Tracing the maternal origin of the common wall lizard (*Podarcis muralis*) on the northern range margin in Central Europe

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ABSTRACT

The maternal origin of isolated populations of the common wall lizard (*Podracis muralis*) in the Czech Republic, representing the north-eastern range border of the species, was addressed. We compared mitochondrial DNA sequences of the cytochrome *b* gene of samples from these populations with those from within the continuous range in Slovakia, the northern Balkan region, and those available from previous studies. We recorded five main haplogroups in the studied region, with all available Central European samples belonging to the same haplogroup. The star-like structure of this haplogroup suggests a scenario of relatively recent, post-glacial population expansion, which is further supported by a coalescent-based demographic analysis. The presence of unique haplotypes in two of the three isolated Czech populations together with close phylogenetic relationships to adjacent Slovak populations suggests either autochthonous origin or human-mediated introductions from geographically and genetically closest populations. We therefore support conservation programs for all three isolated Czech populations.

1. Introduction

Reptiles are ectothermic vertebrates and their spatial and temporal distribution is highly dependent on temperature (Markwick, 1998). In temperate zones of the western Palearctic region they are physiologically adapted to seasonal climate changes, where lower and higher temperature limits determine their distribution (Böhme, 2003; Sommer et al., 2009). Historically, climatic oscillations of glacial and interglacial cycles during the Quaternary caused significant range shifts in the European biota (Davies and Shaw, 2001; Sommer and Zachos, 2009). During the glacials, distributions of many species were restricted to refugia, typically at lower latitudes that later served as sources for colonization of higher latitudes as the climate warmed (Hewitt, 2000; Bennett and Provan, 2008). Given the current global warming trends causing growing temperatures in areas home to many temperate ectotherms, reptiles are interesting group to study distribution dynamics and historical origins of their populations, particularly at the higher latitude boundaries of the present-day ranges. Populations from these areas are good candidates for further expansions northward in the future (Parmesan, 2006).

The region of Central Europe was historically crossed by many expanding vertebrate taxa (Hewitt, 2004). In most cases it is thermophilic ectothermic vertebrates that find their climatic optima in this region since at least the end of Miocene (Böhme, 2003). Among the well-documented examples of vertebrates with wider ranges during the Holocene climatic optimum are e. g. turtle *Emys orbicularis* (Sommer et al., 2009), lizard *Lacerta viridis* (Böhme et al., 2007), and snake *Zamenis longissimus* (Musilová et al., 2010). Numerous records of subfossil specimens showed that these species have become extinct from much of their northern Holocene distribution, which spread as far north as Southern Scandinavia (Sommer et al., 2007; Musilová et al., 2010). Within the recent European ranges, the Central-European populations

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Table 1
Tissue samples and sequences of the Central Balkan clade of *Podarcis muralis* used in this study.

Code	Country	Locality	Haplogroup	Coordinates		Source	GenBank accession number
				N	E		
Pbh07_BA	Bosnia and Hercegovina	Kozara Mts.	I	45.04	16.97	This study	MG851979
P7_L1_BG, P8_L2_BG, P9_L3_BG	Bulgaria	Zgorigrad	V	43.19	23.53	This study	MG851950 MG851951 MG851952
P29_BG	Bulgaria	Vraca	V	43.20	23.53	This study	MG851931
P41_BG	Bulgaria	Bjala	V	42.88	27.88	This study	MG851942
P42_BG	Bulgaria	Ruse	V	43.83	25.97	This study	MG851943
478_BG	Bulgaria	Kralovo	V	42.65	24.81	This study	MG851961
594_BG	Bulgaria	Shumen	V	43.26	26.89	This study	MG851962
mur5	Croatia	Nova Gradiška, Strmac	I	45.25	17.38	Podnar et al. (2007)	DQ001025
P1_CZ, P2_CZ, P3_CZ, P4_L14_CZ, P5_L15_CZ, P6_CZ, P11_CZ, P12_CZ, P13_CZ, P14_CZ, P28_CZ	Czech Republic	Štramberk	I	49.59	18.12	This study	MG851924 MG851932 MG851940 MG851947 MG851948 MG851949 MG851915 MG851916 MG851917 MG851918 MG851930
3547_CZ, 3548_CZ	Czech Republic	Krčmaň	I	49.52	17.33	This study	MG851974 MG851975
3549_CZ	Czech Republic	Brno	I	49.22	16.67	This study	MG851976
HAN1	Germany	Halle an der Saale	I	51.74	11.97	Schulte et al. (2012a)	HQ652958
UU89, 90	Germany	Altenhain	I	51.29	12.70	Schulte et al. (2008)	HQ652886 HQ652887
UU34	Germany	Ammelshain	I	51.29	12.63	Schulte et al. (2008)	HQ652885
P33_HU, P34_HU, P35_HU, P45_HU	Hungary	Esztergom	I	47.80	18.75	This study	MG851935 MG851936 MG851937 MG851944
1961_RO	Romania	Sf. Elena	II	44.67	21.71	This study	MG851969
2415_RO	Romania	Soimos	V	46.10	21.72	This study	MG851970
3420_RO	Romania	Svinita	III	44.50	22.08	This study	MG851973
P30_RS	Serbia	Podujevace	IV	42.98	21.39	This study	MG851933
P32_RS	Serbia	Sokolac	II	44.26	19.43	This study	MG851934
1736_RS	Serbia	Sopotnica	V	43.15	22.13	This study	MG851968
2655_RS	Serbia	Maglič	IV	43.61	20.54	This study	MG851971
3579_RS	Serbia	Bukovi	II	44.15	19.91	This study	MG851977
djz_655_SK, djz_656_SK, djz_657_SK, djz_658_SK	Slovakia	Vlachy	I	49.10	19.48	This study	MG851953 MG851954 MG851955 MG851956
djz_1728_SK, djz_1729_SK, djz_1730_SK, djz_1731_SK	Slovakia	Ihráč	I	48.63	18.95	This study	MG851957 MG851958 MG851959 MG851960
P15_L4_SK	Slovakia	Temešská skala	I	48.87	18.37	This study	MG851919
P16_L5_SK	Slovakia	Bojnice	I	48.78	18.58	This study	MG851920
P17_L6_SK, P18_L7_SK, P19_SK	Slovakia	Bratislava	I	48.15	17.07	This study	MG851921 MG851922 MG851923
P22_L8_SK, P23_L9_SK, P24_SK	Slovakia	Bratislava II	I	48.14	17.07	This study	MG851925 MG851926 MG851927
P26_L10_SK, P27_L11_SK	Slovakia	Trubárka	I	48.88	18.15	This study	MG851928 MG851929
P38_SK	Slovakia	Zádiel	I	48.62	20.83	This study	MG851938
P39_SK	Slovakia	Plešivec	I	48.55	20.47	This study	MG851939
P40_SK	Slovakia	Kečovo	I	48.48	20.50	This study	MG851941
P46_SK	Slovakia	Višňové	I	49.17	18.78	This study	MG851945
P48_SK	Slovakia	Nižná Kamenica	I	48.77	21.53	This study	MG851946
1460_SK, 1462_SK	Slovakia	Šášovské Podhradie	I	48.57	18.90	This study	MG851965 MG851966
1726_SK	Slovakia	Plavecké Podhradie	I	48.49	17.26	This study	MG851967
3052_SK	Slovakia	Kováčov	I	47.82	18.77	This study	MG851972

A

CYTOCHROME *B*
1143 bp
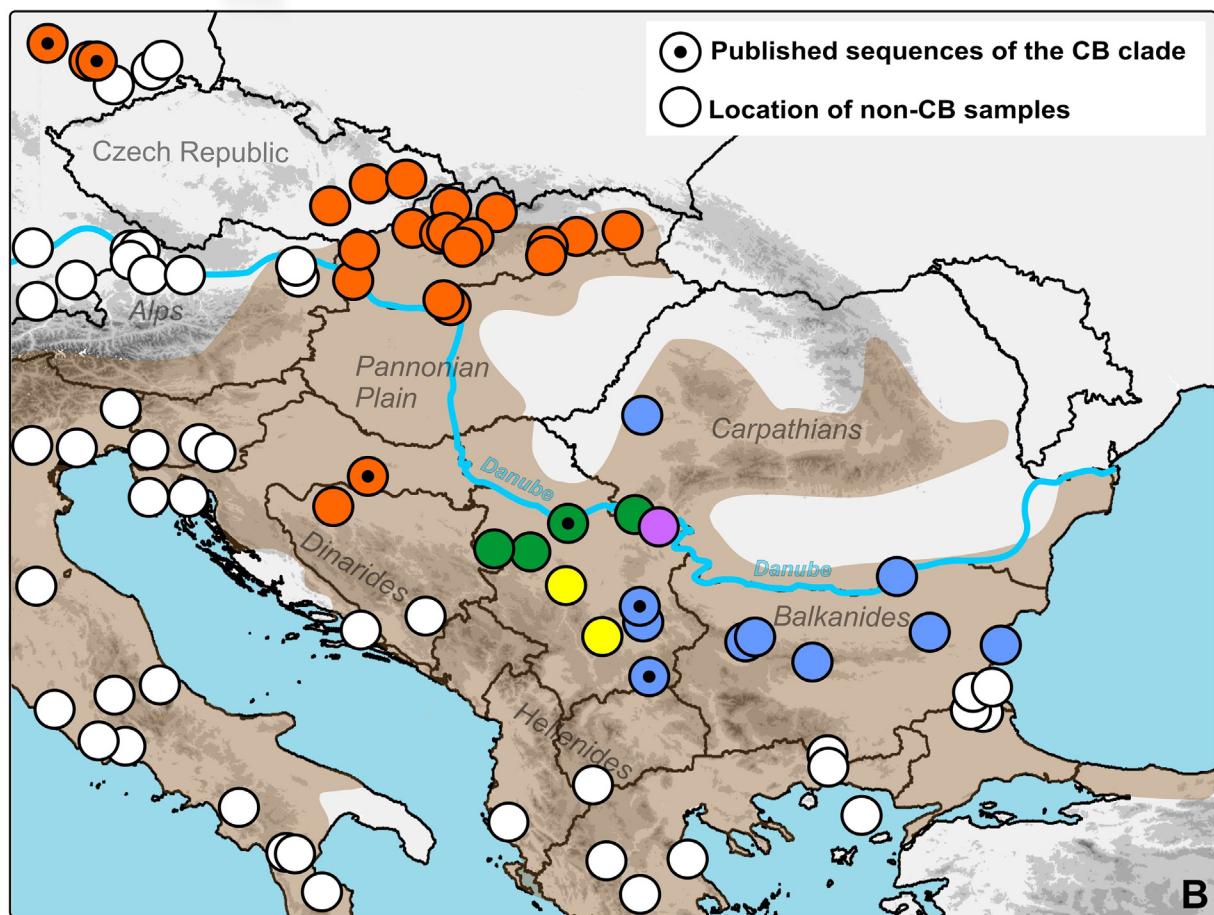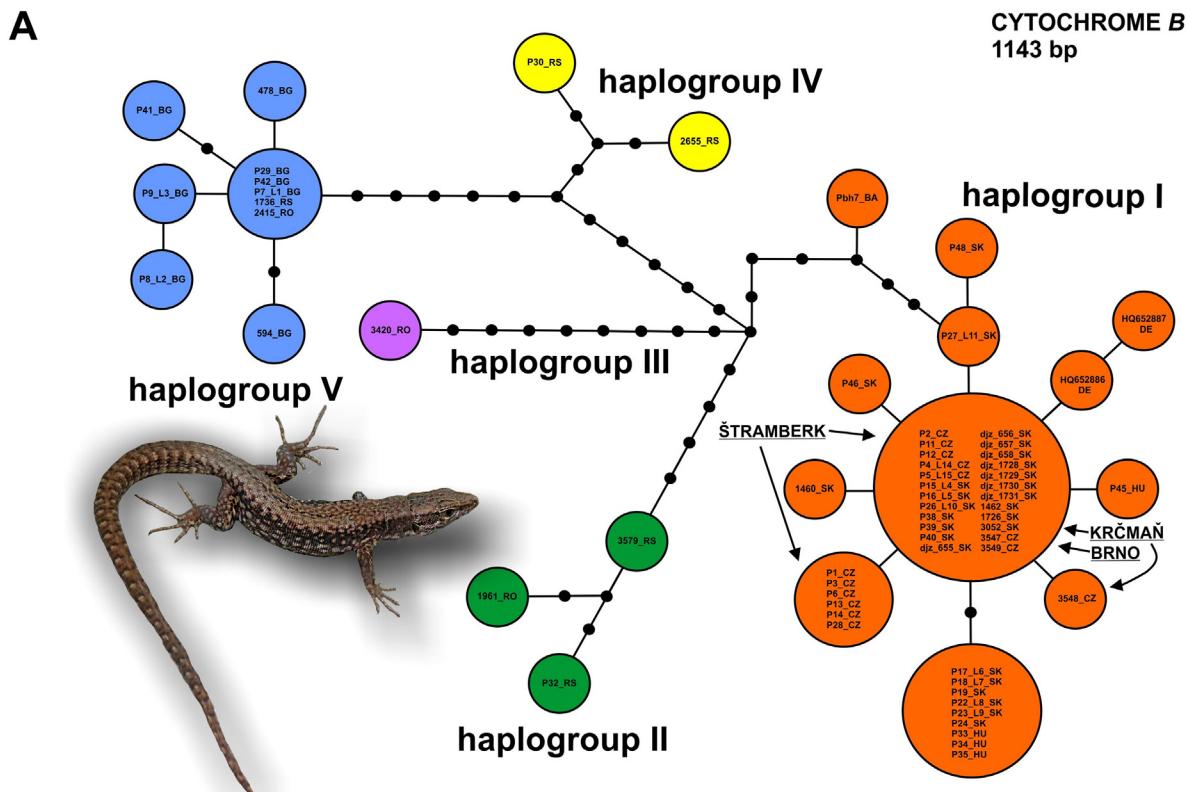
(caption on next page)

Fig. 1. (A) Haplotype network of the Central Balkan (CB) clade of *Podarcis muralis* based on complete cytochrome *b* sequences, and (B) geographical distributions of the main haplogroups of the CB clade in the studied area according to results of the network- and tree-based approaches. Approximate species distribution is given in brown shading (Sillero et al., 2014). The three isolated populations from the Czech Republic are underlined, and positions of their haplotypes are indicated by arrows. Standard country codes are used in the sample coding. Inset: an adult male from Štramberk, Czech Republic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
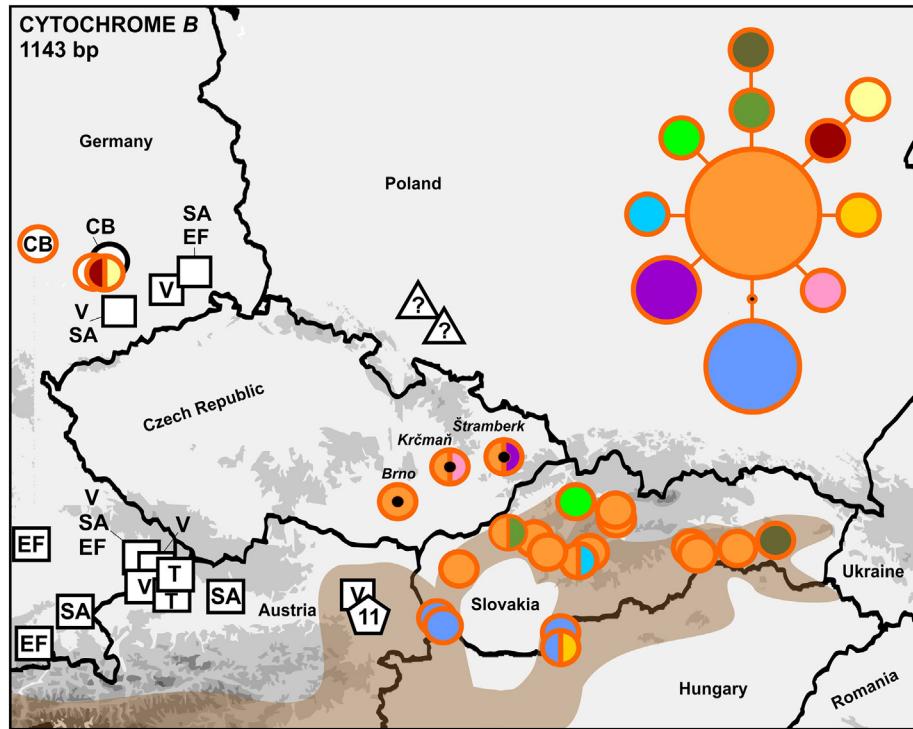

Fig. 2. Haplotype network and distribution of individual haplotypes of the haplogroup I of the Central Balkan clade (CB) in Central Europe (in circles). Approximate species distribution is given in brown shading. Populations of the Central Balkan clade in Germany: circle with orange border and colour haplotype affiliations is locality Altenhain (Saxony, HQ652886-7; Schulte et al., 2008); white circles with orange borders are localities Halle an der Saale (Saxony-Anhalt, HQ652958) and Ammelshain (Saxony, HQ652885) not included in the network analysis due to short sequence lengths; white circle with black border is locality Böhlitz (Saxony; Schulte et al., 2011) without available genetic data. Genetically investigated exotic introduced populations from Germany and Austria (Schulte et al., 2008; Schulte et al., 2011; Schulte et al., 2012a) are given in squares; abbreviations for their phylogenetic origin (*sensu* Schulte et al., 2011; Salvi et al., 2013): EF – East France, SA – South Alps, T – Tuscany, V – Venetian. The presumably autochthonous population belonging to the "clade 11" (*sensu* Salvi et al., 2013; AY185096, DQ001020) in Austria is in pentagon. Recently discovered isolated populations in Poland are in triangles with question marks (genetic data are not yet available; Wirga and Majtyka, 2013). The Czech isolated populations are marked by black dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

show low haplotype diversity of mitochondrial DNA (mtDNA). Furthermore, in each of the mentioned species the post-glacial colonization of Central Europe was derived from two lineages originating from the Balkan refugia (Fritz et al., 2007; Sommer et al., 2009; Musilová et al., 2010).

In the present study, we analyzed the mtDNA phylogeography of another xerothermic reptile species, the Common Wall Lizard, *Podarcis muralis* (Laurenti, 1768), whose actual distribution in Central Europe resembles that of another xerothermic lizard *Lacerta viridis*, or snakes *Natrix tessellata* and *Z. longissimus* (Sillero et al., 2014). The species inhabits almost the entire southern part of the European landmass as it has successfully colonized regions in North-Western and Central Europe far outside its core range in the Mediterranean region (Gassert et al., 2013; Salvi et al., 2013; Michaelides et al., 2015a). Currently, more than twenty geographically restricted mtDNA evolutionary lineages of *P. muralis* are known (Schulte et al., 2012a, 2012b; Gassert et al., 2013; Salvi et al., 2013). More or less isolated populations from the northern range margins raise questions whether they are native and represent autochthonous populations or if they were introduced by humans (Schulte et al., 2008; Schulte et al., 2012a, 2012b). While the status of most populations from the north-western regions has been evaluated recently using a comparative phylogeographic approach (Schulte et al., 2012a, 2012b; Gassert et al., 2013; Michaelides et al., 2015a, 2015b), the relationships and origin of populations at the northern range margin in Central and Eastern Europe remain unknown. Understanding the history of populations in this region becomes particularly important given the fact that the common wall lizard is prone to establishing thriving and self-sustaining populations following human-mediated introductions. To date, > 150 cases of such introductions have been reported, and were the result of either deliberate releases of captive animals, arrival *via* timber trade or rail freight transport (Schulte et al.,

2012c; Michaelides et al., 2015b). High phenotypic variability of *P. muralis* makes it merely impossible to distinguish between native and introduced populations based solely on morphological characters (Bellati et al., 2011; Schulte et al., 2012c).

The aim of this study is to ascertain the origin and population structure of *P. muralis* in the north-eastern range margin of Central Europe, where the common wall lizard as a xerothermic species is considered a reptile rarity. This could be illustrated by an anecdote of its first discovery in a close proximity of the north-western Carpathians in limestone quarries in Štramberk, Czech Republic. The species was first misidentified as the viviparous lizard (*Zootoca vivipara*) by Štěpánek (1955), and correctly re-identified as *P. muralis* not earlier than almost a half century later (Zavadil, 1999; Moravec and Beneš, 2000; Pavlík and Šuhaj, 2000). Currently, three isolated populations of *P. muralis* are known from the Czech Republic. Beside the one from Štramberk, two more were discovered in Brno and Krčmaň in the last decade. However, there are still doubts whether the species is native or was introduced in the Czech Republic. The reasoning behind the latter possibility is that keeping reptiles as pets, including wall lizards, was a popular hobby in the area during the 20th century. Many reptile species were known to be imported to captivity by Central Europeans travelling to the Balkans for holidays or business (see Steinicke, 2000; Schulte et al., 2008). We therefore compared mtDNA sequences of the cytochrome *b* gene (*cytb*) of the three isolated populations from the Czech Republic with those from the continuous range in Slovakia, the northern Balkans (Central Balkan clade *sensu* Schulte et al., 2011, 2012a), and ones available from previous studies to test, whether the Czech populations are (i) native populations at the north-eastern range margin, (ii) introduced populations stemming from one or more source regions, or (iii) mixed populations between native and introduced *P. muralis*. In addition, we present details of the genetic structure of the

Central Balkan clade, and briefly discuss potential conservation measures for the studied populations.

2. Materials and methods

We investigated the three known isolated northernmost populations of *P. muralis* from the Czech Republic (three abandoned quarries with xerothermic vegetation and areas ranging from 0.8 to 15 ha) together with samples from Slovakia, southeastern Europe, and the Balkans (Table 1, Figs. 1, 2). Blood samples or small tail tips were collected from living specimens, muscle tissues from dead individuals (road-kills). Tissue samples were preserved in 96% ethanol or frozen and stored at −25 to −80 °C. No lizards were killed for this study and no voucher specimens were deposited in museum collections.

We used standard phenol-chloroform extraction methods or various DNA extraction kits for DNA isolation. The complete cytochrome *b* gene (*cytb*) was amplified with primers L14910 and H16064 (Burbrink et al., 2000) and the same primers were used for sequencing. The sequencing was performed by Macrogen Inc. (Seoul, South Korea or Amsterdam, Netherlands; http://www.macrogen.com) and new sequences have been deposited in GenBank under the accession numbers MG851915–MG851983 (Table 1 and Additional file 1: Table S1). The final alignment was based on nucleotide sequences of complete *cytb* (1143 bp), which served for the construction of haplotype networks. For phylogenetic analyses we used trimmed sequences of *cytb* (411 bp-long fragment) to facilitate utilization of sequences available in GenBank (see Additional files 1,2: Table S1, Fig. S1).

DNA sequences were checked for their quality and mitochondrial origin by translating them into amino acids. No stop codons were detected when the sequences were translated using the vertebrate mitochondrial genetic code in the program DnaSP 5.10 (Librado and Rozas, 2009). The same program was used to calculate uncorrected *p*-distances among the main haplogroups within the lineage containing the Czech and Slovak populations, and to estimate the haplotype diversity (*Hd*), number of segregating sites (*S*) and nucleotide diversity (π) for selected haplogroups/populations. In order to reconstruct phylogenetic relationships of individuals from the Czech Republic and Slovakia, we applied phylogenetic analyses using the all-individuals dataset supplemented by distinct published haplotypes from the species range. Phylogenetic trees were inferred using the Bayesian approach (BA) and maximum likelihood (ML) by MrBayes 3.2 (Ronquist et al., 2012) and RAxML 8.0. (Stamatakis, 2014), respectively. The best-fit codon-partitioning schemes and the best-fit substitution models were selected for BA and ML analysis separately using PartitionFinder v1.1.1 (Lanfear et al., 2012), and according to the Bayesian information criterion (BIC): K80 + I + G (1st and 2nd codon position), GTR + G (3rd codon position) for BA; and GTR + G in each codon position in ML. The ML clade support was assessed by 1000 bootstrap pseudoreplicates. The MrBayes analysis was set as follows: two separate runs with four chains for each run, 10 million generations with samples saved every 100th generation. The convergence of the two runs was confirmed by the convergence diagnostics (average standard deviation of split frequencies, potential scale reduction factor). First 20% of trees were discarded as the burn-in after inspection for stationarity of log-likelihood scores of sampled trees in Tracer 1.6 (Rambaut et al., 2013) (all parameters had effective sample size > 200). A majority-rule consensus tree was drawn from the post-burn-in samples and posterior probabilities were calculated as the frequency of samples recovering any particular clade.

A haplotype-network approach can be more effective for presentation of intraspecific evolution than the tree-based phylogenetic approaches (Posada and Crandall, 2001). Therefore, we constructed a haplotype network using the 95% limit of parsimony as implemented in TCS 1.21 (Clement et al., 2000) for the Central Balkan clade, in which the populations from Central Europe clustered together (including two GenBank individuals from German Saxony: HQ652886-7; Schulte et al., 2008).

The past population dynamics was inferred using the Bayesian coalescent-based approach of the Bayeasian skyline plot (BSP; Drummond et al., 2005) as implemented in BEAST 2.1 (Bouckaert et al., 2014). This method computes the effective population size through the time directly from sampled sequences and does not require a specific *a priori* assumed demographic model. This method was applied for the haplogroup I (without geographically isolated samples from Bosnia and Hercegovina, and Croatia) of the Central Balkan clade. A uniform prior for the average substitution rate with the initial value 0.013 mutations/site/million years was applied according to Podnar et al. (2014). Preliminary analyses were run using both strict molecular clock and uncorrelated lognormal relaxed molecular clock. Since the parameter of the standard deviation of the uncorrelated lognormal relaxed clock was close to zero, the final analyses were run enforcing the strict molecular clock model. Using PartitionFinder v1.1.1. (Lanfear et al., 2012), all codon positions treated together as one partition and the HKY substitution model were selected as the best-fit partitioning scheme and the best-fit model, respectively. The final BSP analysis was run in duplicates to check for consistency between runs, each run for 10 million generations and sampled every 1000 generations. Convergence, effective sample size (ESS > 200), stationarity, and the appropriate number of generations to be discarded as burn-in (10%) were assessed using Tracer 1.6 (Rambaut et al., 2013). The resulting BSP was also summarized in Tracer 1.6 with the maximum time as the median of the root height parameter. In addition to the BSP, a signature of a possible expansion was assessed also by the neutrality-test statistics of the Fu's F_S (Fu, 1997) and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas, 2002) calculated in DnaSP 5.10 with the estimation of the statistical significance using 10,000 coalescent simulations.

3. Results

We obtained 69 complete *cytb* sequences with no signal of contamination or sequences of nuclear genomic origin. The BA/ML analyses resulted in phylogenetic trees with many distinct lineages but unresolved topology (Fig. S1), which is in a general concordance with previous studies (*e.g.* Salvi et al., 2013). Populations from the Czech Republic and Slovakia clustered with the Central Balkan clade (*sensu* Schulte et al., 2011, 2012a; or "clade 4" *sensu* Salvi et al., 2013), which was divided into four lineages with unresolved mutual relationships (Fig. S1). All samples from the Czech Republic and Slovakia form one lineage together with individuals from northern Bosnia and Herzegovina, north-eastern Croatia (DQ001025), Hungary, and eastern Germany (haplogroup I).

According to the haplotype-network analysis of 1143 bp-long sequence alignment (Fig. 1), 24 haplotypes were detected within the Central Balkan clade structured into five main haplogroups: haplogroup I found in the area from eastern Germany, Czech Republic, Slovakia, Hungary and northern Bosnia and Herzegovina; and four Balkan haplogroups (haplogroups II, III, IV, V) from western Romania, Serbia, and northern Bulgaria. These haplogroups are separated from each other by 0.2–1.2% of uncorrected *p*-distance in their *cytb* sequences. Twelve haplotypes were detected in the haplogroup I, including one slightly distant haplotype from northern Bosnia and Herzegovina (47 sequenced individuals from 21 localities and two GenBank sequences from one German locality). Overall, the Balkan haplogroups (II-V) included 12 haplotypes in 16 sequenced individuals, and the highest haplotype diversity was found in the Central Balkan mountains and neighboring areas (southern Carpathians, eastern Dinarides, Stara Planina Mts.). Three haplotypes were detected in haplogroup II (Serbia and western Romania), one haplotype in haplogroup III (near the Danube River in Romania), two haplotypes in haplogroup IV (two Serbian samples), and six haplotypes in haplogroup V (western Romania, eastern Serbia, and northern Bulgaria). All remaining sequenced individuals analyzed in this study from Albania, southern Bulgaria, southern Bosnia and

Herzegovina, and Italy corresponded to other phylogenetic clades of *P. muralis* (see Additional files 1,2: Table S1, Fig. S1). These clades are out of the focus of this study, therefore their structure and distribution are not discussed (see *e.g.* Salvi et al., 2013).

Focusing on populations from Central Europe, eleven different haplotypes were detected in the area, all within the haplogroup I ($S = 15$; $Hd = 0.71$; $\pi = 0.128$; Fig. 2). The most common haplotype of the haplogroup I is widely distributed across the Czech Republic and western, central, and eastern Slovakia (13 localities, 24 individuals). The second most common haplotype separated by two mutation steps from the most common haplotype was found along the Danube River (4 localities, 9 individuals). Other haplotypes are geographically restricted and some of them supposedly linked to discrete localities: Esztergom (Hungary), Krčmaň, Štramberk (Czech Republic), Nižná Kamenica, Šášovské Podhradie, Trubárka, and Višňové (Slovakia). All three isolated Czech populations share the most common haplotype, with Štramberk ($S = 1$; $Hd = 0.55$; $\pi = 0.048$) and Krčmaň ($S = 1$; $Hd = 1.00$, $\pi = 0.087$) also possessing haplotypes unique to each locality and separated from the most common haplotype by a single mutation step. Two unique haplotypes (one and two mutation steps distinct from the most common haplotype) were also detected in German locality Altenhain (UU89, UU90, HQ652886–7; Figs. 1 and 2). The haplogroup I is also present in other German localities from Saxony and Saxony-Anhalt (Ammelshain, UU34, HQ652885; Halle an der Saale, HAN1, HQ652958), but the sequences were not included in the haplotype networks due to their short lengths. The demographic analysis using the Bayesian skyline plot method (Fig. 3) gave evidence of population growth of the haplogroup I, which commenced at approximately 20 Kya. The neutrality tests significantly rejected the null hypothesis of selective neutrality and constant population size ($R_2 = 0.0557$, $P = 0.037$; $F_S = -5.3962$, $P = 0.003$).

4. Discussion

4.1. The Central Balkan clade: phylogeography and colonization of the northern range margin

Our results confirm previous reports on complex phylogeographic structure among *P. muralis* populations inside the detected mtDNA clades (*e.g.* Salvi et al., 2013). This is also the case of the Central Balkan clade (CB), which was sampled less thoroughly in previous studies (Schulte et al., 2008; Schulte et al., 2012a; Salvi et al., 2013). We uncovered five distinct haplogroups occurring throughout the northern Balkans, southern and northern Carpathians, and a part of the Pannonian Plain. The haplotype diversity markedly increases south of the


Fig. 3. Demographic history (effective population size through the time) of the Central European population of the haplogroup I of the Central Balkan clade of *Podarcis muralis* as estimated by the Bayesian skyline plot.

Danube River, while the populations on the north-western edge of the CB distributional range are relatively uniform. Similar patterns of high haplotype diversity in the south have been found in several other Balkan-Carpathian reptile species that colonized northern areas of Europe and are in concordance with the refugia-within-refugia model (Gómez and Lunt, 2007). Based on the observed pattern, we predict existence of several smaller and isolated refugia in southern Europe. These refugia, which harbored populations of the Central Balkan clade during the Pleistocene climatic oscillations, could be located in the Dinarides, Carpathians, and Balkanides (Stara-Planina Mts.; see distributions of haplogroups in Fig. 1). Populations of *P. muralis* presumably colonized northern parts of Europe by expansion of the haplogroup I, which is supported by a typical star-like structure (Figs. 1, 2) of the haplotype network. Other haplogroups seem to be restricted to the southern Carpathians and eastern Dinarides, regions known as refugia for its specific topography (Jablonski et al., 2016). Areas between main topographic units of the Balkans (Dinarides/Hellenides and Balkanides/Macedonian-Thracian Massif) probably form borders between the Central Balkan clade and other endemic clades found in the southern parts of the Balkans (see Salvi et al., 2013). Similar pattern has also been observed in other reptiles of the peninsula (Jablonski et al., 2016; Marzahn et al., 2016).

Our data provide evidence that common wall lizard populations in the Czech Republic and Slovakia belong to the same *cytb* haplogroup (Figs. 1 and 2) and they are closely related to populations from eastern Germany (Saxony and Saxony-Anhalt) and northern Hungary, and a bit more distantly to populations from northern Bosnia and Herzegovina and north-eastern Croatia (Fig. S1). As our sampling detected the genetically nearest population to Central Europe in the north-eastern Dinarides, we can expect that the population now distributed in Central Europe originated from this area and not from the southern Carpathian refugia, as would be more likely given the phylogeographic patterns of several other reptile species (*e.g.* Ursenbacher et al., 2006; Jablonski et al., 2016). However, due to a sampling gap in the Romanian Carpathians and most of Hungary, these conclusions can only be considered preliminary.

Looking at genetic structure of the haplogroup I, there is usually separation by one mutation step from the most common haplotype, except of some southern Slovak populations (from near the Danube River) possessing a haplotype separated by two steps (Fig. 2). This could suggest historical separation of the Danubian and Carpathian populations. The star-like pattern of the haplotype network of the haplogroup I supports a hypothesis of population expansion, likely postglacial, which is further supported by our demographic analysis (Fig. 3) and neutrality tests. One possible explanation of the phylogeographic pattern in Central Europe is that dispersal in this region occurred primarily along the mountain chain of the northern Carpathians. As all mutations forming the star-like pattern of the haplotype network are synonymous, we do not expect that positive selection played a role in forming this pattern. Furthermore, since the studied populations are located on the range margin, we do not suspect the selective sweep was responsible for the observed genetic structure. However, to understand the colonization history of Central Europe by *P. muralis*, we need a better sampling from the western part of the Pannonian Plain and from the eastern and southern Carpathians.

4.2. Populations in the Czech Republic: origin and conservation implications

Due to the special position of the low number (three) mutually separated *P. muralis* populations within the herpetofauna of the Czech Republic (classified there among the critically endangered species), the question of their origin has been in the conservationist spotlight for a long time. Currently there are three isolated populations, all associated with old limestone quarries: two in northern/central Moravia (Štramberk, Krčmaň) and one in southern Moravia (Brno; all in the eastern part of the Czech Republic). During the early decades of the
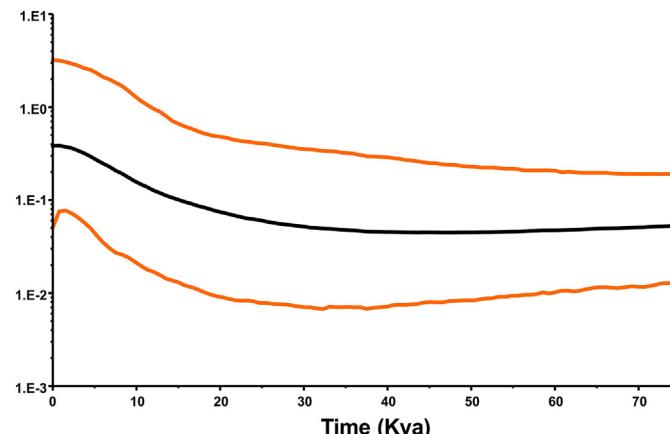
20th century, several anecdotal reports were made on the occurrence of Mediterranean reptiles (*Hemidactylus turcicus*, *Dolichophis caspius*, *Vipera ammodytes*, *V. aspis*) in the area of the present-day north-eastern Czech Republic. These reports were probably results of the activities of enthusiastic herpetofauna fans attempting to establish populations of non-native species by introduction (Adolph, 1922; Pax, 1925; Krátký and Wenig, 1930; Moravec, 2015). Although we do not have clear evidence that *P. muralis* was included in such activities, it is easy to imagine that the species could be a target of similar attempts, especially when a similar problem was recorded in eastern Germany. As presented by Grosse et al. (2015), the first record of *P. muralis* in eastern Germany comes from roughly the same time of the early decades of the 20th century, when Mertens (1917), found a specimen of presumably Italian origin in the city park of Leipzig. Schulte et al. (2008) and Grosse et al. (2015) reported that the populations from Saxony and Saxony-Anhalt carry haplotypes of the Central Balkan clade, and these populations are considered introduced (Steinicke, 2000; Schulte et al., 2008, 2011).

Combining with our sampling of the Central Balkan clade we found out that three populations from eastern Germany belong to the haplogroup I, with two haplotypes found within the two individuals sampled. Both these haplotypes were unique and found in the population from Altenhain (which is probably a population derived from the neighboring Ammelshain population; Schulte et al., 2011). Similarly, the presence of two unique haplotypes has been detected in two Czech populations (Krčmaň, Štramberk; Fig. 2). This rather unexpected finding suggests two possible scenarios: i) the Czech populations (or at least the oldest known from Štramberk) are autochthonous remnants from the past Holocene climatic optimum, which would be similar to the situation found in other reptile species such as *Emys orbicularis*, *Lacerta viridis*, *Natrix tessellata*, or *Zamenis longissimus*; or ii) these populations are allochthonous, and were introduced either from Slovakia or from another yet uncovered geographically nearby region. The latter hypothesis of the allochthonous origin of the Czech population in Štramberk is supported by a high frequency of scale anomalies found in individuals of this population (Veselý et al., 2007; Moravec and Veselý, 2015). These anomalies may be a result of demographic bottlenecks (*i.e.* reduced levels of allelic diversity) resulting from a small founder population, frequently observed in introduced populations of reptiles (Gautschi et al., 2002). These authors demonstrated a significantly bigger loss of allelic diversity in a serially bottlenecked population than in a population that was bottlenecked just once.

On the other hand, our sampling throughout the nearest populations of *P. muralis* in Slovakia revealed close phylogenetic relationships to the Czech populations, which supports the autochthonous hypothesis. In this case, small haplotype differences may suggest long-term isolation of the Czech populations with an occurrence of historical bottlenecks. This hypothesis is further supported by relatively short geographical distance (about 60 km) between Štramberk and the nearest Slovak populations (Trubárka, Višňové; Table 1, Fig. 2). However, these localities are recently separated by a densely forested mountain chain with rather unfavorable habitat and microclimatic conditions. Therefore, a possible explanation is that *P. muralis* populations expanded and colonized suitable localities in the territory of the present-day Czech Republic during the Holocene climatic optimum (*i.e.* around 9000–5000 years ago) as is known for populations of two other ecologically similar reptile species, *Lacerta viridis* (Böhme et al., 2006) and *Zamenis longissimus* (Musilová et al., 2010). However, it is also possible that these "insular" populations might have persisted under more favorable microclimatic conditions during the glacials, and therefore may be older than populations established by later (re)colonization events. All Czech localities are situated in limestone areas and are characterized by Mediterranean-like climate conditions unlike other parts of the Czech Republic, and the regions might have acted as ideal spots for retraction during moderately cold periods, as is also supported by the distribution model based on paleoclimatic data presented by Gassert et al. (2013). To investigate this scenario in detail, employment of additional genetic

markers and dense sampling in the Pannonian-Carpathian region will be necessary.

The localities in the Czech Republic represent isolated populations at the north-eastern range margin of the species distribution adjacent to the presently known indigenous range. Due to special habitat preferences, the species almost exclusively inhabits abandoned limestone quarries and their surroundings as such sites best fit Mediterranean conditions in microclimate, vertical orography, rocky substrate, and xerothermic vegetation. Despite its known genetic nature, we find it difficult to distinguish between alternative hypotheses when tracing mitochondrial origin in these cases. In the light of genetic evidence, we suggest the following scenario: (1) the populations from Štramberk and Krčmaň may represent autochthonous relict populations due to possession of unique haplotypes found nowhere else throughout the sampled range. These findings remain surprising due to the fact that despite Krčmaň has often been visited by zoologists from the nearby Palacký University in Olomouc in the last four decades, *P. muralis* records have appeared much later (Mačát and Veselý, 2009; Zavadil et al., 2015). Even if recent introduction would be the case, maternal lineages still come from the geographically and genetically closest populations; (2) the population from Brno is most likely the result of recent introduction from neighboring Slovak populations or even from Štramberk or Krčmaň.

Even if allochthonous transfers in the near past are considered as the plausible explanation, Czech populations do not represent a serious alien threat to genetic integrity of the geographically closest native populations in Slovakia. This contrasts to the situation well known from the north-western range margin of the species distribution, where often substantively genetically distant forms were introduced (Schulte et al., 2012a, 2012c; Michaelides et al., 2015b). The Czech populations would represent either a new category or a kind of assisted colonization within conservation translocations *sensu* Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC, 2013). Although none of the three localities is seriously threatened by habitat destruction, they all are sensitive to vegetation overgrowth. All three localities are partially protected but only one population (Štramberk) is being regularly monitored for population size. Potential loss of these populations could mean the loss of adaptive genetic variation, which could be important for the survival of the species in the area and further expansion northward in the future (Nielsen et al., 2001; Parmesan, 2006). Considering the unique DNA variation, a conservation program is warranted for all three populations of the common wall lizard in the Czech Republic. To be successful, such program should include cleaning measures focused on control of bushes and trees serving as shelters for young individuals, but later leading to overshading of the habitat. As wall lizards prefer open exposed rocky habitats on limestone substrate, the overshaded places become unsuitable for population survival. On the other hand, it seems that human presence does not pose a serious threat to the populations (Urban et al., 2006). Two of the three localities are often visited by people with no visible impact on wall lizard abundance.

5. Conclusion

Central Europe was historically crossed by many naturally expanding species, including taxa that originated from the Mediterranean. The studied example of the common wall lizard, *Podarcis muralis*, on its north-eastern range margin in Central Europe, in the Czech Republic, demonstrate importance to understand origins of marginal populations. Considering possible human-mediated introductions of the common wall lizard from southern Europe, commonly establishing viable populations in Western Europe, it is important to give evidence that all so far studied Central European isolated populations belong to the same haplogroup as the one widespread within the continuous range in the region. The presence of unique haplotypes in two of the three isolated Czech populations, together with close phylogenetic relationships to

adjacent Slovak populations suggests either an autochthonous origin of these populations or human-mediated introductions from the geographically and genetically closest populations. We are therefore in support of conservation programs for all three known Czech populations.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mito.2018.04.006.

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Additional file 1: Table S1. The list of sequences with GenBank accession numbers and their localities used for phylogenetic analyses (411 bp). For our sequences of the Central Balkan clade used in this study see Table 1.

GenBank Accession Number	Locality	Country	Authors	Reference
AY234155	Benasque	SPAIN	Busack et al.	Amphibia-Reptilia 26: 239-256 (2005)
FJ867366	Trieste	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867389	Mt. Alburni	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867393	Pollino	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867365	Trento	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
JX856985	Abbotsbury, Birdbook, Corfe Castle, Eastbourne, Folkestone, Holmsley, Newton Ferrers, Poole, Seacombe, Shoreham, Shorwell, Ventnor Botanical Garden, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856989	Boscombe, Corfe Castle, Dancing Ledge, Holmsley, Poole, Seacombe, Shorwell, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856990	Winspit, West Worthing	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972516	Cento, Castelarrano, Nonantola, Olina, Pian Di Venola, Vignola, Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972517	Olina, Vignola, Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972518	Nonantola, Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972519	Nonantola, Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972520	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972521	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972523	Castelarrano	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

KP972524	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972525	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972526	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972527	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972528	Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972529	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972530	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972531	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972532	Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972533	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972534	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972535	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972536	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972537	Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972538	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972539	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856991	Abbotsbury, Bristol, Boscombe, Corfe Castle, Dancing Ledge, Folkestone, Holmsley, Poole, Ventnor Botanical Garden, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972522	Castelarrano	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856987	East Portland, Cheyne Weare, Holmsley, Wembdon, Wellington	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856988	Wellington	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856993	East Portland, Cheyne Weare	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP118978	Joselin	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118979	Jersey Is.	UK	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118980	Iles de Chausey, Jersey Is.	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118981	Jersey Is.	UK	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118982	Iles de Chausey	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118983	Nebias, Frontier Cabardes	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118984	Iles de Chausey, Chateau du Guildo, Dinan, Sees, Vitre, Josselin, Pontchateau, Puybelliard, Pouzagues, St. Gervais, St. Michel, St. Lizier, St.	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

	Girons Frontier Cabardes			
KP118985	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118990	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118986	Bastide	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118987	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118988	St. Lizier, Nebias, Frontiers Cabardes	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118989	St. Girons, Bastide	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856983	Bury	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856984	Bury	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856982	Ventnor Town, Holmsley, Ventnor Botanical Garden, Shorwhel	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972490	Prato, Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972492	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972495	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972496	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972497	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972498	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856992	Bristol, Boscombe	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972482	Buti, Travale, Colle di Val'Elsa, Greve in Chianti, Montemassi, Chianni, Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972484	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972499	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972500	Travale	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972501	Travale	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972502	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972503	Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972504	Montemassi, Travale, Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972505	Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972506	Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972507	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972512	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972508	Chianni, Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972509	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972510	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972511	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972513	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

KP972514	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972515	Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972481	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972483	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972485	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972486	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972487	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972488	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972489	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972493	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972494	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972491	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856986	Birdbook, Eastbourne	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972470	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972471	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972477	Badia Polesine	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972478	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972472	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972475	Badia Polesine	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972476	Barbarano Vicentino	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972479	Badia Polesine	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972474	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972473	Bassano Di Grappa (Campesse)	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972480	Bassano Di Grappa (Campesse)	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
AY185096	Bade	AUSTRIA	Podnar et al.	Org. Div. Evol. 4: 307–317 (2004)
DQ001020	Gumpolds-Kirchen	AUSTRIA	Podnar et al.	J. Mol. Evol. 64: 308-20 (2007)
DQ001025	Nova Gradiska, Strmac	CROATIA	Podnar et al.	J. Mol. Evol. 64: 308-20 (2007)
AY185097	Vis isl.	CROATIA	Podnar et al.	Org. Divers. Evol. 4: 307–317 (2004)
AY896135	Kotili, Xanthi	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
AY896136	Xanthi	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
AY896126	Makedonia (Ag. Germanos)	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
KF372239	Pollino National Park	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372240	Pollino National Park	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372241	Pollino National Park	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372242	Fago del Soldato	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372244	Fago del	ITALY	Salvi et al.	BMC Evol. Biol.

	Soldato			doi:10.1186/1471-2148-13-147 (2013)
KF372243	Fago del Soldato	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372191	Vieille-Roche	FRANCE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372192	Vieille-Roche	FRANCE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372212	Montseny	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372213	Montseny	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372214	Montseny	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372216	Meranges	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372217	Meranges	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372193	Palacios de Compludo	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372194	Braña de Sosas	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372195	Señora de Carrasconte	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372196	La Omañuela	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372197	León	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372198	La Candamia	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372199	Valdehuesa	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372200	Valdehuesa	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372202	Tanes	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372203	Matienzo	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)

KF372204	Matienzo	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372205	Oriñon	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372206	Guadarrama	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372207	Guadarrama	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372208	Guadarrama	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372209	Gúdar	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372210	Penyagolosa	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372211	Penyagolosa	SPAIN	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372219	Massif des Maures	FRANCE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372221	Massif des Maures	FRANCE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372220	Valle de Gilly	FRANCE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372230	Ostia Antica	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372231	Paganico	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372235	Bassiano	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372236	Bassiano	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372232	Majelletta	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372233	Majelletta	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372258	Velouxi	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372259	Platanitsa, Achaia	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147

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KF372261	Mainalo, Pelloponnisos	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372245	Ribnica	SLOVENIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372246	Ribnica	SLOVENIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372247	Donja Lamana Draga	SLOVENIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372248	Donja Lamana Draga	SLOVENIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372262	Kisavos mt.	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372256	Metsovo	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372257	Metsovo	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372263	Samothraki isl.	GREECE	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372264	Kapakli	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372265	Kapakli	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372266	Dereköy	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372267	Dereköy	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372268	Dereköy	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372269	Dereköy	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372270	Dereköy	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372271	Pınarözü	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372272	Pınarözü	TURKEY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372237	Fondi	ITALY	Salvi et al.	BMC Evol. Biol.

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KF372238	Fondi	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372249	Zuce	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372250	Zuce	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372251	Zuce	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372252	Sinevo	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372253	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372254	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372255	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372225	Bianzano	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372226	Bianzano	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372227	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372228	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372229	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
HQ652932	Aschaffenburg	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652892	Darmstadt	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652908	Stuttgart-Wangen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652876	Schloß Holte-Stukenbrock	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652880	Duisburg-Hüttenheim	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652886	Ammelshain	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652887	Ammelshain	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652921	Lörrach	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–

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HQ652920	Lörrach	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652958	Halle an der Saale	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652874	Bramsche, Ueffeln	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652917	-	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652919	Inzlingen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652875	Bielefeld	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652918	Inzlingen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
AF248007	Souprosse	FRANCE	Surget-Groba et al.	Mol. Phylogenet. Evol. 18: 449-459 (2001)
MG851983	Manarola	ITALY	P02_IT	This study
MG851982	Riomaggiore	ITALY	P01_IT	This study
MG851981	Ceresole d'Alba, Torino	ITALY	P47_IT	This study
MG851978	Šume	BOSNIA AND HERZEGOVINA	P36_BA	This study
MG851980	NP Llogare	ALBANIA	P31_AL	This study
MG851963	Ropotamo	BULGARIA	612_BG	This study
MG851964	Bjala Voda	BULGARIA	838_BG	This study

Additional file 2: Figure S1. Bayesian phylogeny of Podarcis muralis based on a fragment (411 bp-long) of mtDNA (cytb) showing schematically overal genetic diversity of the species and details of the Central Balkan clade. Numbers at nodes show Bayesian posterior probabilities and ML bootstrap support values.