Phylogenetic relationships of the European lacertid genera Archaeolacerta and Iberolacerta and their relationships to some other ‘Archaeolacertae’ (sensu lato) from Near East, derived from mitochondrial DNA sequences

W. Mayer1 and O. Arribas2

Abstract

Parts of the mitochondrial genes coding for 12S rRNA and 16S rRNA (together about 960 bp) were sequenced for all Mediterranean species of ‘Mountain lizards’ of the genera Archaeolacerta (sensu lato) and Iberolacerta. All subspecies of the Iberian species Iberolacerta cyreni and I. monticola were included in this study. In addition, samples of Apathya cappadocica and Darevskia rudis were analysed to elucidate the relationships of the European ‘Mountain lizards’ to their possible relatives in the Near East. Maximum parsimony and neighbour joining analyses lead to the following major conclusions: (i) the monophyly of the genus Iberolacerta is very well supported; (ii) Archaeolacerta bedriagae (the type species of the genus) is most basal with respect to the ingroup taxa. If we accept Iberolacerta as a genus, Archaeolacerta becomes paraphyletic. Therefore, we propose to restrict Archaeolacerta to the type species and to treat A. mosorenis and A. oxycephala provisionally as members of the collective genus Lacerta; (iii) within the genus Iberolacerta three groups were found: a Pyrenean group, an Iberian group and I. horvathi. The relationships among these groups remain unresolved; and (iv) the Peña de Francia lizards, described originally as a subspecies of I. cyreni, are in fact more closely related to I. monticola.

Key words: Lacertidae – Iberolacerta – Archaeolacerta – Darevskia – Apathya – Podarcis – Lacerta – phylogeny – mitochondrial rRNA sequences

Introduction

The systematics and taxonomy of small lacertids belonging to the so-called ‘Lacerta part II’ (sensu Arnold 1973) is far from being resolved. Some species groups previously assigned to Lacerta Linnaeus 1758 (s.l.) are now grouped in different genera or subgenera, depending on the authors’ points of view. A complete and more stable taxonomy of the whole group is still not accomplished. These taxonomic units are: Apathya Mehely 1907, Archaeolacerta Mertens 1921, Darevskia Arribas 1997, Iberolacerta Arribas 1997, Gallotia Boulenger 1916, Omannosaura Lutz, Bischoff and Mayer 1986, Parvilaocerta Harris, Arnold and Thomas 1998, Podarcis Wagler 1830, Teira Gray 1838, Timon Tschudi 1836 and Zootoca Wagler 1830. Apart from these more or less well defined groups, a heterogeneous assemblage of species still remains in the collective genus Lacerta.

Today the European Mountain lizards (‘Archaeolacertae’) are assigned to the genera Darevskia, Archaeolacerta and Iberolacerta (Arribas 1997a, 1999b). ‘Lacerta’ graeca, a saxicolous species with unresolved relationships but frequently ascribed to this group, remains without clear generic assignment. Archaeolacerta was originally defined by Mertens (1921) as a subgenus of Lacerta (type species: L. bedriagae) for Mehely’s (1907, 1909) ‘Archaeolacerta’, a plethora of small Palaeartic species in which the skull is depressed and only slightly ossified, with fenestrated supraocular osteoderms and frequently without pterygoid teeth. In the last decades the name was repeatedly used at the generic level for different groups of Mountain lizards (first used by Lanza et al. 1977). In the most recent reviews, Arribas (1997a, 1999b) restricted Archaeolacerta to the Tyrrenic species A. bedriagae, and tentatively to the balcanic A. mosorenis and A. oxycephala, but emphasized the necessity of more detailed studies in order to ascertain the degree of relationship between the Tyrrenic and the two Balcanic species.

The genus Iberolacerta (see Arribas 1997a, 1999b) includes the following taxa: Iberolacerta aranica (Arribas 1993 a,b), I. aurelioi (Arribas 1994), I. bonnali (Lantz 1927), I. monticola (Boulenger 1905) (ssp. monticola s.str. and cantabrica Mertens 1928) and I. cyreni (Müller and Hellmich 1937) (with ssp. cyreni s.str., castiliana Arribas 1996 and martinezzicai Arribas 1996), and additionally the east-Alpine and Dinaric species I. horvathi (Mehely 1904). This genus is a group of small sized and moderately saxicolous lizards. Their colourations and patterns are typical for the members of the Eurasian radiation (vertebral stripes, costal bands, etc.). Bellies are usually spotted in various degrees. Within the Eurasian radiation of lacertids (sensu Mayer and Beny 1994) a characteristic of Iberolacerta is the lack of microchromosomes (karyotype composed only of 36 macrochromosomes or less, as some species show Robertsonian fusions of acrocentric chromosomes resulting in biarmed ones). Also, in contrast to other small lacertid genera from the same area, females have greater snout–vent length than males. The number of vertebrae in males is mostly 26, and ranges from 27 to 29 in females (Arribas 1997a, 1998, 1999b).

The intragenic relationships of Iberolacerta have been studied from two different data sets: morphologic, osteologic and karyotypic (Arribas 1997a, 1999b) and allozyme studies (Mayer and Arribas 1996 as well as Almeida et al. 2002). In this paper we present partial DNA sequences of the mitochondrial genes coding for the 12S rRNA and 16S rRNA of European ‘Mountain lizards’ – with special consideration of the members of the genus Iberolacerta – to clarify the phylogeny of this group.

Materials and methods

Localities and abbreviations of the lizard samples investigated are listed in table 1. Podarcis muralis was used as outgroup.

DNA from tissue samples (liver, heart or tail tips) was extracted and purified using a phenol-chloroform standard protocol (Sambrook et al. 1989). For sequence comparisons we amplified sections of the mitochondrial genes coding for 12S rRNA and 16S rRNA which produce a 125 fragment of about 460 bp and a 16S fragment of about 900 bp length (primer see Table 2). Polymerase chain reaction (PCR)
Differences between MP and NJ are found with respect to the position of I. horvathi, the branching order of the Pyrenean group (I. aranica, I. aurelioi and I. bonnali) as well as within the A. cappadocica–A. oxycephala–L. graeca group.

The clade representing Iberolacerta as presently defined is well supported by both tree constructing methods (100% of the MP trees, bootstrap values of 97% in the NJ tree). The

Results

Maximum parsimony analysis resulted in 45 equivalent most parsimonious trees with a length of 639 steps, 279 sites are variable, the number of informative characters is 166, and the consistency index is 0.615. The NJ tree is given in Fig. 1, the MP 50% majority rule consensus tree in Fig. 2.

The main lineages of the trees (Figs 1 and 2) namely (1) Iberolacerta (perhaps including Archaeolacerta mosorenensis), (2) Darevskia (D. rudis), (3) a group with the three otherwise well differentiated taxa Archaeolacerta oxycephala, Apathya cappadocica and L. graeca, and (4) Archaeolacerta bedriagae, are deeply rooted.

---

Table 1. Investigated samples: taxa, localities and GenBank accession numbers

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>12s-rRNA</th>
<th>16s-rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iberolacerta monticola monticola</td>
<td>Estrela, Beira Alta, Portugal</td>
<td>AF440589</td>
<td>AF440604</td>
</tr>
<tr>
<td>I. m. cantabrica</td>
<td>Paderne, La Coruña, Spain</td>
<td>AF440591</td>
<td>AF440606</td>
</tr>
<tr>
<td>I. m. cantabrica</td>
<td>Puerto de Vegarada, León, Spain</td>
<td>AF440590</td>
<td>AF440605</td>
</tr>
<tr>
<td>I. cyreni cyreni</td>
<td>Giadarrama, Segovia-Madrid, Spain</td>
<td>AF440592</td>
<td>AF440607</td>
</tr>
<tr>
<td>I. c. castiliana</td>
<td>Gredos, Avila, Spain</td>
<td>AF440593</td>
<td>AF440608</td>
</tr>
<tr>
<td>I. c. martineciar</td>
<td>Peña de Francia, Salamanca, Spain</td>
<td>AF440594</td>
<td>AF440609</td>
</tr>
<tr>
<td>I. aurelioi</td>
<td>Ordino, Andorra</td>
<td>AF440595</td>
<td>AF440610</td>
</tr>
<tr>
<td>I. aranica</td>
<td>Armeros, Lleida, Spain</td>
<td>AF440596</td>
<td>AF440611</td>
</tr>
<tr>
<td>I. bonnali</td>
<td>Port de Orlà, Lleida, Spain</td>
<td>AF440597</td>
<td>AF440612</td>
</tr>
<tr>
<td>I. horvathi</td>
<td>Mont Perdido, Huesca, Spain</td>
<td>AF440598</td>
<td>AF440613</td>
</tr>
<tr>
<td>Archaeolacerta bedriagae</td>
<td>Col de Vergio, Corse</td>
<td>AF440599</td>
<td>AF440614</td>
</tr>
<tr>
<td>A. mosorenensis</td>
<td>unknown locality in former Yugoslavia</td>
<td>AF440600</td>
<td>AF440615</td>
</tr>
<tr>
<td>A. oxycephala</td>
<td>Hvar, Croatia</td>
<td>AF440601</td>
<td>AF440616</td>
</tr>
<tr>
<td>Archaeolacerta graeca</td>
<td>Lira, Lakonia, Greece</td>
<td>AF440602</td>
<td>AF440617</td>
</tr>
<tr>
<td>Apathya cappadocica</td>
<td>Al Barah, Syria</td>
<td>AF145444</td>
<td>AF149946</td>
</tr>
<tr>
<td>Darevskia rudis</td>
<td>Chernal valley, Adjaria, Georgia</td>
<td>AF238180</td>
<td>AF149938</td>
</tr>
<tr>
<td>Podarcis muralis</td>
<td>Baden, Austria</td>
<td>AF440603</td>
<td>AF440618</td>
</tr>
</tbody>
</table>

Table 2. Primers used for PCR and sequencing. (1) Knight and Mindell (1993); (2) Titus and Frost (1996); (3) Kocher et al. (1989), modified; (4) Reeder (1995), modified; (5) Knight and Mindell (1993), modified

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Used for</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-1091</td>
<td>5'-aaactggtgaattagcccaactact-3'</td>
<td>PCR</td>
<td>(1)</td>
</tr>
<tr>
<td>H-1298</td>
<td>5'-gtcatactgctatgaacacgt-3'</td>
<td>Sequencing</td>
<td>(2)</td>
</tr>
<tr>
<td>L-1318</td>
<td>5'-acgctagcgaagggtatgca-3'</td>
<td>Sequencing</td>
<td>Inverse H-1298</td>
</tr>
<tr>
<td>H-1478</td>
<td>5'-aggtgaaggcggactggt-3'</td>
<td>Sequencing</td>
<td>(3)</td>
</tr>
<tr>
<td>H-1557</td>
<td>5'-gacatactgctagcttgacct-3'</td>
<td>PCR</td>
<td>(1)</td>
</tr>
<tr>
<td>L-2190</td>
<td>5'-gtggctaaagaaagcaacacgagc-3'</td>
<td>PCR</td>
<td>(4)</td>
</tr>
<tr>
<td>L-2510</td>
<td>5'-cegtgttatcaacaaacacgtt-3'</td>
<td>Sequencing</td>
<td>(5)</td>
</tr>
<tr>
<td>H-3056</td>
<td>5'-ccggctgaacctactaagcaacg-3'</td>
<td>PCR</td>
<td>(4)</td>
</tr>
</tbody>
</table>

products separated on agarose gel were purified using QiAquick® spin columns (QIAGEN®, Qiagen Inc., Valencia, California, USA), and reamplified with the same primers. Sequencing of approx. 500 bp of the 16S gene and approx. 460 bp of the 12S gene was performed by the sequence service of MWG-Biotech (Ebersberg, Germany).

The alignment of the concatenated 12S and 16S sequences (ranging from 953 to 969 bp) was performed with CLUSTAL X (Thompson et al. 1997) and corrected by eye. A total of 15–20 bp of each sequence which could not be aligned unambiguously were excluded from the analysis resulting in a final alignment length of 958 positions (including gaps) (the alignment can be viewed under ‘Sequences’ at our website at http://www.nhm-wien.ac.at/NHM/1Zoo/first_zoological_department/web/chemsys/cuhp_25e.html). The neighbour joining tree (NJ, p-distances) was calculated by CLUSTAL X, the maximum parsimony (MP) dendrograms (Heuristic Search, gaps treated as fifth character state) and the MP majority rule consensus tree were produced using PAUP® version 4.0h3a (Swofford 1990) program package. The trees were rooted using PAUP® as an outgroup.

Fig. 1. Neighbour joining (NJ) tree, bootstrap values above 50% (2000 bootstrap resamplings) are given above branches.
relationships between I. horvathi and the Ibero-Pyrenean species are not clearly resolved. Whereas in the MP dendrogram I. horvathi appears as the sister group of all the remaining Iberolacerta (supported by 73% of the 45 most parsimonious trees), in the NJ tree it is the sister species of the Pyrenean group (bootstrap value 71%).

With respect to the Iberian species, we found nearly identical sequences in the three I. monticola samples studied, belonging to the type subspecies (Serra de Estrela) as well as to I. m. cantabrica from Puerto de Vegarada (Cantabrian Mts.) and La Coruña province (sea level populations). The sequences obtained from the samples of the two subspecies of I. cyreni, namely I. c. cyreni from Guadarrama and I. c. castiliana from Gredos, are very similar (0.6% sequence difference) and well differentiated from I. monticola (approx. 6.5%). But surprisingly, I. cyreni martinezricai does not group with I. cyreni but with I. monticola (100% in both trees).

Both samples of I. aranica (from Armeros and Port de Orlá) have shown identical sequences. The Pyrenean group is clearly monophyletic, but the relationships among the three species are not clear. Whereas I. aurelioi and I. bonnali group in the MP tree, I. aranica and I. aurelioi are sister species in the NJ dendrogram. The low support of the respective nodes in both trees implies an unresolved trichotomy of these three species.

All other relationships are not well resolved. Nevertheless, in both cladograms A. mosorensis appears as the taxon most closely related to the Iberolacerta clade, and Apapthya cappadocica, A. oxycephala and 'L.' graeca form a group, although these groupings are only weakly supported. Finally, Archaeolacerta bedriagae and Darevskia rudis belong to clearly different lineages.

Discussion

The position of I. horvathi is uncertain with respect to other Iberolacerta species. In the MP cladogram it appears as the sister species of the whole Ibero-Pyrenean species group, a relationship corroborated by allozyme analysis (Mayer and Arribas 1996; Almeida et al. 2002). In contrast, in the NJ tree I. horvathi is the sister species of the Pyrenean clade only. The results of Harris et al. (1998) and Harris (1999) based on shorter sequences gave equivalent topologies with the same tree constructing methods. In a study of a fair number of taxa from the Eurasian radiation of lacertids based on morphology, osteology and karyology, Arribas (1997a, 1999b) found I. horvathi as sister species of the Pyrenean group. However, if the analysis was restricted to Iberolacerta species only, I. horvathi changed position as sister species of either the Iberian group or of the Pyrenean group, thus giving rise to an unresolved trichotomy in the consensus tree. In any case, in the present mtDNA analyses I. horvathi was never closely related to the Iberian group (I. cyreni and I. monticola). Therefore the designation of a subgenus Pyrenesauro Arribas 1999 is not meaningful as it would leave Iberolacerta s.str. (as presently defined) as a paraphyletic group.

The DNA sequence similarities between I. m. monticola and I. m. cantabrica is in accordance with karyological (Odierna et al. 1996), allozymic (Mayer and Arribas 1996; Almeida et al. 2002) and morphological results (Arribas 1996). Except for differing frequencies of contact of some head scales, no other morphological differences between these taxa were found. The present range of I. monticola is more or less continuous along the Cantabrian Mountains and Galicia, with a southern exclave in the Serra da Estrela. These two groups of populations represent the two subspecies currently recognized (e.g. Salvador 1984; Barbadillo 1987; Perez-Mellado et al. 1993; Arribas 1996; Perez-Mellado 1997, 1998). The discovery of lowland populations from the Galician coast (Galán 1982, 1991, 1999; Elvira and Vigal 1982) raises new questions about the ecological needs and the biogeography of this species, so far considered as a mountain species.

Both lowland and highland populations originated likely in the course of a retreat towards more fresh and wetter conditions as a consequence of the progressive temperature rise during the Holocene. In the Estrela and Cantabrian mountain ranges the populations moved to the heights whereas in the Galician lowlands they retreated to wet and shapy gorges (covered by relic autochthonous deciduous forests which are considered to be an stable habitat from the Tertiary up to the present), as well as to coastal cliffs in areas with high precipitations (see for instance Arribas 1996). Populations from Cantabrian Mountains show a wide ecological valence. They may have retreated to the mountains before the postglacial temperature rise, but lowland populations still persist, especially in the west of Asturias (see Arribas 1996). Even some Cantabrian populations (as from Sierra de la Cabrera Baja and Sanabria area) are morphologically more similar to Portuguese specimens than to those from the main Cantabrian range (O. Arribas, unpublished data). Thus we assume that the present distribution was established postglacially and the subspecific distinction is not justified.

Iberolacerta cyreni is quite different from I. monticola. The Guadarrama and Gredos populations are presently allopatric and considered different subspecies of I. cyreni based on scalation characters (Arribas 1996). But the two populations seem to be poorly differentiated and may also be the result of a Holocene retreat to higher altitudes and the subsequent isolation of these populations. Both populations share the same karyotypic characteristics of I. cyreni (chromosome number and
morphology, Nuclear Organizer Region of Karotype (NOR) position [Odierna et al. 1996] and no differences in osteology were found (Arribas 1997a, 1998).

The Peña de Francia lizards, originally described as *I. c. martinezricai*, represent a very well differentiated taxon which is with certainty more closely related to *I. monticola* than to *I. cyreni*. The ancestors of *I. cyreni* and *I. monticola* possibly split off from Upper Miocene whereas *I. m. martinezricai* probably separated at the beginning of the Pleistocene Cooling and represents a remain of a formerly remote position itself. If *A. mosorensis* lacks microchromosomes (*Iberolacerta autapomorph*) is hitherto unknown.

**Taxonomic implications**

We propose the following taxonomic alterations: (1) the genus *Archaeolacerta* should be restricted to the type species *A. bedriagae*. (2) The species *mosorensis*, *oxycphala* and *graeca* should remain provisionally in the collective genus *Lacerta*. To express the fact that there is no closer relationship between them and the real members of the genus (the green lizards) we propose a spelling between quote marks (e.g., ‘Lacerta mosorensis’). (3) The Peña de Francia lizards, originally described as *I. cyreni martinezricai*, could be treated provisionally as a subspecies of *I. monticola*: *I. monticola martinezricai*. (4) *I. monticola cantabrica* is a synonym of *I. monticola monticola*. (5) *I. cyreni cyreni* and *I. cyreni castiliana* should not be given subspecific status and unified as *I. cyreni*.

**Acknowledgements**

We are grateful to P. Galan (La Coruña), V. Orlova (Moscow), W. Bischoff (Bonn), and P. Keymar (Wien) for some samples used in our study.

**Zusammenfassung**

Die phylogenetischen Beziehungen der europäischen Lacertiden der Gattung *Archaeolacerta* und *Iberolacerta* und ihre Verwandtschaft zu anderen „Archaeolacertae“ aus dem nahen Osten, basierend auf mitochondrialen DNA-Sequenzen


**References**


Concerning the remaining species included in the analysis, *Archaeolacerta*, as recently redefined (Arribas 1997a, 1999b), represents a polyphyletic assemblage with the Tyrrenhan paleoendemic type species *A. bedriagae* as the most basal taxon in our trees. Furthermore, both trees group ‘L.’ *graeca* and *A. oxycphala* with *A. cappadoeca*. Even though this clade is only weakly supported, our results tentatively imply closer relationships among these species. Further studies will be necessary to clarify if these three species form a monophyletic group to be classified as the genus *Apathya*, which so far includes only *A. cappadoeca*. Our results are in accordance with earlier, similar suggestions by Harris et al. (1998) and Harris (1999).

The relationships of *A. mosorensis* are particularly interesting. In our analyses it is the sister group to the *Iberolacerta* clade, a very interesting result although only weakly supported. Further research must clarify whether *A. mosorensis* in fact represents a sister group to *Iberolacerta* or holds a more remote position itself. If *A. mosorensis* lacks microchromosomes (*Iberolacerta autapomorph*) is hitherto unknown.


Arribas, O., 1998: Osteology of the Pyrenaean Mountain Lizards and comparison with other species of the collective genus Archaeolacerta Mertens, 1921 s.l. from Europe and Asia Minor (Squamata: Lacertidae). Herpetozoa, Wien, 11, 47–70.


Author’s addresses: W. Mayer, Naturhistorisches Museum Wien, Molecular Systematics, Burgring 7, A-1014 Wien, Austria. E-mail werner.mayer@nhm-wein.ac.at. Oscar Arribas (for correspondence), Avda. Fco. Cambó 23, E-08003 Barcelona, Spain. E-mail: oarribas@pie.xtec.es