya ul., 26, 300002 Tula, RUSSIA	E-mail: rept@tula.net Tel.: +7 (0872) 77-53-92 Fax: +7 (0872) 77-53-92
Lebedinsky, Andrei	Nizhny Novgorod State University	Malinovskogo ul., 7-43, 603136 N. Novgorog, RUSSIA	E-mail: lebedinsky@mail.nnov.ru Tel.: +7 (8312) 68-89-75 Fax: +7 (8312) 39-70-45
Leontyeva, Olga	Department of Biogeography Faculty of Geography Moscow State University	Leninskie Gory, 119992 Moscow, RUSSIA	E-mail: bio-geog@mtu-net.ru Fax: +7 (095) 932-88-36
Litvinchuk, Spartak	Institute of Cytology Russian Academy of Sciences	Tikhoretskii prosp., 4, 194064 St. Petersburg, RUSSIA	E-mail: slitvinchuk@yahoo.com
Litvinov, Nikolay	Perm State Pedagogical University	Sibirskaya ul., 32-39, 614000 Perm, RUSSIA	E-mail: Ganshchuk@mail.ru Tel.: +7 (3422) 12-92-69
Lukin, Yuri	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 550-10-83
Lyapkov, Sergei	Biological Evolution Department Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: lyapkov@mail.ru Tel.: +7 (095) 939-35-01
Lymberakis, Petros	Natural History Museum University of Crete	Knossos Av., P.O. Box 2208, Irakleio, Crete Irakleio 71409, GREECE	E-mail: lyberis@nhmc.uoc.gr Tel.: +30 (2810) 39-32-66 Fax: +30 (2810) 32-43-66
Maletzky, Andreas	Institute of Zoology University of Salzburg	Hellbrunnerstrasse 34, A-5020 Salzburg, AUSTRIA	E-mail: a_maletzky@aon.at Tel.: +43 (676) 750-93-50 Fax: +43 (662) 80-44-56-98
Malimonov, Vladimir	Institute of Plant and Animal Ecology Ural Branch Russian Academy of Science	8 Marta ul., 202, 620144 Yekaterinburg, RUSSIA	E-mail: malimonov@ipae.uran.ru Fax: +7 (343) 229-41-61
Malysheva, Daria	Institute of Gene Biology Russian Academy of Science	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	E-mail: fleur-de-lys@newmail.ru Tel.: +7 (095) 964-04-30 +7 (095) 135-98-64 Fax: +7 (095) 135-41-05 +7 (095) 330-49-62
Manilo, Valentina	National Museum of Natural History National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01030 Kyiv, UKRAINE	E-mail: manilo@zoomus.freenet.kiev.ua Tel.: +38 (044) 234-70-16
Mantziou, Georgia	Natural History Museum University of Crete	Knossos Av., P.O. Box 2208, Irakleio, Crete Irakleio 71409, GREECE	E-mail: mantziou@nhmc.uoc.gr Tel.: +30 (2810) 39-32-66 Fax: +30 (2810) 32-43-66
Manuilova, Olga	Herpetological Laboratory Research Institute of Water and Landscape Ecosystems of Ukraine	Lenina vul., 20, UA-72315 Melitopol', UKRAINE	E-mail: olga_manuilova@ua.fm Tel.: +38 (06192) 6-84-96 +38 (06192) 6-87-87
Mappes, Tapio	University of Jyväskylä	P.O. Box 35, Jyväskylä FIN-40014, FINLAND	E-mail: tmappes@bytl.jyu.fi Tel.: +358 (40) 586-09-78 Fax: +358 (14) 260-23-21
Marchenkovskaya, Alexandra	Institute of Biology Dnipropetrovs'k National University	Naukova vul., 13, UA-49050 Dnipropetrovs'k, UKRAINE	E-mail: vgasso@mail.dsu.dp.ua vgasso@ua.fm Tel.: +38 (0562) 46-92-82 Fax: +38 (0562) 46-55-23
Marco, Adolfo	Department of Evolutionary Biology Donana Biological Station (Estación Biológica de Donana) CSIC	Apartado 1056, E-41013 Sevilla, SPAIN	E-mail: amarco@cica.es Tel.: +34 (954) 23-23-40 Fax: +34 (954) 62-11-25
Martirosyan, Irena	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	E-mail: Irena_M@rambler.ru Tel.: +7 (095) 330-49-62 +7 (095) 135-98-64 Fax: +7 (095) 330-49-62 +7 (095) 135-41-05
Maslova, Irina	"Khankaiskii" Nature Reserve	Ershova ul., 10, Spassk-Dal'nii, 692245 Primorskii Krai, RUSSIA	E-mail: Irinarana@yandex.ru Tel.: +7 (43252) 2-57-93 Fax: +7 (42352) 2-31-38
Mayer, Werner	Molecular Systematics Vienna Natural History Museum	Burgring 7, A-1014 Vienna, AUSTRIA	E-mail: werner.mayer@nhm-wien.ac.at Tel.: +43 (1) 52-17-75-93
Maymin, Michael	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61 +7 (812) 557-57-82
Mazanaeva, Lydmila	Dagestan State University	Batiraya ul., 8, 367025 Makhachkala, RUSSIA	E-mail: Mazanaev@mail.ru Tel.: +7 (8722) 67-95-46 Fax: +7 (8722) 68-23-26

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Mebert, Konrad	Department of Biological Sciences Old Dominion University	ADDRESS??? 23529 USA	E-mail: kmebe001@odu.edu kmebe001@yahoo.com Tel.: +41 (41) 781-47-07 (in Switzerland)
Mednikov, Dmitriy	Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: sevin@orc.ru Tel.: +7 (095) 954-32-62 Fax: +7 (095) 954-55-34
Melnikov, Daniel	Department of Zoology Faculty of Biology Rostov State University	B. Sadovaya ul., 105, 344006 Rostov-on-Don, RUSSIA	E-mail: melnikovda@yandex.ru Tel.: +7 (8632) 77-52-56 Fax: +7 (8632) 64-52-55
Miaud, Claude	University of Savoie	F-73376 Technolac, FRANCE	E-mail: claude.miaud@univ-savoie.fr Tel.: +33 (4) 79-75-88-86 Fax: +33 (4) 79-75-88-86
Mikulíček, Peter	Biodiversity Research Group Department of Zoology Charles University Institute of Vertebrate Biology Academy of Sciences of the Czech Republic	Vinická 7, CZ-128 44 Prague 2, CZECH REPUBLIC	E-mail: petermikulicek@pobox.sk mikulice@natur.cuni.cz Tel.: +42 (5) 962-7959 Fax: +42 (5) 962-7959
Milto, Konstantin	Collection manager of Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Mirsalikhova, Firuza	Tashkent Zoo	J. Abidov str., 101, Tashkent, UZBEKISTAN	E-mail: emili@tkt.uz Tel.: +998 (17) 162-56-18
Mishagina, Jeanne	Siberian Zoological Museum Institute of Systematic and Ecology of Animals Siberian Branch Russian Academy of Sciences	Frunze ul., 11, 630091 Novosibirsk, RUSSIA	E-mail: jmish@ngs.ru Tel.: +7 (3832) 73-32-53
Misyura, Andrew	Institute of Biology Dnipropetrovsk National University	Naukova vul., 13, UA-49050 Dnipropetrovsk, UKRAINE	E-mail: vgasso@mail.dsu.dp.ua vgasso@ua.fm Tel.: +38 (0562) 46-92-82 Fax: +38 (0562) 46-55-23
Moravec, Jirí	Department of Zoology National Museum Prague	CZ-115 79 Praha 1, CZECH REPUBLIC	E-mail: jiri.moravec@nm.cz Tel.: +42 (2) 224-497-272
Morozova, Yekaterina	Prokopiev's Private Herpetocultural Laboratory	Kurchatova vul., 10 apt. 215, UA-61108 Kharkiv, UKRAINE	E-mail: chamaeleolizard@hotmail.com
Morozov-Leonov, Svyatoslav	Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: morleone@aport.ru morleone@rambler.ru Tel.: +38 (044) 234-13-95 Fax: +38 (044) 234-15-69
Nagy, Zoltan	Department of Pharmacy and Molecular Biotechnology University of Heidelberg	Im Neuenheimer Feld 364, D-69120 Heidelberg, GERMANY	E-mail: lustimaci@yahoo.com Tel.: +49 (6221) 54-48-47 Fax: +49 (6221) 54-48-84
Nazarov, Roman	Vertebrate Zoology Department Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: r_nazarov@mail.ru Tel.: +7 (095) 202-67-82 (w) +7 (095) 431-37-29 (h)
Nechaeva, Marina	Koltzov Institute of Developmental Biology Russian Academy of Sciences	Vavilova ul., 26, 119991 Moscow, RUSSIA	E-mail: Mnechaeva@proxima.idb.ac.ru Tel.: +7 (095) 135-54-27 Fax: +7 (095) 135-80-12
Nekrasova, Oksana	Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: oneks@mail.ru Tel.: +38 (044) 235-51-87 Fax: +38 (044) 234-15-69
Nemilov, Alexander	Department of Herpetology Leningrad Zoo	Aleksandrovskaia park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61
Nikitina, Natalia	Department of Herpetology and Ornithology Zoological Institut Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru
Nilson, Göran	Department of Zoology University of Göteborg	P.O. Box 463, S-405 30 Göteborg, SWEDEN	E-mail: goran.nilson@reptilia.se Tel.: +46 (30) 374-1895
Niskanen, Martti	Department of Biological and Environmental Science University of Jyväskylä	P.O. Box 35, FIN-40351 Jyväskylä, FINLAND	E-mail: maeranni@cc.jyu.fi Tel.: +358 (50) 535-84-55 Fax: +358 (14) 260-23-21
Novitsky, Ruslan	Institute of Zoology National Academy of Sciences of Belarus'	Akademicheskaya vul., 27, 220072 Minsk, BELARUS'	E-mail: nramphi@mail.ru Tel.: +375 (17) 263-80-08 (h) +375 (17) 284-21-92 (w) Fax: +375 (17) 284-10-36
Ogurtsov, Sergei	Department of Vertebrate Zoology Faculty of Biology Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: sk-ogurtsov@mtu-net.ru Tel.: +7 (095) 249-09-37

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Ohler, Annemarie	Département de Systématique et Evolution Reptiles et Amphibiens Muséum National d'Histoire Naturelle	25 rue Cuvier, F-75005 Paris, FRANCE	E-mail: ohler@mhnh.fr Tel.: +33 (1) 40-79-34-86 Fax: +33 (1) 40-79-34-88
Okulova, Natalia	Severtzov's Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: natmichok@mtu-net.ru Tel.: +7 (095) 318-40-00
Oliveira, José Miguel	Institute for the Nature Conservation	Mata Nacional do Choupal Coimbra, 3000 Coimbra, PORTUGAL	E-mail: jmcbo@mail.pt Tel.: +351 (239) 49-90-21 Fax: +351 (239) 49-90-29
Olmo, Ettore	Istituto di Biologia e Genetica University of Ancona	Via Breccie Bianche, I-60131 Ancona, ITALY	E-mail: olmoet@unian.it Tel.: +39 (071) 220-46-15 Fax: +39 (071) 220-46-09
Orlov, Nikolai	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: azemiops@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Orlova, Valentina	Zoological Museum Moscow State University	B. Nikitskaya ul., 6, 103009 Moscow, RUSSIA	E-mail: orlova@zmmu.msu.ru val_orlova@mail.ru Tel.: +7 (095) 202-67-82
Ortmann, Daniel	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: Ortmannda@compuserve.de Tel.: +49 (228) 249-57-94 +49 (228) 912-22-49 Fax: +49 (1212) 526-714-382
Osipenko, Elena	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61 +7 (812) 388-07-40
Pagano, Alain	Animal Ecology University of Angers	Campus Belle, F-49045 Beille Angers, FRANCE	E-mail: alain.pagano@univ-angers.fr Tel.: +33 (241) 73-50-82 Fax: +33 (241) 73-53-52
Pancisin, Lubomir	Department of Ecology Faculty of Natural Sciences Comenius University	Mlynska Dolina B-2, SK-842 15 Bratislava, SLOVAKIA	E-mail: pancisin@sci.muni.cz pancisin@fns.uniba.sk Tel.: +421 (2) 602-96-257 Fax: +421 (2) 654-29-064
Papenfuss, Theodore	Museum of Vertebrate Zoology University of California, Berkeley	Berkeley, CA 94720, USA	E-mail: asiaherp@uclink4.berkeley.edu Tel.: +1 (510) 643-77-06 Fax: +1 (510) 643-82-38
Pappel, Piret	Institute of Zoology and Hydrobiology University of Tartu	Vanemuine str. 46, EST-51014 Tartu, ESTONIA	E-mail: piprap@ut.ee Tel.: +372 (5) 286-826
Parham, James	Museum of Paleontology University of California, Berkeley	1101 Valley Life Sciences Building, Berkeley, CA 94720, USA	E-mail: Parham@socrates.berkeley.edu Tel.: +1 (510) 643-21-09 Fax: +1 (510) 642-18-22
Patrakov, Sergei	Tomsk State University	Lenina prosp., 36, 634050 Tomsk, RUSSIA	E-mail: parahod777@mail2000.ru Tel.: +7 (3822) 42-61-43 +7 (3822) 26-12-34 Fax: +7 (3822) 41-03-53
Paunovic, Ana	Natural History Museum	Njegoseva 51, 11000 Belgrad, SERBIA AND MONTENEGRO	E-mail: paunmchi@eunet.yu Tel.: +381 (11) 344-21-49 Fax: +381 (11) 344-22-65
Pavlov, Aleksey	Ecological Faculty Kazan State University	Kremlevskaya ul., 18, 420008 Kazan, RUSSIA	E-mail: zilant@mi.ru Tel.: +7 (8432) 31-51-79 +7 (8432) 31-51-46 +7 (8432) 57-81-76
Perälä, Jarmo	Ecological Genetics Research Unit Department of Ecology and Systematics University of Helsinki	P.O. Box 65 (Biocenter 3, Viikinkaari 1) FIN-00014 Helsinki, FINLAND	E-mail: jarmo.perala@helsinki.fi jsj.perala@luukku.com Tel.: +358 40747761 +358 400747761 (GSM) Fax: +358 919157694
Peregontsev, Evgeny	UzZooComplex	Djangokh str., 9 apt. 18, 700128 Tashkent, UZBEKISTAN	E-mail: eperegon@mail.ru Tel.: +998 (71) 137-56-30 Fax: +998 (71) 144-12-89
Peskov, Vladimir	Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: olekstyto@yahoo.com Tel.: +38 (044) 244-83-49 (h)
Peskova, Tatiana	Kuban State University	Stavropol'skaya ul., 224/1-49, 350040 Krasnodar, RUSSIA	E-mail: peskova@kubannet.ru Tel.: +7 (8612) 33-39-00
Pestov, Mark	The N. Novgorod Society for Protection of Amphibians and Reptiles	Kostina ul., 2, of. 157, P.O. Box 631, 603000 N. Novgorod, RUSSIA	E-mail: vipera@dronr.ru Tel.: +7 (8312) 30-25-07 Fax: +7 (8312) 30-28-81

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Pinho, Catarina	Centro de Investigaçao em Biodiversidade e Recursos Genéticos (CIBIO) Universidade do Porto	Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vila do Conde, PORTUGAL	E-mail: catarina@mail.icav.up.pt Fax: +351 (252) 66-17-80
Piombo, Roberta	University of Genoa	Via Col di Lana 5/44, I-16152 Genova, ITALY	E-mail: roberta.piombo@libero.it Fax: +39 (010) 601-32-31
Pisanets, Eugeny	Zoological Museum National Museum Natural History	B. Khmel'nits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: zoomus@profit.net.ua zoomuseum@svitonline.com Tel.: +38 (044) 234-70-16
Piskurek, Oliver	Faculty of Bioscience & Biotechnology Tokyo Institute of Technology	4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, JAPAN	E-mail: piskurek@bio.titech.ac.jp Tel.: +81 (45) 924-57-44 Fax: +81 (45) 924-58-35
Polynova, Galina	Department of Ecology Russian Peoples' Friendship University	Pavlova ul., 8/5, 113093 Moscow, RUSSIA	E-mail: gvpolynova@mail.ru Tel.: +7 (095) 249-35-64 Fax: +7 (095) 952-89-01
Postolachi, Vlad	National Museum of Ethnographi and Natural History	M. Kogilniceanu str., 82, MD-2012 Chishinau, MOLDOVA	E-mail: som@as.md vpostolachi@rambler.ru Tel.: +373 (2) 23-88-42
Poulakakis, Nikos	Natural History Museum University of Crete	Knossos Av., P.O. Box 2208, Irakleio, GREECE	E-mail: Poulakakis@nhmc.uoc.gr Tel.: +30 (2810) 39-32-66 Fax: +30 (2810) 32-43-66
Poyarkov, Nikolai	Department of Vertebrate Zoology Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: oyarkov@orc.ru Tel.: +7 (095) 939-33-02 (dpt.) +7 (095) 244-78-75 (contact)
Prokopiev, Sergey	Prokopiev's Private Herpetocultural Laboratory	Kurchatova vul., 10 apt. 215, UA-61108 Kharkiv, UKRAINE	E-mail: chamaeleolizard@hotmail.com Tel.: +38 (0572) 19-61-85
Racca, Laura	The Durrell Institute of Conservation and Ecology University of Canterbury	Flat 2, 4 Norfolk Terrace, 65 Rouge Bouillon str. Helier, Jersey JE2 3ZB, UK	E-mail: auracal@yahoo.it Tel.: +44 1534629264
Rannap, Riinu	Conservation Department Ministry of the Environment Nature	Toompuiestee 24, EST-15172 Tallinn, ESTONIA	E-mail: riinu.rannap@ekm.envir.ee Tel.: +372 (0) 62-62-889 Fax: +372 (0) 62-62-901
Rastegar-Pouyani, Nasrullah	Department of Biology Faculty of Science Razi University	Kermanshah 67149, IRAN	E-mail: nasrullah_r@hotmail.com Tel.: +98 (831) 836-0914 Fax: +98 (831) 422-3308
Ratnikov, Viatcheslav	Voronezh State University	Universitetskaya pl., 1, 394000 Voronezh, RUSSIA	E-mail: gfkig207@main.vsu.ru Tel.: +7 (0732) 53-04-20 Fax: +7 (0732) 78-99-89
Recuero-Gil, Ernesto	Museo Nacional de Ciencias Naturales (CSIC)	C/José Gutiérrez Abascal, 2, E-28006 Madrid, SPAIN	E-mail: ernestorecuero@hotmail.com Tel.: +34 (91) 411-1328 Fax: +34 (91) 564-5078
Redcozubov, Oleg	Institute of Zoology Academy of Sciences of Moldova	Tr. Academiei 1, MD-2028 Chishinau, MOLDOVA	E-mail: som@as.md Tel.: +373 (2) 73-75-09 Fax: +373 (2) 73-12-75
Ribakova, Anna	Department of Herpetology Leningrad Zoo	Aleksandrovskaia park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61
Rikhvanov, Leonid	Tomsk Polytechnic University	Pugacheva prosp., 1-65, 634015 Tomsk, RUSSIA	E-mail: leorikh@b10.tpu.edu.ru Tel.: +7 (3822) 41-89-10 Fax: +7 (3822) 41-89-10
Rogovin, Konstantin	Severtzov Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: rogovin@orc.ru Tel.: +7 (095) 951-61-37 Fax: +7 (095) 954-55-34
Roitberg, Evgeni		Oldendorpstr. 7, D-18059 Rostock, GERMANY	E-mail: Roitberg@fhn-dummerstorf.de
Ruchin, Alexander	Biological Faculty Mordovian State University	Bol'shevnikov ul., 68, 430000 Saransk, RUSSIA	E-mail: biotech@moris.ru Fax: +7 (8342) 32-25-23
Rudyk, Alexandr	University of California, San Francisco	MRII0409 Box 05064, SF Campus 0462, San Francisco, CA 94143, USA	E-mail: olmrudyk@yahoo.com Fax: +1 (415) 822-37-68
Ryabina (Kuvshinova), Svetlana	Department of Zoology and Ecology Moscow Pedagogical State University	Kibal'chicha ul., 6, 129366 Moscow, RUSSIA	E-mail: natmichok@mtu-net.ru Tel.: +7 (095) 437-24-12
Ryabinina, Natalia	Engelhardt Institute of Molecular Biology Russian Academy of Sciences	Vavilova ul., 32, 119991 Moscow, RUSSIA	E-mail: nryabinina@hotmail.com Tel.: +7 (095) 750-02-95 +7 (095) 135-97-80 Fax: +7 (095) 135-14-05
Ryabov, Sergei	Basic Labor of Zoological Institute Tula Regional Exotarium	Oktyabr'skaya ul., 26, 300002 Tula, RUSSIA	E-mail: rept@tula.net Tel.: +7 (0872) 77-53-92

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Ryskov, Aleksei	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	E-mail: Ryskov@mail.ru Tel.: +7 (095) 257-25-35 +7 (095) 135-87-41 Fax: +7 (095) 135-41-05
Saarikivi, Jarmo	President of the Herpetological Society of Finland University of Helsinki	Tursontie 12-14 D5, FIN-00610 Helsinki, Finland	E-mail: jarmo.saarikivi@helsinki.fi Tel.: +358 (40) 509-85-72
Saber, Samy	Zoology Department Faculty of Science Al Azhar University	Assiut 71511, EGYPT	E-mail: samy_nn@yahoo.com Tel.: +20 27422156 Fax: +20 88325436
Sadek, Riyad	Biology Department American University of Beirut	P.O. Box 11-0236, Riad El-Solh 1107-2020, LEBANON	E-mail: rsadek@aub.edu.lb Tel.: +961 (3) 72-68-56 Fax: +961 (7) 24-03-36
Sartaeva, Halima	International Kazakh-Turkish Pedagogical Institute		E-mail: emili@tkt.uz
Savisko, Vladimir	Department of Herpetology Leningrad Zoo	Aleksandrovskii park, 1, 197198 St. Petersburg, RUSSIA	E-mail: herpzoo@RH4397.spb.edu Tel.: +7 (812) 319-47-61 +7 (812) 157-02-69
Schabetsberger, Robert	Institute of Zoology University of Salzburg	Hellbrunnerstrasse 34, A-5020 Salzburg, AUSTRIA	E-mail: Robert.Schabetsberger@sbg.ac.at Tel.: +43 (662) 87-38-33 Fax: +43 (662) 804-45-698
Schmidt, Peter	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: p_e_schmidt@yahoo.de Tel.: +49 (228) 249-57-94 +49 (228) 912-22-49 Fax: +49 (1212) 526-714-382
Schmidt, Nicole	Zoologisches Forschungsinstitut und Museum Alexander Koenig	Adenauerallee 160, D-53113 Bonn, GERMANY	E-mail: nicoleschmidt78@gmx.de Tel.: +49 (228) 249-57-94 +49 (228) 912-22-49 Fax: +49 (1212) 526-714-382
Schweiger, Silke	Vienna Natural History Museum	Burgring 7, A-1014 Vienna, AUSTRIA	E-mail: ammo1@gmx.at Tel.: +43 (1) 521-77-593 Fax: +43 (1) 521-775-235-254
Semenov, Dmirty	Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: dsemenov@orc.ru Tel.: +7 (095) 334-82-60 Fax: +7 (095) 952-35-84
Semiglazov, Konstantin	The Nizhny Novgorod Society for Protection of Amphibians and Reptiles	Kostina ul., 2, of. 157, P.O. Box 631, 603000 N. Novgorod, RUSSIA	E-mail: vipera@dronr.ru Tel.: +7 903-604-50-83 Fax: +7 (8312) 30-28-81
Semiglazova, Ludmila	The Nizhny Novgorod Society for Protection of Amphibians and Reptiles	Kostina ul., 2, of. 157, P.O. Box 631, 603000 N. Novgorod, RUSSIA	E-mail: vipera@dronr.ru Tel.: +7 (903) 604-50-83 Fax: +7 (8312) 30-28-81
Semyonova, Svetlana	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	Tel.: +7 (095) 257-25-35 +7 (095) 135-87-41 Fax: +7 (095) 135-41-05
Severtsov, Alexsey	Biological Evolution Department Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: Severtsova@mail.ru Tel.: +7 (095) 939-42-47 Fax: +7 (095) 939-43-09
Severtsova, Helen	Biological Evolution Department Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: Severtsova@mail.ru Tel.: +7 (095) 939-35-01 Fax: +7 (095) 939-43-09
Sevinç, Murat	Department of Biology Science and Art Faculty Uludag University	TURKEY	E-mail: smurat@uludag.edu.tr Tel.: +90 (224) 442-92-56 Fax: +90 (224) 442-81-36
Shabanov, Dmitry	Karazin Kharkov National University	Svobody md., 4, UA-61077 Khar'kiv, UKRAINE	E-mail: shabanov@sky.net.ua Tel.: +38 (0572) 45-71-72
Shahparonov, Vladimir	Department of Vertebrate Zoology Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: wshakh@yandex.ru Tel.: +7 (095) 939-01-11
Shiryayev, Konstantin	Tula Regional Exotarium	Oktyabr'skaya ul., 26, 300002 Tula, RUSSIA	E-mail: rept@tula.net Tel.: +7 (0872) 77-53-92 Fax: +7 (0872) 77-53-92
Shishkin, Michael	Paleontological Institute Russian Academy of Sciences	Profsoyuznaya ul., 123, 117997 Moscow, RUSSIA	E-mail: shishkin@paleo.ru schsz@ore.ru Tel.: +7 (095) 339-19-88 (w) +7 (095) 433-97-79 (h) Fax: +7 (095) 339-12-66
Shlyakhtin, Gennadiy	Saratov State University	Astrakhanskaya ul., 83, 410026 Saratov, RUSSIA	E-mail: biofac@sgu.ru Tel.: +7 (8452) 51-16-30 Fax: +7 (8452) 51-16-35

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Sideleva, Olga	Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: osideleva@mail.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Smirina, Ella	Koltsov Institute of Developmental Biology Russian Academy of Sciences	Vavilova ul., 26, 119991 Moscow, RUSSIA	E-mail: elsmir@mail.ru Tel.: +7 (095) 952-30-07 +7 (095) 952-24-23 Fax: +7 (095) 952-30-07
Smirnov, Sergei	Severtzov Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: evol_morphol_lab@newmail.ru Tel.: +7 (095) 954-32-62
Smirnova, Julia	Collection manager of Department of Herpetology and Ornithology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: lacerta@zin.ru Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Smit, Gerard	Department of Herpetology Zoological Museum Amsterdam	P.O. Box 94766, Amsterdam 1090 GT, THE NETHERLANDS	E-mail: gsmit@science.uva.nl Tel.: +31 (20) 525-66-24
Söderman, Fredrik	Department of Population Biology Uppsala University	Norbyvägen 18, D, S-752 36 Uppsala, SWEDEN	E-mail: fredrik.soderman@ebc.uu.se Tel.: +46 (18) 471-2651 Fax: +46 (18) 471-6424
Sokolov, Alexei	Department of Vertebrate Zoology Biological Faculty Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: aquazoon@mtu-net.ru Tel.: +7 (095) 335-49-12
Sokolov, Alexandr	Tambov State University	Internatsional'naya ul., 33, 392622 Tambov, RUSSIA	E-mail: taf@tsu.tmb.ru Tel.: +7 (0752) 74-58-39 Fax: +7 (0752) 71-03-07
Sorochinskiy, Georgiy	Director of "Zoocomplex" Ltd.	Chilanzar-26, 19 apt. 40, 700128 Tashkent, UZBEKISTAN	E-mail: eperegona@mail.ru Tel.: +998 (71) 107-04-90
Sperone, Emilio	Ecology Department Calabria University	Via P. Bucci-Rende, I-87036 Cosenza, ITALY	E-mail: esperone@libero.it Tel.: +39 (0984) 49-29-72 Fax: +39 (0984) 49-29-86
Spodarets, Dmitry	Institute of Biology Dnipropetrovsk National University	Naukova vul., 13, UA-49050 Dnipropetrovsk, UKRAINE	E-mail: vgasso@mail.dsu.dp.ua vgasso@ua.fm Tel.: +38 (0562) 46-92-82 Fax: +38 (0562) 46-55-23
Stanyukovich, Maria	Laboratory of Parasitology Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: laelaps@zin.ru Tel.: +7 (812) 235-24-14 Fax: +7 (812) 328-29-41
Stepanyan, Ilona	Institute of Zoology National Academy of Science of Armenia	Paruir Sevak, 7, 375014 Yerevan, ARMENIA	E-mail: ilona_e@pochtamt.ru Tel.: +374 (1) 28-17-71 (w) +374 (1) 42-94-62 (h) Fax: +374 (1) 28-68-87
Stuart, Bryan	Division of Amphibians and Reptiles Department of Zoology The Field Museum	1400 S. Lake Shore Dr., Chicago, IL 60605-2496, USA	E-mail: bstuart@fmnh.org Tel.: +1 (312) 665-76-73 Fax: +1 (312) 665-76-97
Stumpel, Anton	Alterra, Green World Research	Postbus 47, 6700 AA, THE NETHERLANDS	E-mail: anton.stumpel@wur.nl Tel.: +31 (317) 47-87-63 Fax: +31 (317) 42-49-88
Sultanova, Zulfiya	Faculty of Biology Dagestan State University	Batiraya ul., 8, 367025 Makhachkala, RUSSIA	E-mail: Mazanaev@mail.ru Tel.: +7 (8722) 67-95-46 Fax: +7 (8722) 68-23-26
Surova, Galina	Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: avs@avs.bio.msu.su surova@hotmail.ru Tel.: +7 (095) 244-07-06 Fax: +7 (095) 244-07-06
Sviridenko, Helen	Department of Zoology National Taurida Vernadsky University	Yaltinskaya ul., 4, Simferopol', UA-98500/95007 Crimea, UKRAINE	E-mail: nmkovb@tnu.crimea.ua Tel.: +38 (0652) 23-03-93
Sytnik, Alexey	Department of Biology Shevchenko National University	Volodymyrska vul., 64, UA-01033 Kyiv, UKRAINE	E-mail: Sytnik77@hotmail.com Tel.: +38 (044) 234-82-16
Sztatecsny, Marc	Institut für Zoologie Universität Wien	Althanstrasse 14, A-1090 Vienna, AUSTRIA	E-mail: marc.sztatecsny@univie.ac.at Tel.: +43 (1) 427-754-496 Fax: +43 (1) 427-79-544
Tabachishin, Vasilij	Saratov State University	Astrakhanskaya ul., 83, 410026 Saratov, RUSSIA	E-mail: biofac@sgu.ru Tel.: +7 (8452) 51-16-30 Fax: +7 (8452) 51-16-35
Tagirova, Valentina	Khabarovsk State Teachers Training University	K. Marksa ul., 68, 680000 Khabarovsk, RUSSIA	E-mail: valtix@mail.ru Tel.: +7 (4212) 30-82-50

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Tokarskaya, Olga	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	Tel.: +7 (095) 257-25-35 +7 (095) 135-87-41 Fax: +7 (095) 135-41-05
Toktamyssova, Zauze	Institute of Zoology	al-Faraby, 93, Akademgorodok, 480060 Almaty, KAZAKHSTAN	E-mail: Zhamal@arna.kz Tel.: +7 (327) 48-09-19 Fax: +7 (327) 48-19-50
Tomasevic, Natasa	Faculty of Biology Institute of Zoology	Studentski Trg 6, Belgrade 11000, SERBIA AND MONTENEGRO	E-mail: tosic@yubc.net
Tomovic, Ljiljana	Faculty of Biology Institute of Zoology University of Belgrade	Studentski Trg 6, Belgrade 11000, SERBIA AND MONTENEGRO	E-mail: lili@bf.bio.bg.ac.yu Tel.: +381 (11) 18-72-66 Fax: +381 (11) 63-85-00
Tripepi, Sandro	Calabria University Ecology Department	Via P. Bucci-Rende, I-87036 Cosenza, ITALY	E-mail: s.tripepi@unical.it Tel.: +39 (0984) 49-29-71 Fax: +39 (0984) 49-29-86
Truveler, Kirill	Faculty of Biology Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, Russia	E-mail: truveler@mail.ru Tel.: +7 (095) 939-35-02
Tsellarius, Elena	Zoological Institute Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	Tel.: +7 (812) 323-31-40
Tsinenko, Olga	Department of Herpetology and Ornithology Zoological Institut Russian Academy of Sciences	Universitetskaya nab., 1, 199034 St. Petersburg, RUSSIA	E-mail: 12SEH@zin.ru tsinenko@yahoo.com Tel.: +7 (812) 323-31-40 Fax: +7 (812) 328-29-41 +7 (812) 114-04-44
Tsurcanu, Vladimir	Institute of Zoology Academy of Sciences of Moldova	Tr. Academiei 1, MD-2028 Chishinau, MOLDOVA	E-mail: som@as.md Tel.: +373 (2) 73-75-09 Fax: +373 (2) 73-12-75
Tuniyev, Boris	Caucasian State Nature Biosphere Reserve	K. Marksa ul., 8, 354340 Sochi, RUSSIA	E-mail: adlerzap@sochi.ru Tel.: +7 (8622) 94-23-46 +7 (8622) 44-44-31 Fax: +7 (8622) 69-20-03
Tytov, Oleksiy	International Solomon University	Sholudenko vul., 1-b, UA-01135 Kyiv, UKRAINE	E-mail: olekstytov@yahoo.com Tel.: +38 (044) 540-15-86 (h)
Ursenbacher, Sylvain	Laboratory for Conservation Biology Institut d'Ecologie Université de Lausanne	IE-ZEA, CH-1015 Lausanne, SWITZERLAND	E-mail: sylvain.ursenbacher@unil.ch sylvain.ursenbacher@ie-zea.unil.ch Tel.: +41 (21) 692-41-63 Fax: +41 (21) 692-41-65
Ushakov, Michail	Galich'ya Gora Nature Reserve Voronezh State University	Donskoe, Zadonskii Raion, 399240 Lipetsk Oblast', RUSSIA	E-mail: ushakov@dev-reserve.vsu.ru Tel.: +7 (07471) 3-33-65 +7 (07471) 3-34-13 Fax: +7 (07471) 3-33-65
Uteshev, Viktor	Institute of Cell Biophysics Russian Academy of Sciences	Pushchino, 142290 Moscow Oblast', RUSSIA	E-mail: Gakhova@icb.psn.ru
Vashetko, Emiliya	Institute of Zoology Uzbek Academy of Sciences	Navoyi str., 1, 700095 Tashkent, UZBEKISTAN	E-mail: emili@tkt.uz Tel.: +998 (71) 246-07-18
Vassiliev, Boris	Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: evol_morphol_lab@newmail.ru vassil@vassil.bio.msu.su Tel.: +7 (095) 939-33-02
Vassilieva, Anna	Moscow State University	Leninskie Gory, 1/12, 119899 Moscow, RUSSIA	E-mail: vassil@vassil.bio.msu.su Tel.: +7 (095) 939-33-02
Vershinin, Vladimir	Institute of Plant and Animal Ecology Ural Branch Russian Academy of Science	8 Marta ul., 202, 620144 Yekaterinburg, RUSSIA	E-mail: wow@ipae.uran.ru ecom@ipae.uran.ru Tel.: +7 (343) 222-85-70 Fax: +7 (343) 229-41-61
Vorobjova, Emilia	Institute of Ecology and Evolution Russian Academy of Sciences	Leninsky prosp., 33, 119071 Moscow, RUSSIA	E-mail: sevin@orc.ru Tel.: +7 (095) 954-32-62 Fax: +7 (095) 954-55-34
Voronov, Alexander	Institute of Gene Biology Russian Academy of Sciences	Vavilova ul., 34/5, 119334 Moscow, RUSSIA	E-mail: cuprum@atrus.ru Tel.: +7 (095) 135-98-64 Fax: +7 (095) 135-41-05
Vörös, Judit	Department of Zoology Hungarian Natural History Museum	Baross u. 13, H-1088 Budapest, HUNGARY	E-mail: jvoros@zoo.zoo.nhmus.hu Tel.: +36 (1) 267-34-62 Fax: +36 (1) 267-34-62
Wahlgren, Richard	International Society for the History and Bibliography of Herpetology	Prennegatan 23B, S-223 53 Lund, SWEDEN	E-mail: Richard.Wahlgren@Skanska.se Tel.: +46 (40) 144-652 Fax: +46 (40) 144-460
Westerström, Alexander	Stockholm University	Rusthållarvägen 151, Bagarmossen, S-128 44 Stockholm, SWEDEN	E-mail: bulgardaghica@hotmail.com Tel.: +46 (73) 990-5069

Name	Affiliation or Institution	Mailing Address	E-mail, Telephone, Fax
Wycherley, Julia	University of Sussex	Falmer, Brighton BN1 9QG, UK	E-mail: julia.wycherley@virgin.net Tel.: +44 (1273) 60-67-55 Fax: +44 (1273) 67-84-33
Yanchukov, Alexey	Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine	B. Khmelnits'kogo vul., 15, UA-01601 Kyiv, UKRAINE	E-mail: oneks@mail.ru Tel.: +38 (044) 235-51-87 Fax: +38 (044) 234-15-69
Yanchurevich, Olga	Yanka Kupala State University of Grodno	Ozheshko vul., 22, 230023 Grodno, BELARUS'	E-mail: yanch@grsu.by oyanch@mail.ru Tel.: +375 (152) 78-50-02 +375 (152) 31-48-22 Fax: +375 (152) 73-80-30 +375 (17) 210-85-99
Zamaletdinov, Renat	Institute of Ecology of Natural Systems Academy of Sciences of Republic Tatarstan	Daur'skaya ul., 28, 420087 Kazan, RUSSIA	E-mail: i.ricinus@rambler.ru Tel.: +7 (8432) 61-67-03 Fax: +7 (8432) 99-35-12
Zavialov, Evgeny	Saratov State University	Astrakhanskaya ul., 83, 410026 Saratov, RUSSIA	E-mail: biofac@sgu.ru Tel.: +7 (8452) 51-16-30 Fax: +7 (8452) 51-16-35
Zhdanova, Nadezhda	Koltzov Institute of Developmental Biology Russian Academy of Sciences	Vavilova ul., 26, 119991 Moscow, RUSSIA	E-mail: Nzhdanova@rambler.ru Tel.: +7 (095) 952-24-23 Fax: +7 (095) 952-30-07
Zhukova, Tatiana	Kuban State University	Stavropol'skaya ul., 224/1-49, 350040 Krasnodar, RUSSIA	E-mail: peskova@kubannet.ru Tel.: +7 (8612) 33-39-00
Zinenko, Oleksandr	Museum of Nature Karazin Kharkiv National University	Trinklera vul., 8, UA-61022 Kharkiv, UKRAINE	E-mail: zinenkoa@yahoo.com zinenko@au.ru Tel.: +38 (0572) 43-28-86 (h) +38 (0572) 47-60-05
Zuffi, Marco A. L.	Museum Natural History and Territory University of Pisa	Via Roma 79, Calci, I-56011 Pisa, Italy	E-mail: marcoz@museo.unipi.it Tel.: +39 (050) 221-29-67 Fax: +39 (050) 221-29-75
Zuiderwijk, Anna	Department Herpetology Zoological Museum Amsterdam	P.O. Box 94766, Amsterdam 1090 GT, THE NETHERLANDS	E-mail: zuiderwijk@science.uva.nl Tel.: +31 (20) 525-66-24

AUTHOR'S INDEX

- A**
- Adnagulov E. V. 117
 Ananjeva Natalia B. 5, 93, 291
 Anjubault E. 270
 Aprea G. 47
- B**
- Bahiani M. 249
 Bakiev A. G. 252
 Bannikova A. 44
 Baranovskaja N. V. 167
 Bassu L. 27
 Bauer L. 13, 15
 Bernardi F. De 140
 Bleyzac P. 254
 Bonacci A. 113, 120
 Borkin L. J. 16, 53, 57
 Bosbach G. 238
 Bozhansky A. T. 123
 Brizzi R. 258, 263
 Brovko A. U. 100
 Bulakhova N. A. 171, 201
 Böhme W. 74
- C**
- Camargo A. 90
 Capriglione T. 47, 80
 Carlino P. 113
 Castro M. J. 126
 Chernyshov K. I. 20
 Citi S. 245
 Corbett K. F. 219
 Corti C. 27, 113
- D**
- de Sá R. O. 35, 90
 Delfino G. 258
 Dmitrieva E. 130
 Dolmen D. 134
 Dotsenko I. B. 29
 Drabkin P. L. 109
 Dunce I. 138
 Džukić G. 16, 57
- E**
- Estabel J. 267
 Exbrayat J.-M. 249, 254, 267, 270
- F**
- Ficetola G. F. 140
 Fornasiero S. 113
 Fresi C. 27
 Fritz U. 40
- G**
- Gakhova E. 233
 Ganshchuk S. 179
- Gernigon-Spychalowicz T. 249
 Giusti M. 245
 Gollmann B. 143
 Gollmann G. 143
 Guicking D. 40
 Gvoždík V. 32
- H**
- Hachtel M. 146, 238
 Heyer W. R. 35, 90
 Hinchliffe J. R. 274
- I**
- Iizuka K. 279
 Iohanssen L. 310
 Ishchenko V. G. 153
- J**
- Jantra S. 258
- K**
- Kalezić M. L. 16, 57
 Kamelin E. R. 158
 Khalturin M. D. 57
 Korsunen A. 40
 Kotenko T. 43
 Kotserzhynska I. 161
 Krechetova O. A. 171
 Krivosheev V. 164
 Kruzhkova Yu. I. 286
 Kupfer A. 146
 Kupriyanova L. 47, 80
 Kupriyanova N. S. 105
 Kuranova V. N. 167, 171, 201
- L**
- Lada G. A. 53
 Lapid R. 44
 Lazareva O. 176
 Litvinchuk S. N. 16, 53, 57
 Litvinov N. 179
 Lukin Yu. A. 158
 Lyapkov S. M. 183, 187
 Lyapustin S. N. 191
- M**
- Manilo V. V. 61
 Martin R. 193
 Maslova I. V. 117, 191
 Mazanaeva L. F. 44, 57
 Mednikov D. N. 274
 Mezhzherin S. 77
 Miaud C. 193
 Milto K. D. 64
 Monney J.-C. 102
 Moravec J. 32, 74
- Morozov-Leonov S. 77
- N**
- Nakazato T. 279
 Nekrasova O. 77
 Nikitina Natalia G. 291
- O**
- Odierna G. 47, 80
 Ogurtsov S. V. 198, 209
 Oliveira J. M. 126
 Olmo E. 47, 80
 Orlov N. 93
 Orlova V. F. 201
 Ortman D. 146
- P**
- Patrakov S. V. 171
 Pavlov A. 87
 Pereshkolnik S. 44
 Peskov V. N. 100
 Peskova T. Y. 296
 Pidancier N. 193
- R**
- Racca L. 205
 Rettig A. 35
 Rikhvanov L. P. 167
 Rizzuti M. T. 120
 Roitberg E. S. 298
 Rosanov J. M. 16, 57
 Rossi M. 102
 Rudyk O. M. 109
 Ryabov S. 93
 Ryskov A. P. 105
- S**
- Sander U. 146, 238
 Sasu I. 102
 Satta M. G. 27
 Sauer-Gürth H. 40
 Schmidt P. 146, 238
 Semyenova S. 44
 Sessions S. K. 279
 Sevinç M. 263
 Shakhparonov V. V. 209
 Shiryaev K. A. 213
 Shishkin M. A. 301
 Sideleva O. 93
 Skorinov D. V. 57
 Smirina E. M. 298
 Smirnov S. V. 304
 Sokolov A. S. 96
 Sokolov A. Yu. 307
 Sperone E. 113, 216

<i>Dermophis mexicanus</i>		Helminths	252
Development	254, 270	Hematological indexes	296
Diagnostic characters	77	<i>Hemilaelaps</i>	310
Discriminant analysis	102	Hemipenes structure	64
Dispersal	143, 193	Herpetology	13
Distribution	64, 117, 241	<i>Hierophis viridiflavus</i>	113
DNA	40	Home range	171
DNA extraction	230	Hormones, thyroid	304, 312
DNA flow cytometry	16	Hybrids	93
DNA, microsatellite	205	<i>Hyla</i>	15, 16
	E	<i>Hyla arborea</i>	138
Early Triassic	301	<i>Hyla savignyi</i>	32
Eastern Europe	57, 64	Hylidae	32
Ecological niche	171	<i>Hynobius</i>	279
Ecology	143, 205, 216		I
Egg and tadpole translocation	183	Iguania	291
Egg density	130	Illegal export	191
Egg size	238	Integument	291
<i>Eirenis</i>	29	Interdigital membrane	279
Elapidae	307	Internal transcribed spacers	105
Embryogenesis	130	Interspecific breeding	93
Embryonic development	120	Italy	113
<i>Emys orbicularis</i>	43, 245	Italy, Calabria	216
Europe	219		J
Europe, Eastern	57, 64	Jaw apparatus	307
Evolution	47, 304	Jersey, UK	205
ex-USSR	310	Juveniles	193
	F		K
Far East, Russia	117, 191	Karyotype	61
Faunal evolution	301	Karyotype variations	47
Fecundity	201, 238		L
Femur bone thickness	298	<i>Lacerta agilis</i>	100, 109, 241
Fish introduction	140	<i>Lacerta agilis boemica</i>	298
Form-formation	47	<i>Lacerta strigata</i>	298
Frog	176	Laelapidae	310
Frogs	153	Larval limb development	274
Functional morphology	307	<i>Leptodactylus fuscus</i>	90
	G	<i>Leptodactylus pentadactylus</i> species cluster	35
Gekkonidae family	291	Life history	143
Gekkota	291	Life-history characteristics	183
Genetic cryobank	233	Limb development	279
Genetic differentiation	40	Liming	134
Genome size	16, 57	Lizard	249
Geochemical monitoring	167	Lizards	105, 171, 298
Geographic variation	43, 113, 298	Local adaptation	193
Geographical and local variation	187	Longevity	176
Germany	146, 238	Long-legged forms	161
Glutamate receptors	267	Long-term study	146, 150
Gonads	270	<i>Lophopus Tschudi, 1838</i>	15
Green frog	20		M
Growth rate	187	Macedonia	16
Growth	153	Macronyssidae	310
Gymnophiona	254, 270	Mapping	219
	H	<i>Maticora</i>	307
Habitat assessment	219	Mediterranean, Western	27
Habitat differentiation	161		

Meiosis	61		
<i>Mertensiella</i>	263		
Mesostigmata	310		
Metamorphosis	304, 312		
Microsatellite DNA	205		
Microstructure	291		
Migration	143		
Minimum age	146		
Minimum survival rates	146		
Mitosis	61		
Molecular analyses	90		
Molecular methods	230		
Molecular phylogeography	43		
Monitoring	150		
Moor frog	235		
Morphogenesis	235		
Morphological analysis	64		
Morphological characters	96		
Morphological deviations	235		
Morphological variation	77		
Morphology	57, 100, 102		
Morphometrics	53		
Mortality	130		
		N	
Native pond	198		
Native pond odor	209		
ND1	90		
Newt	120		
Newts	140		
Nikolsky's viper	64		
Nomenclature	13		
North of area	87		
Norway	134		
		O	
<i>Onychodactylus fisheri</i>	274		
<i>Onychodactylus</i>	279		
<i>Ophionyssus</i>	310		
Orientation	193, 209		
		P	
Palpation	245		
Parasitic gamasid mites	310		
Pattern	29		
<i>Pelobates</i>	16		
<i>Pelobates fuscus</i>	53		
<i>Pelodiscus sinensis</i>	117, 191		
Periods of learning	209		
Pesticides	296		
Pholidosis	29, 100		
Phylogenetic analyses	90		
Phylogeny	29		
Polyploidization	61		
Pond-type	279		
Population dynamics	150, 205		
Population recovery	183		
		Q	
<i>Quinzhyla</i>	15		
		R	
Radiography	245		
<i>Rana</i>	16, 258		
<i>Rana arvalis</i>	161		
<i>Rana dalmatina</i>	146, 150, 205, 238		
<i>Rana dybowskii</i>	191		
<i>Rana esculenta</i> complex	77		
<i>Rana kurtmuelleri</i>	241		
<i>Rana ridibunda</i>	296		
<i>Rana temporaria</i>	134, 146, 230, 238		
RAPD	93		
RAPD-PCR	40, 230		
Rat snake	93		
Red Data Book	164		
Reintroduction	138		
Release calls	20		
Replacement name	15		
Reproduction	238, 245		
Reproductive biology	213		
Reproductive characteristics	187		
Reproductive isolation	35		
Reproductive structures	263		
Reptiles	164, 219, 301, 310		
Reptilia	286, 307, 310		
Reptilian resources	123		
Return rates	146		
Ribosomal DNA	105		
Russia, Astrakhan' Oblast'	123		
Russia, Far East	117, 191		
Russia, Volga – Kama Region	87, 252		
		S	
Salamander larva	279		
<i>Salamandrella keyserlingii</i>	274		
Sauria	291		
Scalation	74		
Seasonal variations	249		
Sensitive period	198		
Sequence data	90		
Serbia and Montenegro	16		
Serpentes	74, 96, 158, 307		
Sex chromosomes	47, 80		
Sex ratio	74		
Sexual segment of the kidney	249		
Sexual size differences	298		
Short-legged forms	161		
Sibling species	90		
Size, genome	16		
Skeletochronological method	230		
Skeletochronology	153, 298		
Skin organs	291		
Speciation	35, 47, 80		
Sperm storage	158		
Status	219		
Stream-type	279		
Subspecies	87, 109		
Survivorship	187		
Sympatry	171		

Systematics	57, 64	Ultrasonography	245
T		Urban ecology	126, 235
Tadpole	130	Urban growth	126
Taxonomical position	241	Urodela	312
Taxonomy	13, 96	Urodeles	304
Temperature influence	120	USSR, ex-	310
<i>Teratoscincus</i>	291	V	
<i>Testudo</i>	40	Variability	201, 286
<i>Testudo hermanni</i>	27	Variation	32
Thermopreferenda	179	Variety	100
Thumb pad glands	258	<i>Vipera</i>	213
Thyroid hormones	304, 312	<i>Vipera berus</i>	64, 96, 102, 241
Toad	130	<i>Vipera nikolskii</i>	64
Toadlets	209	<i>Vipera renardi bashkirovi</i>	87
Trade-off	238	Viperidae	64, 96, 158
Triassic, Early	301	Volga-Kama Region, Russia	87, 252
<i>Trimeresurus albolabris</i>	158	W	
<i>Trimeresurus erythrurus</i>	158	Western Mediterranean	27
<i>Triturus</i>	57	Wetland features	140
<i>Triturus alpestris</i>	150, 241	X	
<i>Triturus alpestris inexpectatus</i>	120	<i>Xenopus laevis</i> tadpoles	267
<i>Triturus cristatus</i>	146	Z	
<i>Triturus italicus</i>	216	<i>Zamenis situla</i>	74
<i>Triturus vittatus</i>	274	Zone of intergradation	109
<i>Triturus vulgaris</i>	134, 274	<i>Zootoca vivipara</i>	47, 201
<i>Typhlonectes compressicauda</i>	254, 270		
U			
Ukraine	43, 100, 109		

CONTENTS

History and Anniversary Dates of Russian Herpetology in St. Petersburg <i>Natalia B. Ananjeva</i>	5
--	---

SYSTEMATICS, PHYLOGENY, BIOGEOGRAPHY, AND GENETICS OF AMPHIBIANS AND REPTILES

Plural Names Earn and Need Plural Verbs <i>L. Bauer</i>	13
A Replacement Name for <i>Lophopus</i> <i>L. Bauer</i>	15
Genome Size Variation in the Balkan Anurans <i>L. J. Borkin, S. N. Litvinchuk, J. M. Rosanov, G. Džukić, and M. L. Kalezić</i>	16
Variability of Advertisement Calls and Release Calls of Green Frogs in the Moscow Oblast', Russia <i>K. I. Chernyshov</i>	20
Preliminary Data on Body Size Differences in Adults of <i>Testudo hermanni hermanni</i> Gmelin, 1789: Comparison Between Two Western Mediterranean Insular Populations and the Continental Population of Southern Tuscany <i>C. Corti, M. A. L. Zuffi, L. Bassu, C. Fresi, and M. G. Satta</i>	27
Taxonomic Value of the Pholidosis and Pattern Characters in Systematic and Phylogeny of Genus <i>Eirenis</i> <i>I. B. Dotsenko</i>	29
Variation of <i>Hyla savignyi</i> : A Color Pattern of Cypriote and Mainland Populations <i>V. Gvoždik and J. Moravec</i>	32
Sibling Species, Advertisement Calls, and Reproductive Isolation in Frogs of the <i>Leptodactylus pentadactylus</i> Species Cluster (Amphibia, Leptodactylidae) <i>W. R. Heyer, R. O. de Sá, and A. Rettig</i>	35
DNA Polymorphism and Genetic Differentiation of <i>Testudo graeca</i> L. <i>A. Korsunenko, V. Vasilyev, S. Pereshkolnik, L. Mazanaeva, R. Lapid, A. Bannikova, and S. Semyenova</i>	40
First Data on the Geographic Variation of <i>Emys orbicularis</i> in Ukraine: mtDNA Haplotypes, Coloration, and Size <i>T. Kotenko, O. Zinenko, D. Guicking, H. Sauer-Gürth, M. Wink, and U. Fritz</i>	43
Chromosomal Changes and Form-Formation, Subspeciation in the Wideranged Euroasian Species <i>Zootoca vivipara</i> (Evolution, Biogeography) <i>L. Kupriyanova, G. Odierna, T. Capriglione, E. Olmo, and G. Aprea</i>	47
Morphological Variation in Two Cryptic Forms of the Common Spadefoot Toad (<i>Pelobates fuscus</i>) from Eastern Europe <i>G. A. Lada, L. J. Borkin, and S. N. Litvinchuk</i>	53

Geographic Differentiation in Newts (<i>Triturus</i>) of Eastern Europe: Genome Size, Allozymes, and Morphology <i>S. N. Litvinchuk, L. J. Borkin, J. M. Rosanov, D. V. Skorinov, M. D. Khalturin, G. Džukić, M. L. Kalezić, and L. F. Mazanaeva</i>	57
Cases of Mixoploidy in Brown Frogs of Ukraine <i>V. V. Manilo</i>	61
Distribution and Morphological Variability of <i>Vipera berus</i> in Eastern Europe <i>K. D. Milto and O. I. Zinenko</i>	64
Morphological Variation and Sex Ratio in the Leopard Snake (<i>Zamenis situla</i>) from Sozopol (Bulgaria) <i>J. Moravec and W. Böhme</i>	74
Diagnostic Traits in the Morphology of Green Frogs (<i>Rana esculenta</i> Complex) in the Middle Dnepr Basin <i>O. Nekrasova, S. Mezhzherin, and S. Morozov-Leonov</i>	77
The Contribution of Cytogenetics to the Systematics of Reptiles <i>E. Olmo, T. Capriglione, G. Odierna, and L. Kupriyanova</i>	80
Steppe Viper (<i>Vipera renardi</i>) in the Northern Point of Its Area <i>A. Pavlov</i>	87
Are <i>Leptodactylus didymus</i> and <i>L. mystaceus</i> Phylogenetically Sibling Species (Amphibia, Anura, Leptodactylidae)? <i>R. O. de Sá, W. R. Heyer, and A. Camargo</i>	90
Paternal Inheritance of Molecular Markers in Rat Snakes Interspecies Breeding <i>O. Sideleva, S. Ryabov, N. Ananjeva, and N. Orlov</i>	93
On the Taxonomic Status of the Common Adder of the Partially Wooded Steppe of the Oka – Don Plain <i>A. S. Sokolov</i>	96
Taxonomical Analysis of Morphological Variety of the Sand Lizard (<i>Lacerta agilis</i>) in Ukraine <i>O. A. Tytov, V. N. Peskov, and A. U. Brovko</i>	100
Are There Morphological Differences Between Two Genetically Differentiated Clades in the Adder <i>Vipera berus berus</i> ? <i>S. Ursenbacher, I. Sasu, M. Rossi, and J.-C. Monney</i>	102
Molecular-Genetic Characteristics of Lizard Ribosomal DNA <i>A. S. Voronov, G. A. Voronova, N. S. Kupriyanova, and A. P. Ryskov</i>	105
Contact Zone Between Two Subspecies of the Sand Lizard: <i>Lacerta agilis exigua</i> Eichw., 1831 and <i>Lacerta agilis chersonensis</i> Andr., 1832 in Three Regions of the Left-Bank Ukraine <i>O. I. Zinenko, P. L. Drabkin, and O. M. Rudyk</i>	109
Geographical Comparison and Body Size Differentiation in the European Whip Snake, <i>Hierophis viridiflavus</i> , from Central and Southern Italy <i>M. A. L. Zuffi, S. Fornasiero, P. Carlino, A. Bonacci, C. Corti, E. Sperone, and S. Tripepi</i>	113

ECOLOGY, FAUNISTIC, CONSERVATION, AND CAPTIVE BREEDING OF AMPHIBIANS AND REPTILES

On the Distribution of <i>Pelodiscus sinensis</i> (Wiegmann, 1834) (Testudines: Trionychidae) in the Russian Far East <i>E. V. Adnagulov and I. V. Maslova</i>	117
<i>Triturus alpestris inexpectatus</i> : Normal Developmental Stages Morphology and Temperature Influence <i>A. Bonacci, M. T. Rizzuti, and S. Tripepi</i>	120
Conservation of Resources of Reptiles in Astrakhan' Oblast' (Russia). Astrakhan' Oblast' Reptilian Resources Conservation (experience of regional realization) <i>A. T. Bozhansky</i>	123
Conflicts Between Urban Growth and Species Protection: Can Midwife Toads (<i>Alytes obstetricans</i>) Resist the Pressure? <i>M. J. Castro, J. M. Oliveira, and A. Tari</i>	126
The Effects of Density on Mortality and Development of the <i>Bufo bufo</i> Eggs and Tadpoles <i>E. Dmitrieva</i>	130
The Amphibian Decline in Norway – Reasons and Remedy (Case: Acidic Precipitation) <i>D. Dolmen</i>	134
The European Tree Frog Reintroduction in Latvia <i>I. Duncce and J. Zvirgzds</i>	138
Influence of Hydroperiod, Sun Exposure and Fish Presence on Amphibian Communities in a Human Dominated Landscape <i>G. F. Ficetola and F. De Bernardi</i>	140
Postmetamorphic Growth and Movements in Yellow-Bellied Toads, <i>Bombina variegata</i> : Approaching Life-Path Analysis <i>G. Gollmann and B. Gollmann</i>	143
Return Rates and Long-Term Capture History of Amphibians in an Agricultural Landscape Near Bonn (Germany) <i>M. Hachtel, D. Ortmann, A. Kupfer, U. Sander, P. Schmidt, and K. Weddeling</i>	146
Eleven Years of Monitoring: Amphibian Populations in an Agricultural Landscape near Bonn (Germany) <i>M. Hachtel, P. Schmidt, U. Sander, D. Tarkhnishvili, K. Weddeling, and W. Böhme</i>	150
Growth of Brown Frogs of Fauna of Russia: Some Problems of Study of Growth in Amphibians <i>V. G. Ishchenko</i>	153
Sperm Storage in Two Species of Snakes: Asian Pit Vipers <i>Trimeresurus albolabris</i> (Gray, 1842) and <i>Trimeresurus erythrurus</i> (Cantor, 1839), Bred at the Leningrad Zoo Terrarium <i>E. R. Kamelin and Yu. A. Lukin</i>	158
Habitat Variation in <i>Rana arvalis</i> of Northeastern Ukraine <i>I. Kotserzhynska</i>	161
The Red Data Book of Ul'yanovsk Oblast': Amphibians and Reptiles <i>V. Krivosheev</i>	164

The Content of Chemical Elements in the Organism of Anura, Amphibia, as an Indicator of the Environmental Conditions <i>V. N. Kuranova, N. V. Baranovskaja, and L. P. Rikhvanov</i>	167
The Study of the Ecological Niche Segregation for Sympatric Species of Lizards <i>Lacerta agilis</i> and <i>Zootoca vivipara</i> <i>V. N. Kuranova, S. V. Patrakov, N. A. Bulakhova, and O. A. Krechetova</i>	171
A Comparative Skeletochronological Analysis of Demography of Four Amphibian Species (Anura, Ranidae) from Ivanovo Oblast', European Russia <i>O. Lazareva</i>	176
Environment and Body Temperatures of Reptiles in Volga–Ural Region <i>N. Litvinov and S. Ganshchuk</i>	179
Conservation and Recovery of Rare Amphibian Species of European Russia: Development of Basic Principles and Effective Practical Measures <i>S. M. Lyapkov</i>	183
Geographical and Local Variation of Reproductive and Demographic Characteristics in Brown Frogs <i>S. M. Lyapkov</i>	187
Illegal Export of Amphibians and Reptiles from the Russian Far East to Countries of the Asian Region: The Situation in 2003 <i>I. V. Maslova and S. N. Lyapustin</i>	191
Preliminary Results on the Genetic Control of Dispersal in Common Frog <i>Rana temporaria</i> Froglets <i>C. Miaud, J. Sérandour, R. Martin, and N. Pidancier</i>	193
Basis of Native Pond Fidelity in Anuran Amphibians: the Case of Chemical Learning <i>S. V. Ogurtsov</i>	198
Some Aspects of Reproductive Biology of <i>Zootoca vivipara</i> (Jacquin, 1787) in the Asian Part of Its Area <i>V. F. Orlova, V. N. Kuranova, and N. A. Bulakhova</i>	201
Conservation of the Agile Frog — the Rarest Amphibian in the British Isles? <i>L. Racca</i>	205
The Role of the Native Pond Odor in Orientation of the Green Toad (<i>Bufo viridis</i> Laur.) Youngs-of-the-Year <i>V. V. Shakhparonov and S. V. Ogurtsov</i>	209
New Data on Reproductive Biology of Caucasian Species of the Genus <i>Vipera</i> <i>K. A. Shiryayev</i>	213
Ecological Preferences of the Italian Newt <i>Triturus italicus</i> (Peracca, 1898) in Calabria <i>E. Sperone and S. Tripepi</i>	216
Mapping Important Herpetofaunal Areas in Northern Europe <i>A. H. P. Stumpel and K. F. Corbett</i>	219
An Access to the Females as a Resource of Male's Territory in <i>Lacerta saxicola</i> <i>A. Yu. Tsellarius and E. Yu. Tsellarius</i>	222
Interrelations Between Sexes in <i>Lacerta saxicola</i> <i>E. Yu. Tsellarius and A. Yu. Tsellarius</i>	226

Rapd Markers in Application to <i>Rana temporaria</i> Genotype Characterization (preliminary data of population study) <i>O. V. Tsinenko</i>	230
Gene Cryobanks for Conservation of Endangered Amphibian Species <i>V. Uteshev and E. Gakhova</i>	233
Morphological Deviations in Population <i>Rana arvalis</i> Nilss. on Urbanized Territories: Spectrum, Topography, Frequency <i>V. L. Vershinin</i>	235
Egg Size Versus Clutch Size: Variation and Trade-off in Reproductive Output of <i>Rana dalmatina</i> and <i>R. temporaria</i> in a Pond Near Bonn (Germany) <i>K. Weddeling, G. Bosbach, M. Hachtel, U. Sander, P. Schmidt, and D. Tarkhnishvili</i>	238
Some Notes on the Herpetofauna of Western Bulgaria <i>A. Westerström</i>	241
Assessment of Reproductive Frequency in the European Pond Turtle (<i>Emys orbicularis</i>) Using Manual Palpation, Ultrasonography, and Radiography <i>M. A. L. Zuffi, S. Citi, M. Giusti, and A. Teti</i>	245

MORPHOLOGY, DEVELOPMENTAL BIOLOGY, PARAZITOLOGY

Structure and Ultrastructure of the Sexual Segment of the Kidney in the Diurnal Saharan Lizard <i>Uromastix acanthinurus</i> , Bell 1825 <i>M. Bahiani, T. Gernigon-Spychalowicz, and J.-M. Exbrayat</i>	249
Helminths and Trophic Relations of Colubrid Snakes (Colubridae) in the Volga – Kama Region <i>A. G. Bakiev</i>	252
Some Aspects of the Ontogenesis of the Immune System Organs of <i>Typhlonectes compressicauda</i> (Duméril et Bibron, 1841), Amphibia, Gymnophiona <i>P. Bleyzac and J.-M. Exbrayat</i>	254
Structure and Functional Significance of the Nuptial Thumb Pads in <i>Rana esculenta</i> and <i>R. perezi</i> <i>R. Brizzi, G. Delfino, and S. Jantra</i>	258
Reproductive Structures in <i>Mertensiella caucasica</i> and <i>M. luschani</i> (Amphibia: Salamandridae) <i>R. Brizzi, M. Sevinç, and G. Tanteri</i>	263
Ampa Receptors Localization by Immunohistochemistry in <i>Xenopus</i> Tadpoles <i>J. Estabel and J.-M. Exbrayat</i>	267
The Development, Differentiation and Growth of Gonads in <i>Typhlonectes compressicauda</i> (Amphibia, Gymnophiona) <i>J.-M. Exbrayat and E. Anjubault</i>	270
Diversity in the Timing of Digit Ossification within the Limb Developmental Bauplan in Caudata <i>J. R. Hinchliffe, E. I. Vorobyeva, and D. N. Mednikov</i>	274

A Comparative Study of the Form and Evolutionary Implications of the Interdigital Membrane of Larval Hynobiid Salamanders <i>K. Iizuka, S. K. Sessions, S. Yasugi, T. Nakazato, and Y. Takeuchi</i>	279
The Distal Limb Part's Variability in Amphibia and Reptilia <i>Yu. I. Kruzhkova</i>	286
The Skin Sense Organs of Lizards of <i>Teratoscincus</i> Genus (Squamata: Sauria: Gekkonidae) <i>Natalia G. Nikitina and Natalia B. Ananjeva</i>	291
Hematological Indexes of <i>Rana ridibunda</i> in Clean and Contaminated Ponds <i>T. Y. Peskova and T. I. Zhukova</i>	296
The Relationship Between Body Length and Femur Bone Thickness in <i>Lacerta agilis boemica</i> and <i>L. strigata</i> . Implications for Growth Inferences from Skeletochronological Data <i>E. S. Roitberg and E. M. Smirina</i>	298
The Patterns of Evolution of Early Triassic Herpetofauna in Europe and Gondwana: Comparison and Implications <i>M. A. Shishkin</i>	301
The Urodele Metamorphosis: Regulatory Mechanisms and Evolution <i>S. V. Smirnov</i>	304
On the Morphofunctional Peculiarities of the Jaw Apparatus of Ophiophagous Elapid Snakes and on the Some Stages in Evolution of Elapidae <i>A. Y. Sokolov</i>	307
Observations on the Gamasid Mites (Parasitiformes, Gamasina, Macronyssidae, Laelapidae) Parasitizing Reptiles (Reptilia) from Russia and Adjacent Countries (ex-USSR) <i>M. Stanyukovich and L. Iohanssen</i>	310
The Dental System of Urodelan Amphibians and the Role of Thyroid Hormones in Its Metamorphic Remodeling <i>A. B. Vassilieva</i>	312
List of Participants	315
Author's Index	330
Keywords' Index	331