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Phylogeography of western Palaearctic reptiles – Spatial and temporal speciation patterns ☆

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Abstract

A phylogeographic analysis of eight species complexes of European reptiles was performed using different molecular methods. While mitochondrial genes (mainly cytochrome *b* sequences) enabled conclusions about phylogeography and differentiation, additional application of bisexually inherited markers provided information about speciation stages. As species with similar distribution patterns in southern and Central Europe were selected, matching phylogeographic patterns are useful for drawing general conclusions:

(1) The species complexes are in different stages of speciation. In some cases, cryptic species were detected.
(2) Highest genetic diversity occurs in southern Europe, the Near East and the Caucasus, regions corresponding with glacial refuges in the Iberian, Apennine and Balkan Peninsulas as well as in Turkey and the Caucasus. Often, several microrefugia must have existed in close neighbourhood. Additional microrefugia were located in southern France and in the Carpathian Basin.
(3) North Africa and the Middle East did not serve as glacial refuges for Central or northern European lineages and are typically inhabited by independent clades.
(4) Evidence for multiple range retractions and expansions, which were postulated for the times of Pleistocene climatic oscillations, could be found in the Balkans, but in Central Europe their traces have been wiped out by the last glacial. Only the Holocene invasion has left imprints in the genomes from this area.
(5) Central and northern Europe were recolonized from Balkan and Pontic refugia in the Holocene.

☆ Modified version of a lecture was presented at the 48th Phylogenetic Symposium – on Historical Biogeography – at Dresden, November 24–26, 2006. We dedicate this publication to the late Peter Lenk (1964–2005).

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(6) Groups from the Iberian and Apennine Peninsulas rarely conquered other regions. This limitation can be attributed to the barrier function of the Pyrenees and the Alps.

Keywords: Phylogeography; *Emys*; *Lacerta*; *Zamenis*; *Hierophis*; *Natrix*; *Vipera*; Genetic diversity; Genetic structure; Quaternary refugia; Postglacial recolonization; Review

1. Introduction

Phylogeography is a comparatively recent biological discipline. Basically, it aims at reconstructing phylogenetic trees in space and time at low (infrageneric and infraspecific) taxonomic levels (Avise et al. 1987; Avise 2000, 2004). By applying molecular genetic methodology, phylogeography bridges the gap between population genetics and biogeography.

Classical biogeographical models of species differentiation in European fauna have postulated causal relationships between speciation and Pleistocene climatic fluctuations. Accordingly, in cold periods (glacials) thermophilic species should have been restricted to refuge areas which have been postulated in the Mediterranean Region as well as the Pontic and Caspian Regions (de Lattin 1967). In these small, isolated refugia, new mutations could accumulate while selection and genetic drift modified the gene pool. In warm periods (interglacials), such as the present one, those differentiated species would recolonize the northern parts of their former territory and emigrants from different refugia would ultimately meet, forming contact or hybrid zones. In such a scenario, central Europe would have been colonized from two directions, from the southwest and from the southeast (Taberlet et al. 1998; Fig. 1). East–west species pairs such as shrews (*Sorex coronatus* and *S. araneus*) and hedgehogs (*Erinaceus europaeus* and *E. roumanicus*) seem to follow this general pattern (Sudhaus et al. 1997; Hewitt 1999; Santucci et al. 1998; Seddon et al. 2001, 2002).

Molecular genetic methodology provides tools to test such hypotheses. As

(i) genetic markers, such as mitochondrial gene sequences, have apparently evolved with a more or less constant average rate in closely related animals, and
(ii) in a given area, climatic fluctuations should influence different but ecologically similar species in the same way,

it can be hypothesized that the resulting phylogeographic differentiation pattern should be similar in different species of one taxonomic/ecological group.

In general, reptiles are good indicator organisms for phylogeographic patterns. Their mobility is moderate, and geomorphological barriers (sea straits, mountains) are effective for them. We review here results of our team on the phylogeography of a set of European/West Asian reptile species complexes: European pond turtles (*Emys orbicularis* complex), water snakes of the genus *Natrix*, European whip snakes (*Hierophis viridiflavus*), Aesculapian snakes (*Zamenis longissimus*/*Z. lineatus*), adders (*Vipera berus* complex), green lizards (*Lacerta viridis*/*L. bilineata*) and sand lizards (*Lacerta agilis* complex). These species complexes were selected so that similar phylogeographic patterns are expected.

Such a comparative approach, involving species sharing a similar distribution pattern but representing two ecological groups (aquatic/terrestrial), seemed appropriate for revealing correlations between phylogeographic patterns and the history of the species and their habitats. If shared phylogeographic patterns were detected, the next question to be asked is whether intrinsic factors (such as common ancestry) were responsible, or whether extrinsic (geomorphological or ecological) factors shaped these patterns. In the latter case, generalized conclusions concerning speciation processes, glacial refugia, biogeographical barriers in the Pleistocene and Holocene migration routes should be possible.

2. Molecular methods

Whenever possible, we analyzed both mitochondrial and nuclear markers to avoid misinterpretations caused by the 'gene tree and species tree problem' (Avise 2004). Mitochondrial DNA, predominantly the cytochrome *b* gene, was sequenced, while applied nuclear markers were either allozymes and plasma proteins, or the DNA stretches between microsatellites, visualised as ISSR fingerprints. Sampling and laboratory techniques are described in detail in the references given below.

The used mitochondrial and nuclear genomic markers supplement each other and, in accord, allow for inferring phylogeographical history. Mitochondrial DNA sequences are powerful tools for revealing subsequent multiple splitting events using standard phylogenetic analyses such as Maximum Parsimony, Maximum Likelihood (Swofford 2002), or Bayesian Inference (Huelsenbeck and Ronquist 2001). Moreover, geographical partition of genetic differentiation may be inferred by calculating haplotype networks (e.g., using

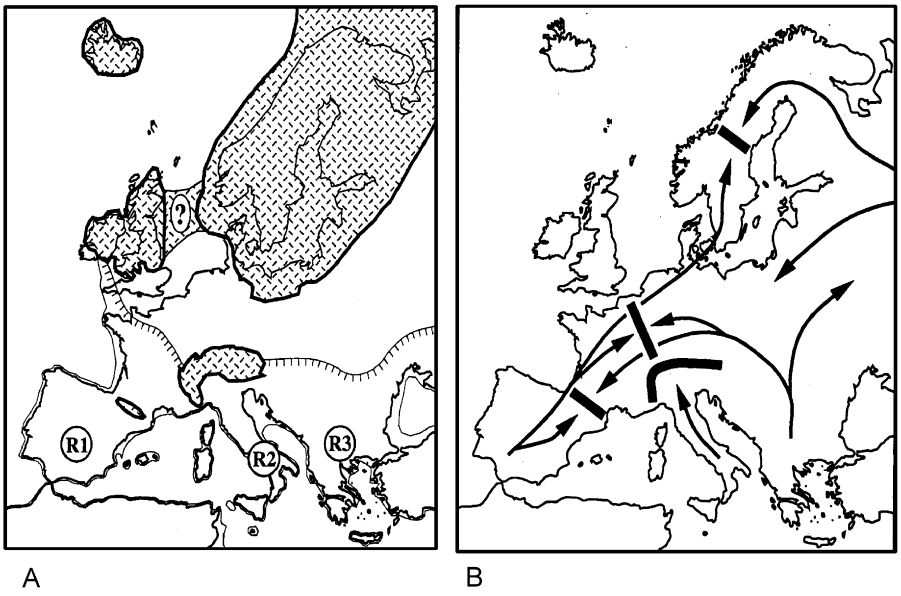

Fig. 1. Hypothetical glacial refugia and post-glacial (re-)immigration routes in Europe: (A) the three main refuges in southern Europe (R1–R3, others have been postulated in the Pontic and Caspian Regions). The southern limit of permafrost soil and the maximum extent of glaciation in the last glacial, 20,000–18,000 yr BP are shown. (B) Hypothetical main post-glacial immigration routes to central and northern Europe; contact zones of different immigration routes (black bars) and main barriers (Pyrenees, Alps) indicated (modified from Taberlet et al. 1998).

statistical parsimony algorithms as implemented in TCS software; Clement et al. 2000). As the mitochondrial genome is inherited only maternally, without inter-individual recombination, coexistence of distinct matri-lineages provides evidence for recent secondary contact. However, matrilineages may be lost in hybrid zones, for instance due to bottlenecks or asymmetrical gene flow. Therefore, hybridization cannot necessarily be detected using mitochondrial DNA and, moreover, differentiation patterns of the nuclear genome could be distinct from mitochondrial differentiation, which is why the additional application of bisexually inherited nuclear markers is often imperative.

Allozyme electrophoresis is a standardized method for describing heterozygosis of populations and measuring genetic distances and gene flow between populations (Murphy et al. 1991). An advantage is the comparability of standard values (such as Nei's D) among different species (e.g., Amann et al. 1997). A disadvantage is the necessity to maintain frozen samples, whereas DNA samples may be ethanol-fixed.

Inter simple sequence repeats (*ISSR*) uses polymerase chain reaction (PCR) with a single microsatellite primer (e.g., $[GACA]_4$, $[GAA]_5$, $[GGAT]_4$). Therefore, only DNA strands are amplified positioned between two identical, but inverted microsatellites. If high annealing temperatures are applied, PCR products are reproducible. They are separated using highly resolving polyacrylamide gel electrophoresis. Typically bands are species-specific; some may be gender- or population-specific (see Wink et al. 1998, 2001).

3. Results

3.1. European pond turtles (*Emys orbicularis* complex)

Altogether more than 1100 pond turtles from localities covering most of the range were studied. Using the mitochondrial cytochrome *b* gene, 56 haplotypes were identified, representing nine clades or lineages (Fritz et al. 2007; Figs. 2–4). Turtles likely to be introduced (mainly from western Germany) were excluded from the general phylogeographic analysis and treated separately (Fritz et al. 2004; Fig. 5).

Lineage I, which includes 10 haplotypes (Ia–Ij), is distributed in eastern Europe and adjacent northern Asia, in the Aegean Region and in Anatolia. North of the Black Sea and the Crimea, basically only haplotype Ia is found, the others occurring further south. The distribution of endemic lineage I haplotypes along the Black Sea coasts suggests several microrefuges there. Most likely, Holocene range expansion occurred from one of these refuges both eastwards and westwards, leading to recolonization of the northern parts of eastern Europe and adjacent western Asia (Lenk et al. 1999; Fritz et al. 2007).

Lineage II, the sister taxon of lineage I, occupies a crescent-shaped range from north-eastern Greece across the Danube lowlands, parts of Central Europe and France to north-eastern Spain. The haplotype with the widest distribution, IIa, is the direct ancestor of all other haplotypes within this lineage, except IIf which is found at the south-eastern margin of the lineage's range. This

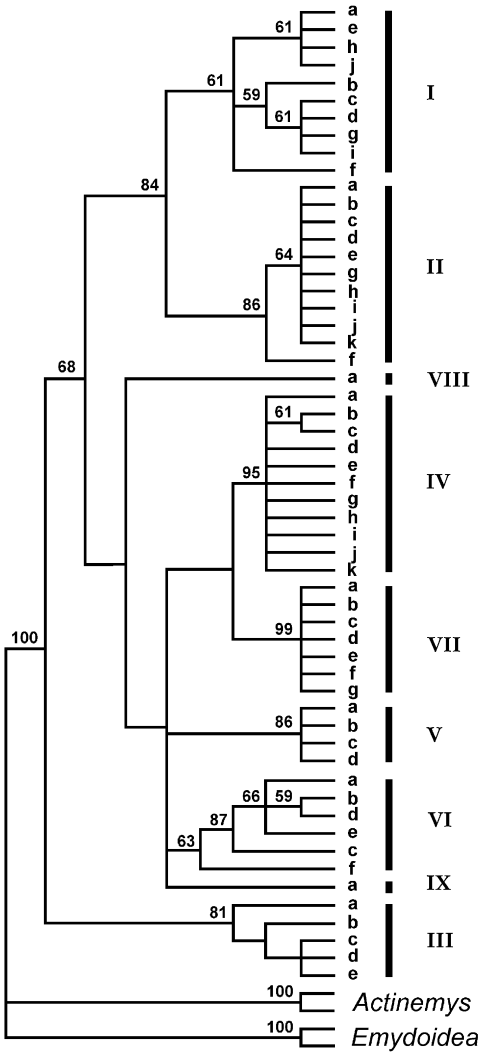

Fig. 2. Strict consensus of six equally parsimonious trees of *Emys* cytochrome *b* haplotypes routed with *Actinemys marmorata* and *Emydoidea blandingii*. Numbers along branches are bootstrap values greater than 50; Roman numerals denote mitochondrial lineages. Lineages I–II, IV–IX, *Emys orbicularis*; lineage III, *E. trinacris* (modified from Fritz et al. 2007).

indicates the likely location of the Pleistocene refuge from where IIa spread along the Danube valley, circumventing the Alps in the east (Fritz 1996; Fritz et al. 2007). Haplotype IIb, restricted to eastern Germany and adjacent Poland, differs from IIa by one mutational step, like other local haplotypes (Fig. 4). The question of whether certain German pond turtle populations comprising haplotype IIa individuals are native or not, is highly debated among nature conservationists. The occurrence of many other haplotypes in a strange mixture in western Germany, and that pet turtles were collected in the past mainly within the range of haplotype IIa turtles, favours that the turtles were introduced (Fritz et al. 2004).

Lineage III inhabits Sicily and perhaps the extreme south of the Italian Peninsula. As turtles harbouring lineage III haplotypes differ in nuclear and mitochondrial genomic markers consistently from all other European pond turtles, they were described as the distinct species *Emys trinacris* (Fritz et al. 2005). It must have remained in isolation for a long time.

Lineage IV is found around the Adriatic Sea, from southern Greece to Italy, east of the Apennines. The northernmost parts of its range are occupied by haplotype IVa only. Lineage V replaces lineage IV west of the Apennines. It is distributed along the coast of the Tyrrhenian Sea to north-eastern Spain and inhabits also Sardinia and Corsica. In the north of its range, only haplotype Va is found. In north-eastern Spain it occurs in mixed populations with haplotypes of lineage II and VI, in the southern Italian mainland with haplotypes of lineage IV. Lineage VI is confined to the Iberian Peninsula and north-western Africa. Its most ancestral haplotypes, VIc and VIf, occur in Morocco. North Africa should therefore be considered as the probable area of origin for lineage VI. Lineage VII is restricted to the south coast of the Caspian Sea and the central Caucasus, and lineage VIII to southern Anatolia. The geographic origin of lineage IX is unknown (Fritz et al. 2007).

A subset of 20 haplotypes of lineages I–VII was used in a partial Mantel test to compare the plausibility of different causal hypotheses to explain the observed distribution patterns (Table 1). Different ecological parameters could not be correlated with the current distribution. A partial correlation was found with geographic distances and with the main refuges postulated by de Lattin (1967). The best explanation for the current phylogeographical pattern among *Emys* haplotypes was found in a model which further subdivides de Lattin's refugia into 'microrefugia' (Lenk et al. 1999; Fritz et al. 2007), implying that geomorphological structures act as important barriers. Apparently local physical barriers such as the Apennines are sufficient to prevent gene flow.

3.2. Green lizards (*Lacerta viridis*/*L. bilineata*)

Hybridization experiments (Rykena 1991, 1996, 2001) and allozyme analyses (Amann et al. 1997; Joger et al. 1998, 2001) revealed that the former *L. viridis* is composed of two biological species, *L. viridis* and *L. bilineata*, the hybrids of which show reduced fertility. Amann et al. (2001) identified a hybrid zone in north-eastern Italy and the adjacent part of Slovenia, in which restricted and asymmetrical gene flow (predominantly from the east to the west) was detected. Such a hybrid zone of 'species in statu nascendi' remains spatially restricted, but may be stable for a long time, if

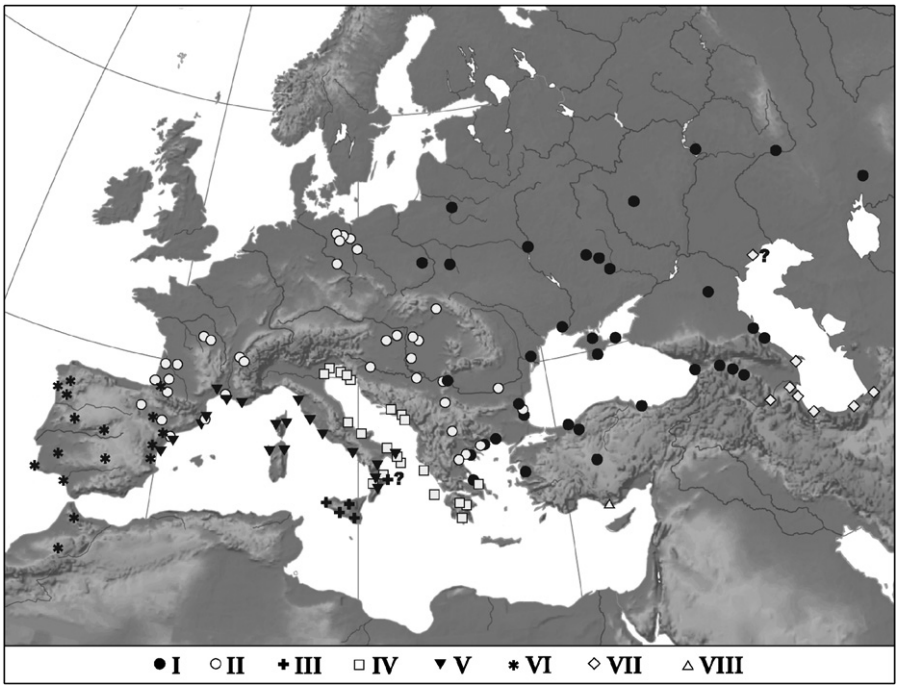

Fig. 3. Distribution of mitochondrial lineages of *Emys* (only natural populations). Note that only lineages I and II occur north of the Alps. Lineages I–II, IV–VIII, *Emys orbicularis*; lineage III, *E. trinacris*. Range of lineage IX of *E. orbicularis* unknown. Syntopic occurrences of distinct lineages indicate secondary contact zones (modified from Fritz et al. 2007).

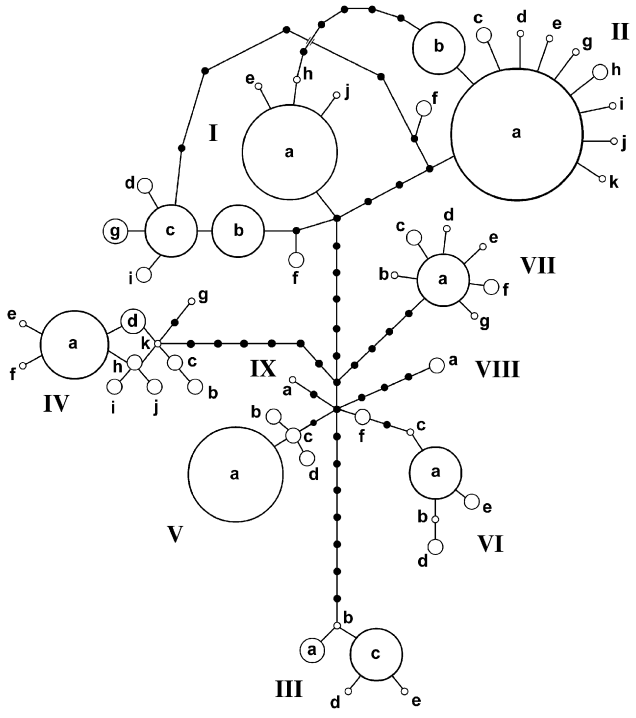

Fig. 4. TCS parsimony network of *Emys* haplotypes. Open circles symbolize known haplotypes, circle size corresponds to frequency of haplotypes; dots, missing haplotypes. Each line connecting identified or hypothetical haplotypes, one mutational step (modified from Fritz et al. 2007).

it is stabilized by equivalent invasion and evasion, hybridization and selection (Barton and Hewitt 1985).

Comparing the allozyme tree (Fig. 6) with the cytochrome *b* tree (Fig. 7), a concordant split of two main clusters – the two species *L. bilineata* and *L. viridis* – is observed. Both trees assign populations from the hybrid zone (Udine, Trieste) to *viridis*, which contradicts a recent analysis of mtDNA sequences by Böhme et al. (2007). These authors found out that populations from this region (and Slovenia) belong to a distinct 'Western Balkan Clade', distributed along the Balkan west coast south to Greece. Phylogenetic analyses suggested that this 'Western Balkan Clade' could be allied with *L. bilineata* rather than with *L. viridis* (Böhme et al. 2007). Mayer and Beyerlein (2001), using 12S and 16S RNA genes, also found western Greek lizards associated with *bilineata*, while Brückner et al. (2001), using cytochrome *b* sequences from the same samples, found them clustering with *viridis*. However, none of the teams studied Turkish *L. viridis*. In our tree (Fig. 7), with Turkish lizards included, the sequences from Trieste and Udine are embedded within *viridis*. The lizards from Turkey appear as basal branch of *viridis*. Yet, *viridis* is made up of three different clades which must have been differentiated in separate glacial refugia: one in Turkey and two in the Balkans.

The Croatian population from the island of Cres clusters with the western *bilineata*. This was also found

by Brückner et al. (2001) and by Godinho et al. (2005). Cres could harbour a relict population of an once more easterly distributed *bilineata*, recently restricted by a western advance of *viridis*.

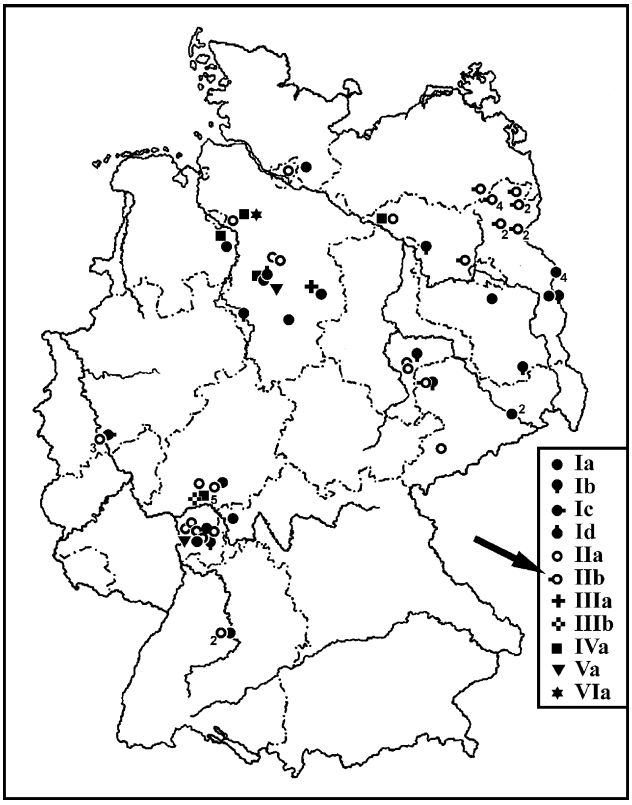

Fig. 5. Haplotypes of *Emys* specimens caught in Germany. Most turtles are introduced aliens. Numbers by symbols indicate more than one individual; arrow, native haplotype IIb (modified from Fritz et al. 2004).

3.3. Sand lizards (*Lacerta agilis* complex)

L. agilis is a widespread species in Europe, northwestern and central Asia, with a distinctive geographical subspecific structure (Bischoff 1988). A phylogeographic analysis based on cytochrome *b* sequences (Kalyabina et al. 2001) confirmed most of the subspecies as distinct lineages, and ranked them in a hierarchical branching order (Fig. 8). If the branching events are linked with the geographical distribution of the subspecies, the Caucasian/Black Sea Region emerges as the group's most probable area of origin, for the following reasons:

- The most ancestral clade is *L. a. boemica* from the eastern Caucasus. It may deserve species status.
- The next branching event corresponds with two major clades, a western and an eastern one.
- The western clade is distributed from western Russia to the Pyrenees, and the most basal internal branches represent the easternmost subspecies, suggesting that this clade moved gradually westwards.
- The eastern clade (from the Caucasus west to the Crimea and east to Lake Baikal) comprises a distinct Balkan subspecies (*L. a. bosnica*) and many populations from the eastern part of the species' range being only slightly differentiated genetically, except the Crimean population. It was recently revalidated as the distinct subspecies *L. a. tauridica* (Kalyabina-Hauf et al. 2004a). This branching pattern implies that the north-eastern Black Sea Region was populated by the eastern clade during both warm and cold Pleistocene periods, whereas the vast eastern part of the distribution is the result of a Holocene invasion from Caucasus, where two subspecies of this clade are still found.

Table 1. Partial Mantel test for causal analysis of the actual distribution pattern of *Emys orbicularis* and *E. trinacris*: Partial regression coefficients and error probabilities for rejection of the null hypothesis (*P*-values) for the partial regressions of pairwise genetic distances between populations and different causal hypotheses and subspecies

Hypotheses	Partial regression coefficient	*P*-value	Significance after Bonferroni correction ($a = 5\%$)
Geographic distance	−15.3	0.0037	Significant
Geographic distance and barriers	0.13	0.0020	Significant
Start of spring	0.05	0.0180	–
Extent of cold period	−0.022	0.9058	–
Extent of warm period	0.18	0.3545	–
Subspecies	1.01	0.0019	Significant
Macrorefugia	2.08	0.0009	Significant
Herpetochorology	−1.37	0.0132	–
Microrefugia	6.14	0.0001	Highly significant ($a = 1\%$)
Amphibian chorology	1.37	0.0132	–

A Bonferroni correction was applied at the 5% significance level (from Lenk 1997).

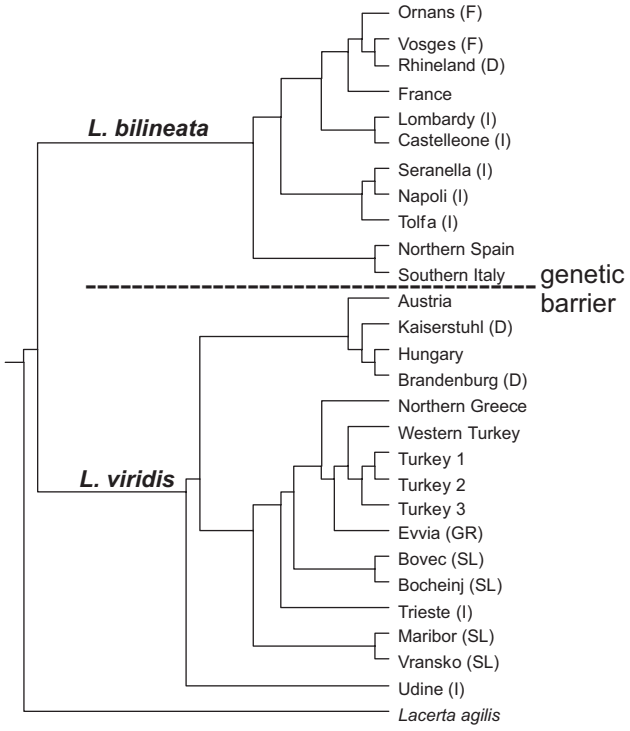

Fig. 6. Evolution of green lizards: Maximum Parsimony tree, derived from allozyme genetic distances. Two species – a western one (*Lacerta bilineata*) and an eastern one (*L. viridis*) – appear separated by a genetic barrier. In Germany and Italy both species occur. In this tree, the Udine population is basal for *L. viridis*. This is a calculation artefact due to its hybrid nature, as both *bilineata* and *viridis* alleles occur in this population (modified from Joger et al. 2001).

As *L. a. bosnica* is sister of the other lizards in the eastern clade, while the western clade branched off from the common stem earlier, the most probable phylogeographic hypothesis must take into account several waves of colonization from east to west. A molecular clock places these waves into different periods, from upper Pliocene to middle Pleistocene (Kalyabina et al. 2001).

3.4. European whip snakes (*Hierophis viridiflavus*)

This colubrid snake is distributed over most of France, Italy and Slovenia and reaches into southwestern Switzerland, Luxemburg, Belgium, northernmost Spain and Croatia. Distinction of any subspecies, originally based on coloration differences, was refuted by Schätti and Vanni (1986). However, our molecular analysis, based on both cytochrome *b* sequences and ISSR fingerprints, revealed a considerable geographic structure suggesting subspecific differentiation (Nagy et al. 2002). Basically, a western and an eastern clade are discernable, the border between them formed by the Apennine chain (Figs. 9 and 10). A third clade, restricted to Sicily and southern Calabria, is sister to the eastern clade. ISSR fingerprints (Fig. 11) revealed distinctive loci for these three clades, but also identified several specimens of hybrid origin which share bands characteristic of eastern and western clade.

For this species the phylogeographic history can be elucidated also with fossil records, discovered in Austria, southern Germany, the Czech Republic and Poland (Szyndlar and Böhme 1993; Ivanov 1997). These areas were reached in warmer periods of the Pleistocene, as well as in the Pliocene, but in glacial periods the distribution area shrunk into its Italian core area.

3.5. Aesculapian snakes (*Zamenis longissimus* complex)

These colubrid snakes, formerly called *Elaphe longissima*, are distributed in France, Italy, Austria, the Balkans and Turkey, with isolated relict populations in southern Germany, the Czech Republic and Poland. Related species occur in Spain and France (*Rhinechis scalaris*), Italy and Greece (*Zamenis situlus*), Turkey and Iran (*Z. hohenackeri*, *Z. persicus*). Compared to these species, *Z. longissimus* seems to be less thermophilic and hence occupies a more northern distribution area.

Protein electrophoretic comparisons (Lenk and Joger 1994) revealed only slight geographic structuring in *Z. longissimus* which, for example, showed an association of the Central European relicts to the Balkan populations and not to the Italian populations (refuting the hypothesis that the Romans had imported the Aesculapian snake to Germany). However, a strong difference in electrophoretic patterns of blood proteins was found between southern Italian Aesculapian snakes and all others. A morphological analysis (Lenk and Wüster 1991) concluded that the southern Italian population must be regarded as distinct species, *Zamenis lineatus*. Phylogenetic analyses of cytochrome *b* sequence variation (Figs. 12 and 13) also provided evidence for the species status of *Z. lineatus* (sequence difference of 7% to *Z. longissimus*). Moreover, it revealed that within *Z. longissimus* an eastern haplotype group (found along the Aegean and Black Seas) can be distinguished from a western group. Both groups nearly meet in Greece. They are separated by the Pindos Mts and must have had different Pleistocene refuges. The western haplotype group is composed of a Danubian haplotype (which includes the German populations) and a Mediterranean haplotype, from which a western haplotype (hitherto found in France and in Tuscany) are derived (Fig. 13). This suggests three distinct microrefuges for the western haplotype group.

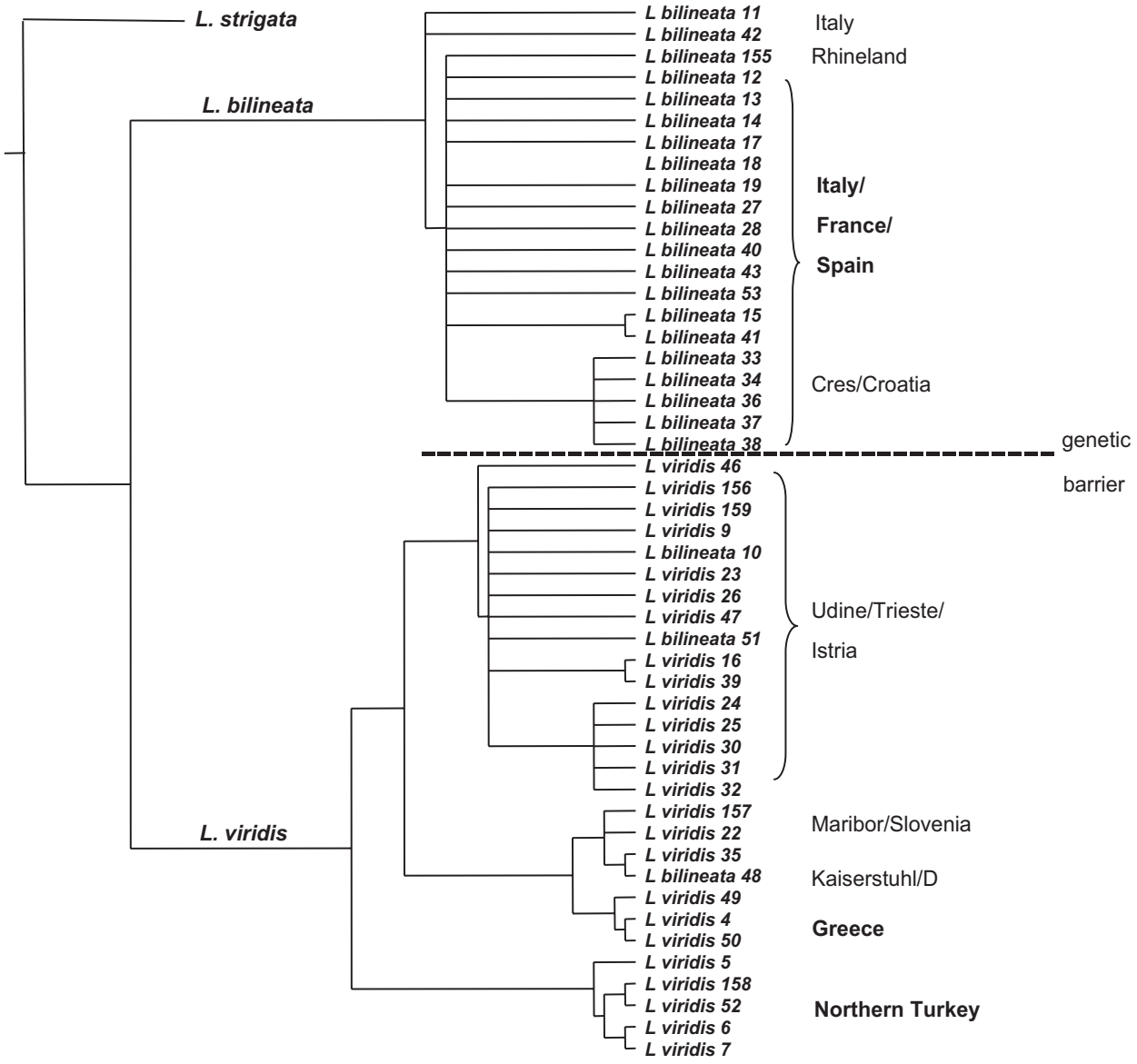

Fig. 7. Maximum Parsimony tree derived from mitochondrial cytochrome *b* sequences of green lizards. The main split is concordant with the one in Fig. 6, supporting the existence of two distinct species. Note that several individuals were initially determined as *Lacerta bilineata* (from Trieste, Italy, and Kaiserstuhl, Germany), but yielded haplotypes of *L. viridis*, whereas lizards from the Croatian island of Cres are unambiguously *L. bilineata* (modified from Joger et al. 2006).

3.6. Viperine snakes (*Natrix maura*)

This aquatic snake is distributed in the western part of the Mediterranean, from Italy to Morocco and Tunisia, north to central France and Lake Geneva. A phylogeographic analysis using cytochrome *b* sequences and ISSR fingerprints (Guicking et al. in press) resulted for both data sets in congruent differentiation patterns (Figs. 14 and 15).

Four major haplotype groups were found – two from North Africa (Tunisia, Morocco) and two from Europe (Cádiz in southern Spain, all other European sequences). Both European groups are part of one clade, while either the Moroccan or the Tunisian type is sister to the European clade (depending on the method of tree reconstruction). In any case, the origin of the species is most probably North African; Europe was colonized via the strait of Gibraltar. A similar phylogeographical pattern exists in the stripe-necked terrapin *Mauremys leprosa* (Fritz et al. 2006a). A standard molecular clock dates the colonization of Europe by viperine snakes to the Pliocene (approx. 3 myr BP). The fact that one of the Tunisian haplotypes is also found in Sardinia may be due to human translocation, perhaps in Roman times. While other taxa of North African origin, such as butterflies of the genus *Melanargia* (Habel et al. in press)

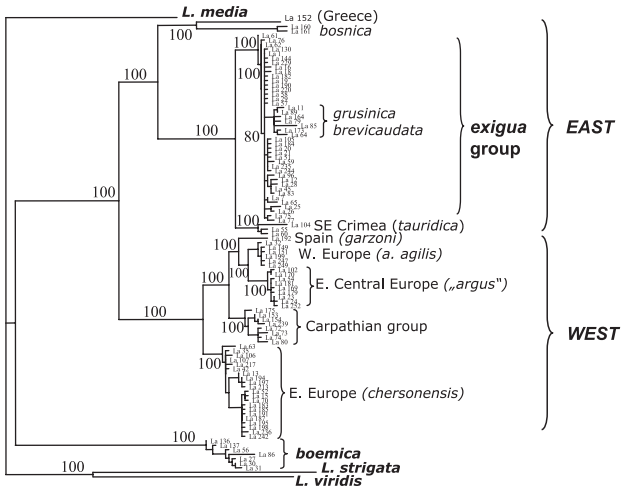

Fig. 8. Maximum Parsimony phylogram of the *Lacerta agilis* complex derived from mitochondrial cytochrome *b* sequences and routed with *L. media*, *L. strigata* and *L. viridis*. Bootstrap values indicated. The *exigua* group comprises subspecies from Caucasus (*L. a. grusinica*, *L. a. brevicaudata*) as well as south-eastern European *exigua* sensu stricto and various central Asian *agilis* (modified from Joger et al. 2006).

may have reached Italy from Tunisia, this seems unlikely for *N. maura*, because the snake is absent from southern Italy and Sicily. The viperine snakes of the old island of Mallorca were probably introduced, too, as they harbour a haplotype also occurring on the French and Spanish mainland (Guicking et al. 2006a).

In Europe, genetic diversity is lowest among north-eastern populations (Italy, France, Switzerland, NE Spain), while distinct genetic groups occur in southern Spain and Portugal (Extremadura, Tejo; Fig. 16). This is paralleled by other Iberian taxa, such as salamanders (Joger and Steinfartz 1994). Southern Iberian populations are more isolated and most differentiated. Northern Iberian populations are the sources of Holocene colonizations of France and Italy, but an additional microrefuge in southern France or western Italy cannot be excluded.

3.7. Dice snakes (*Natrix tessellata*)

Dice snakes resemble ecologically and morphologically viperine snakes but are phylogenetically closer to *N. natrix* (Guicking et al. 2006b). They replace viperine snakes in the east, occupying a vast distributional range from Germany, Switzerland and Italy to Central Asia, Iran and Arabia. The phylogeographic analysis of cytochrome *b* sequence data revealed 10 distinct haplotype groups, two of which occur in Greece and only one in the rest of Europe. The Middle East is the most probable area of origin for this species. Early branching events led to highly differentiated haplotype

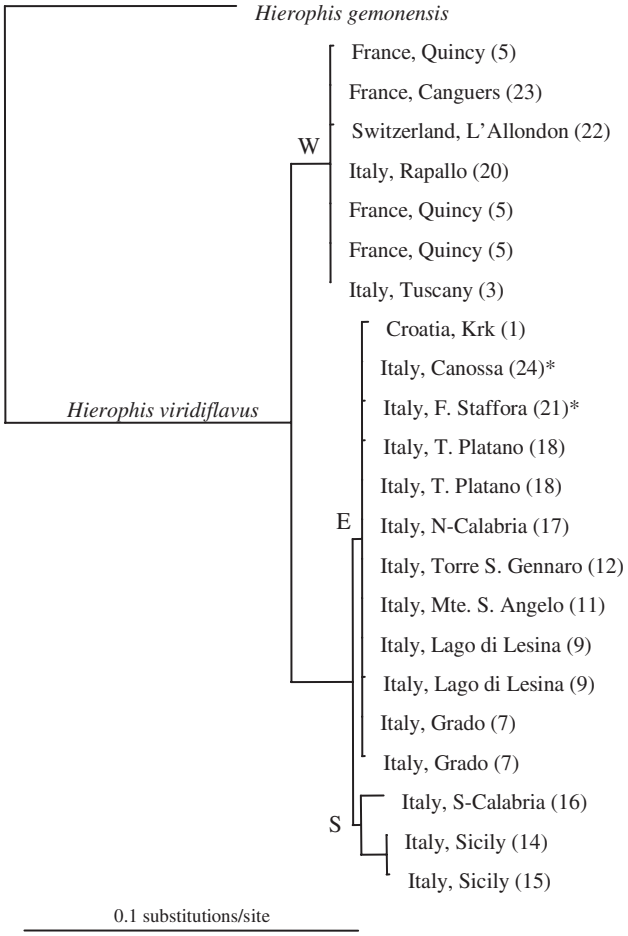

Fig. 9. Maximum Likelihood tree of *Hierophis viridiflavus* derived from cytochrome *b* sequences. W = western populations; E = eastern populations; S = southern populations; * = populations east of the Apennines sharing alleles with the western group according to ISSR-PCR (from Nagy 2004).

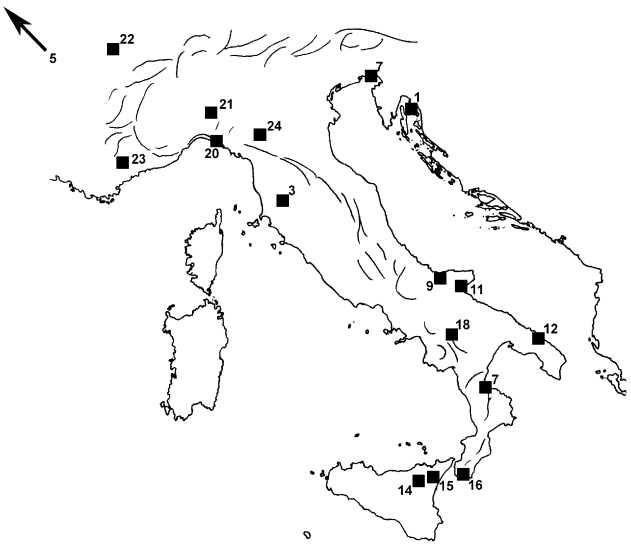

Fig. 10. Sampling sites for *Hierophis viridiflavus*. For locality codes (numbers), see Fig. 9 (modified from Nagy et al. 2002).

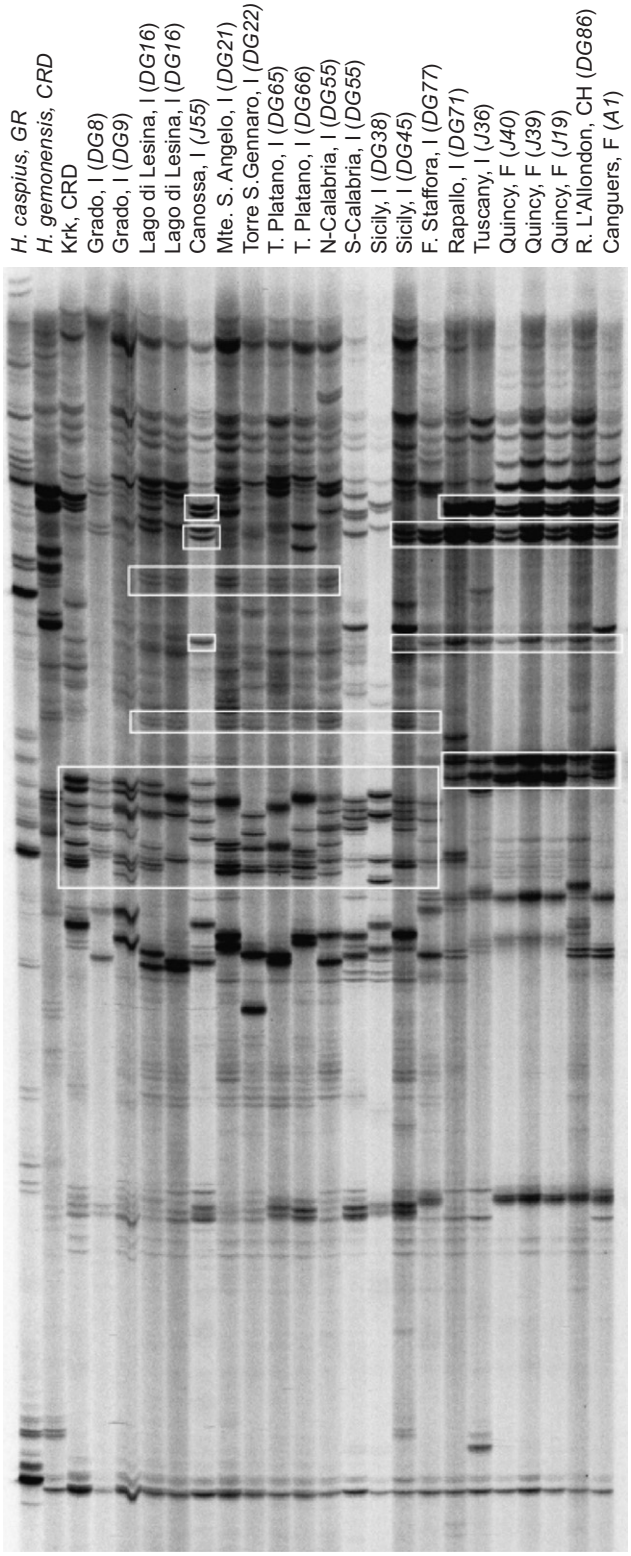

Fig. 11. ISSR-PCR genomic fingerprint of *Hierophis viridiflavus* populations, using the primer $(GACA)_4$. White lines denote bands diagnostic either for the eastern group (left columns) or the western group (right columns). Specimens from Canossa, F. Staffora and Sicily (DG45) are heterozygous with bands from both groups; suggestive of gene flow. The mixed bands in DG45 may, however, be a methodical artefact (modified from Nagy et al. 2002).

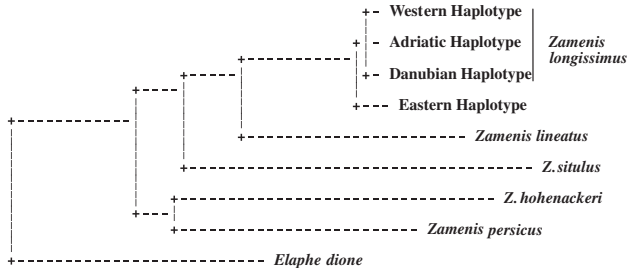

Fig. 12. Neighbor joining phylogram of Aesculapian snakes and relatives (*Zamenis* spp.) derived from cytochrome *b* sequences (modified from Joger et al. 2006).

groups found in Iran (up to 10% sequence difference), Greece and northern Arabia. Four haplotype groups occurring around the Black Sea, in Turkey and Central Asia are related to each other; ISSR fingerprints provide evidence that populations of these groups hybridize. A distinct group is found in Crete, and a rather homogeneous group occupies all the European range except Greece (Figs. 17–19).

In the ISSR tree (Fig. 19) the three European groups appear closer than in the cytochrome *b* tree. A western Turkish haplotype clusters with the haplotype groups from Crete and Greece. Although no hybrids were detected and no group-specific ISSR band is shared by the three groups, the coincidence between geographical and genetic proximity suggests gene flow in the past.

Two distinct Pleistocene refuges in the southern Balkans can be deduced from the data. Only one of them served as source for the recolonization of the rest of Europe. The genetic diversity among European populations is low and with little geographic structuring. The invasion of Italy was dated back to the last interglacial at most, and, thus, a separate microrefuge in the Apennine Peninsula is possible for the last glacial (Guicking 2004). A number of other microrefuges must have existed in Anatolia, in the Caucasus and in western Central Asia.

3.8. Adders (*Vipera berus* group)

In contrast to the species treated before, adders have a more northern distribution, occupying a vast territory from northern Spain to the Polar Circle in Scandinavia, and to the Pacific Ocean in the east. Nevertheless, most genetic diversity is concentrated to the southern margin of the range, and several endemic taxa occur there. The Iberian Adder, *Vipera seoanei* Lataste, 1879, has been regarded as distinct species for long. From the Black Sea Region, *Vipera nikolskii* Vedmederya, Grubant and Rudaeva, 1986 (Ukraine, southern Russia) and *Vipera barani* Joger and Böhme, 1984 (Turkey) were described, but their species status is doubtful. From the Balkans, the subspecies *V. berus bosniensis* Boettger, 1889, is

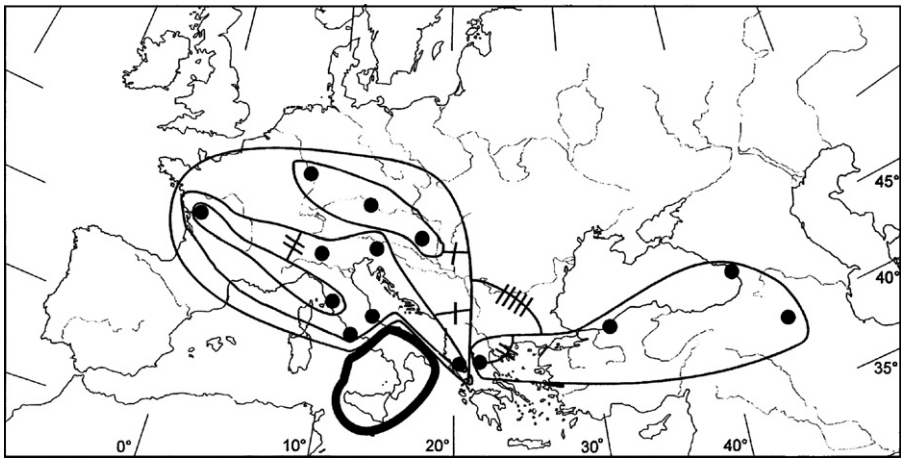

Fig. 13. Phylogeography of Aesculapian snakes (*Zamenis longissimus* and *Z. lineatus*) derived from mitochondrial cytochrome *b* sequences. Narrow lines denote distribution of haplotypes of *Z. longissimus* with number of mutations (dashes) indicated; bold line, *Z. lineatus* (modified from Joger et al. 2006).

known, and in the Far East, *V. berus sachalinensis* Tsarevski, 1916 has been distinguished. The relationships between these taxa were studied by Kalyabina-Hauf et al. (2004b) and Ursenbacher et al. (2006a).

In the cytochrome *b* tree (Fig. 20), *V. seoanei* appears as sister to all other adders of the *V. berus* complex. *V. nikolskii* and *V. barani* occur in a weakly supported clade being embedded within *V. berus.* A particularly stunning result is the separation of Alpine *V. berus* from the rest of the species, appearing as sister taxon to all other *V. berus* plus *V. barani* and *V. nikolskii*. This highly distinct status of Alpine *berus* is also supported by ISSR fingerprint data and by mitochondrial control region sequences (Ursenbacher et al. 2006a). Obviously, these adder populations must have had glacial refugia south of the Alps (probably in Italy, where a population of lowland adders became extinct just several decades ago; Scali and Gentilli 1999). The refuges of northern *berus* are difficult to determine, but the isolated position of the Hungarian sample points to the Carpathian Basin as possible refuge. According to Ursenbacher et al. (2006a), additional refugia may have been located in France, Slovakia or southern Russia/Ukraine. Scandinavia was recolonized post-glacially from two sides, west and east, suggesting several refugia for this fairly cold-tolerant snake.

4. Discussion

General patterns in the phylogeography of all treated reptile groups are obvious. These patterns are not restricted to particular taxonomic groups (turtles, lizards, or snakes). Therefore, extrinsic (geographical or ecological) factors, and not intrinsic, factors have shaped the phylogeographical patterns. No obvious differences were found in phylogeographic patterns of aquatic versus terrestrial reptiles. Mountains serve as barriers for both ecological types, while river valleys serve as immigration routes for both.

Regardless of ecological differences, the highest degree of genetic differentiation occurs always near the southern margin of the range. Here, the glacial refugia have to be postulated, in accordance with the classical view of de Lattin (1967), in particular in the Mediterranean peninsulas. The refugia there must have been the source of repeated colonizations of more northerly areas. Contrary to the northern areas, where climatic drawbacks wiped out colonies sooner or later, the southern peninsulas were continuously inhabited and therefore served as sites of genetic differentiation. Mountain ranges, such as the Apennines and the Balkan mountains, separated populations which differentiated by gene drift and mutation.

4.1. Speciation

In the course of our studies we found speciation processes completed in southern Italy (*Emys trinacris*, *Zamenis lineatus*). Speciation in southern Italy is an interesting case as no barriers limit north–south migration within the peninsula. However, it seems that Sicily, today separated from mainland Italy by the Strait of Messina, served as distinct speciation centre for these species. The role of Sicily as independent speciation centre is further corroborated by the occurrence of a newly discovered, endemic green toad (*Bufo viridis* complex; Stöck et al. 2006) as well as by two other extant (*Crocidura sicula*, *Podarcis wagleriana*) and many extinct vertebrate species endemic to Sicily (and the glacially connected Maltese islands; Fritz et al. 2006b). Likewise, the distinct Sicilian clade of *Hierophis viridiflavus* (Nagy et al. 2002) is to be mentioned here, although reflecting an earlier stage of speciation.

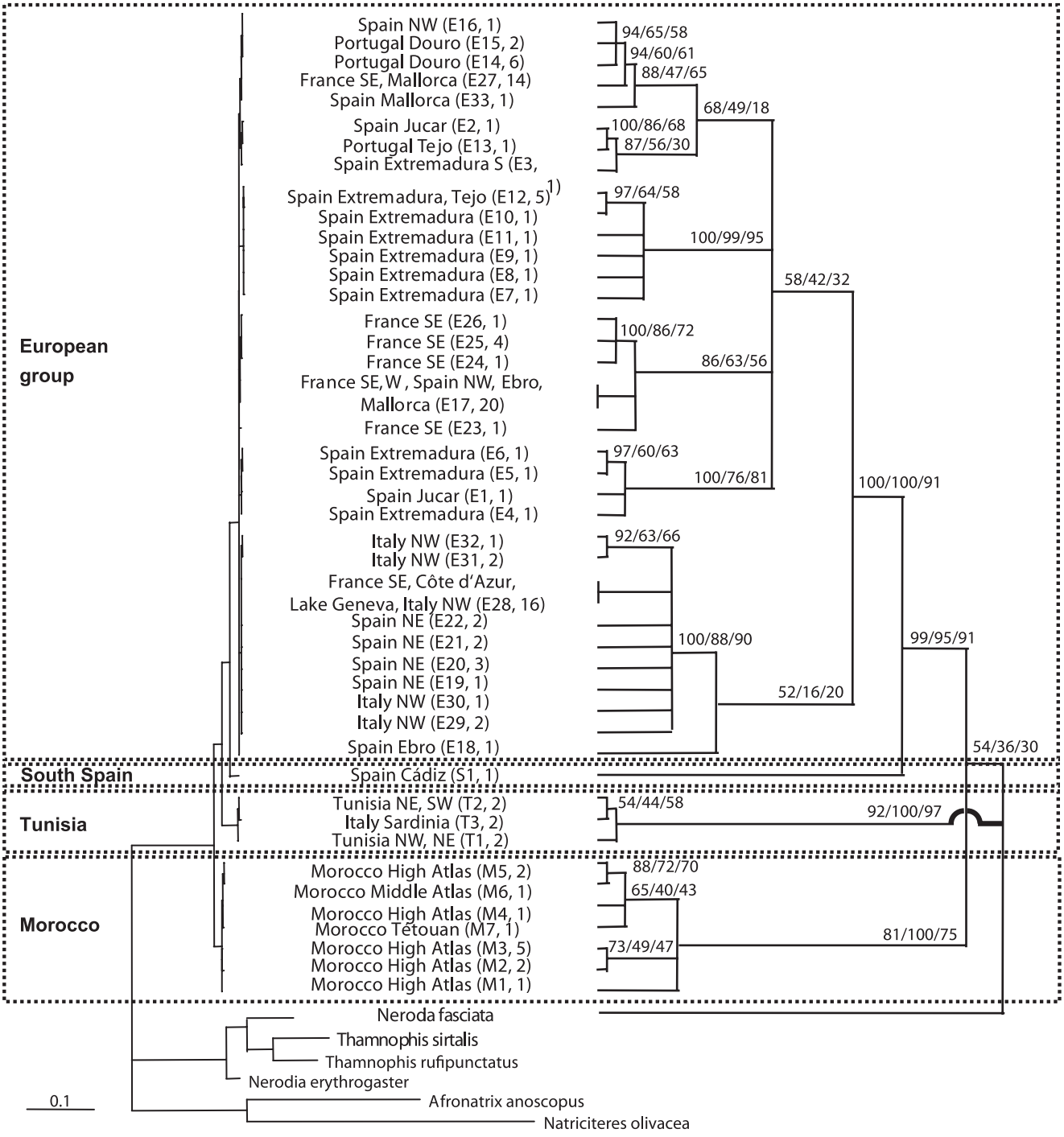

Fig. 14. Phylogenetic trees of *Natrix maura* reconstructed from cytochrome *b* haplotypes, left: Maximum Likelihood phylogram, right: Bayesian cladogram. Support values are indicated: Bayesian posterior probabilities/ML bootstrap values/MP bootstrap values (modified from Guicking et al. in press).

In the Iberian Peninsula, endemic species had been recognized already by earlier authors, who described species like *Vipera seoanei* Lataste, 1879 (*V. berus* complex) and *Lacerta schreiberi* Bedriaga, 1878 (*L. viridis* complex). A high degree of endemism of Iberian taxa, coupled with low colonization potential, is also observed in other groups too, e.g. in butterflies (Habel et al. 2005).

The situation in the Caucasus is comparable: *Lacerta strigata* Eichwald, 1831; *L. [agilis] boemica* Sukhov, 1926 and *Zamenis persicus* (Werner, 1913) represent there the *L. viridis* and *L. agilis* complexes and *Z. longissima*, respectively.

Also in the southern Balkan Peninsula, but not in the north, differentiated taxa and lineages like *L. agilis*

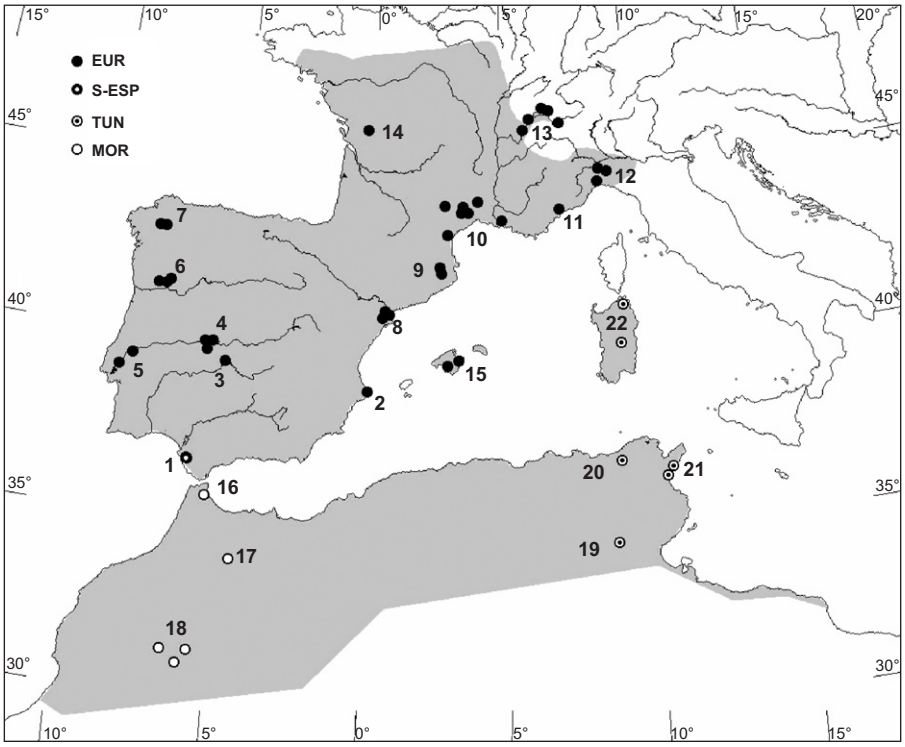

Fig. 15. Geographical distribution of the main haplotype groups of cytochrome *b* in *Natrix maura* (modified from Guicking et al. in press).

bosnica, *L. viridis/bilineata* (*L. viridis* ssp. and western Balkans group), *V. berus bosniensis*, and *Natrix tessellata* (Greek group) occur, indicating the existence of glacial refuges there.

Vipera aspis and its closest relatives, *V. ammodytes* and *V. latastei*, shall be added here as they show a nearly exclusive European distribution (extending into northern Turkey and Georgia) and have completed speciation in the classical Mediterranean refugia: Iberian Peninsula and Northwest Africa (*V. latastei*), Apennine Peninsula (*V. aspis*), Balkan Peninsula to Caucasus (*V. ammodytes*). *Vipera aspis* has been divided into a number of subspecies. The hypothesis of Zuffi (2002) that some of these subspecies represent full species was not accepted by Joger et al. (2004). Ursenbacher et al. (2006b) produced a molecular tree, in which two main groups were clearly separated: an Italian group and a French-Swiss-northern Spanish group. The western Alps separate the two groups, which probably split in the Pliocene. It is uncertain whether speciation has been completed in this case. During the Pleistocene, both groups split into two subspecies each.

While the maternally inherited mitochondrial genes are not helpful to decide whether different biological species have been evolved, nuclear genomic markers can provide valuable information on the existence of hybrid zones and the extent of gene flow across potential species borders. Avise (2000, 2004), as well as Joger et al. (1998), regard the concordance of trees derived from different methodological approaches, as a strong indicator of an intrinsic (genetic) barrier. Such a situation occurs with respect to the concordance between the allozyme tree and the mitochondrial tree in *L. viridis/bilineata*, suggesting that speciation has reached the 'point of no return', although hybridization still occurs. However, hybridisation experiments provide in this case evidence for a limited fitness of hybrids (Rykena 1991, 1996, 2001), further underlining the species status of *L. viridis* and *L. bilineata*.

A similar concordance between nuclear and mitochondrial trees is observed in the first three branching events in *N. maura* and in *N. tessellata*, respectively. Thus, the major clades in these snakes may deserve species status, too. On the other hand, less divergent

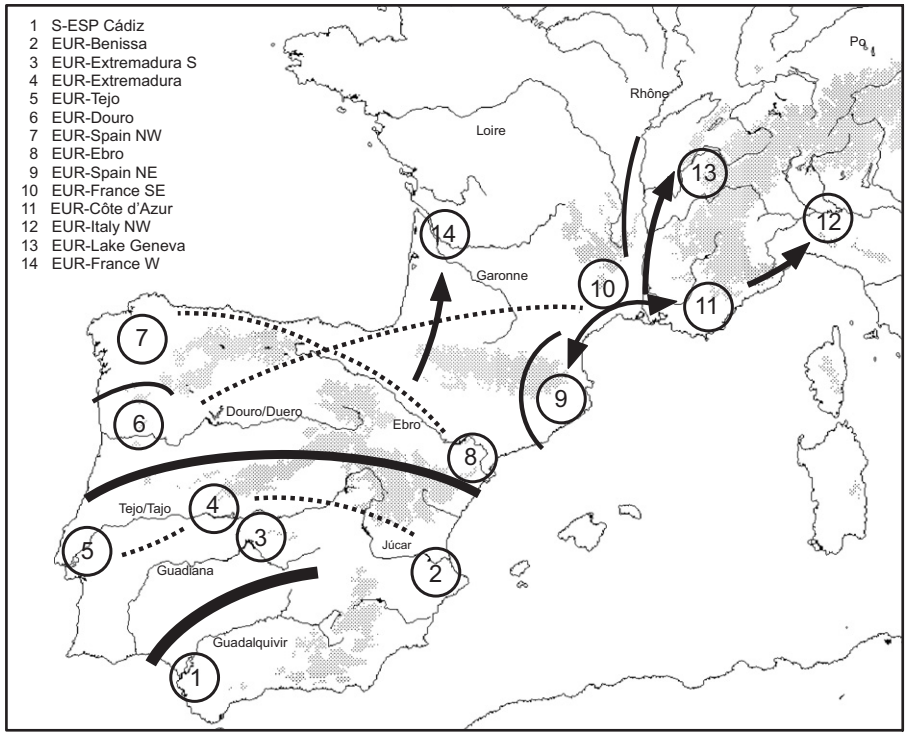

Fig. 16. Phylogeographic differentiation of the viperine snake (*Natrix maura*) in Europe. Main drainage systems and mountain chains indicated. Black lines: barriers for gene flow; dotted lines: long-distance gene flow; arrows: Holocene range expansion (modified from Joger et al. 2006).

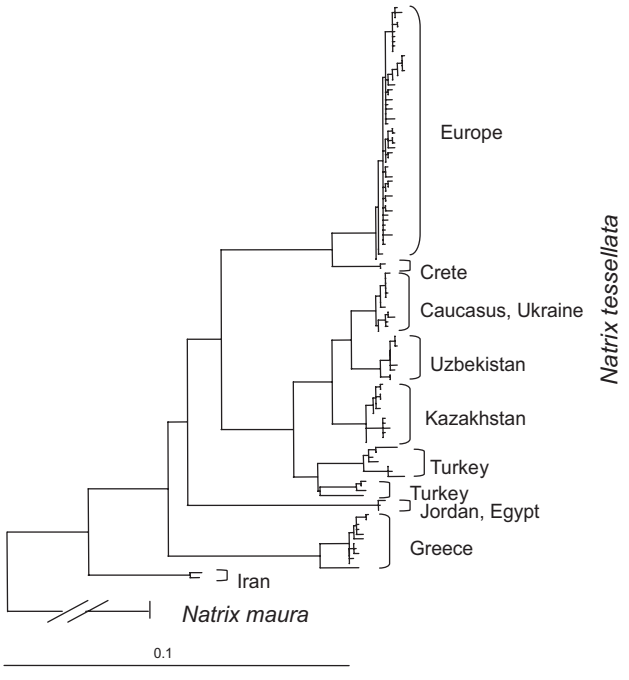

Fig. 17. Maximum Likelihood phylogram for *Natrix tessellata*, based on complete cytochrome *b* sequences and routed with *N. maura*. Distinct haplotype groups indicated (modified from Joger et al. 2006).

clades in *N. tessellata* show evidence of gene flow between geographically neighbouring haplotype groups, when the nuclear ISSR tree is considered, and nuclear and mitochondrial trees are discordant.

4.2. The time scale of evolution

The average evolutionary rate for the mitochondrial cytochrome *b* gene of true vipers (Viperidae) has been estimated to 1.4% sequence difference per 1 million year (Ursenbacher et al. 2006a). For *Natrix*, we calculated a similar rate of 1–1.35% per 1 million year (Guicking et al. 2006b). For *L. agilis*, we estimated a higher rate of up to 2.5% per 1 million year (Kalyabina et al. 2001), but for *Emys*, a distinctly lower level of 0.3–0.4% per 1 million year was suggested (Lenk et al. 1999). These differences are likely to reflect different metabolic rates, being lowest in turtles and highest in lizards; life expectancy is reciprocally correlated.

If these estimates are correct, minimum sequence differences of 1% in turtles, 3% in snakes, and 6% in lizards are due to pre-Pleistocene splits and, hence, cannot be associated with Ice Age refugia. All species groups investigated here originated, accordingly, in the Tertiary and speciation events date back to the Pliocene

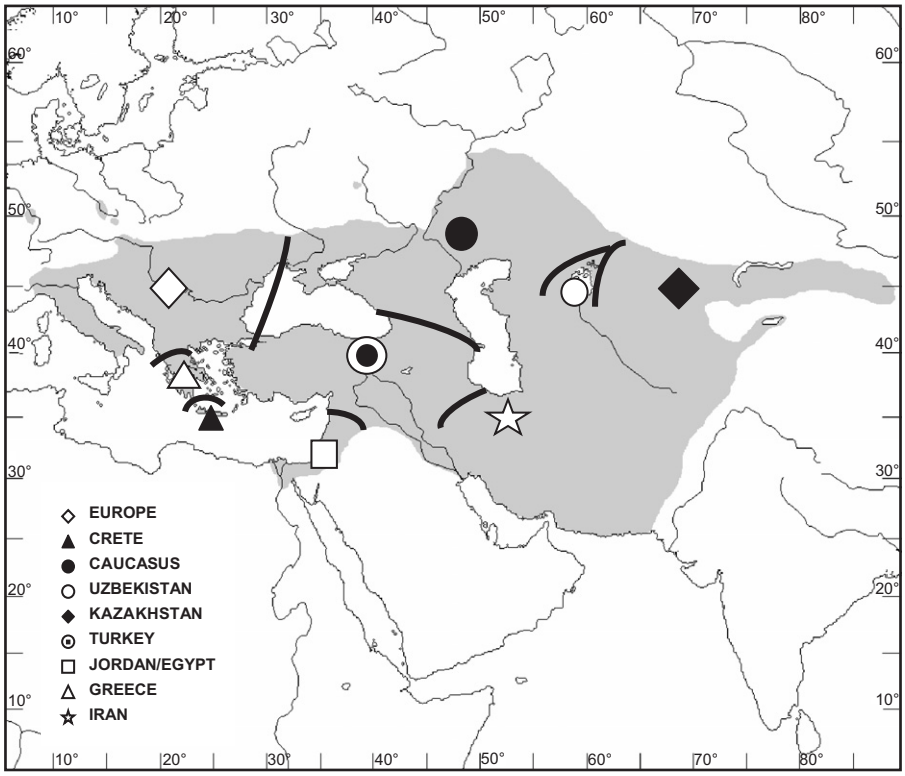

Fig. 18. Distribution of haplotype groups in *Natrix tessellata* (modified from Guicking 2004).

(Table 2). This is also supported by the fossil record, as Pliocene fossils of snakes and turtles resemble recent species so much that they are thought to be conspecific (Szyndlar and Böhme 1993; Fritz 1995). In the genus *Natrix*, the separation between the recent three species was even dated to early Miocene (Guicking et al. 2006b). It is possible that the oldest clades of *N. tessellata*, from Iran and Arabia, and perhaps also the Greek clade – all of which are of late Miocene age – represent distinct species. In *N. maura*, the three main clades are also dated to the late Miocene, the southern Spanish clade to the Pliocene, and the split between the lineages of the main European clade is thought to be of Pleistocene age. On the other hand, as snake species in Table 2 appear generally older than lizard species, the time calibration may be debated. Nagy et al. (2003) estimated the evolutionary rate of mitochondrial genes for colubrid snakes as half of the rate assumed here, hence, being of the same magnitude as in *Lacerta*. If we accept this alternative, *Hierophis* and *Zamenis* would show a similar temporal branching pattern for their sister species like *Lacerta* (Pliocene), and only speciation of *Natrix* would still be of Miocene age. Remarkably, a mid-Miocene fossil of *Natrix* was considered as direct ancestor of the extant *N. natrix*, suggesting that the three clades in *Natrix* might have already been separated in the middle Miocene (Ivanov 2001).

Apart from *Natrix* and *Emys*, most of the major intraspecific clades are of late Pliocene or Pleistocene age. As pronounced climatic oscillations started already in late Pliocene, it seems plausible to consider this intraspecific diversification as the result of climate change and associated range restrictions.

4.3. Pleistocene refugia and re-colonization

Table 3 summarizes putative Pleistocene refugia. The classical Mediterranean refugia (Iberia, Italy and the Balkans) were used by all species groups, but in many cases more than one microrefugium was located on each peninsula. Remarkably, North African and Asian refuges do not play any role for Holocene recolonization of northern and Central Europe, but have acted as independent speciation centres.

Among the studied species, the two most cold-tolerant (*Vipera berus* and *Lacerta agilis*) probably survived in non-Mediterranean refuges, like central France and the Carpathian Basin. A glacial refuge in France was also inferred for two species related to the aforementioned, *Vipera aspis* (Ursenbacher et al. 2006b) and *Lacerta* (*Zootoca*) *vivipara* (Guillaume et al. 2000). Likewise, a glacial refuge in the Carpathian Basin was suggested for some other species of considerable cold-tolerance (*Rana*

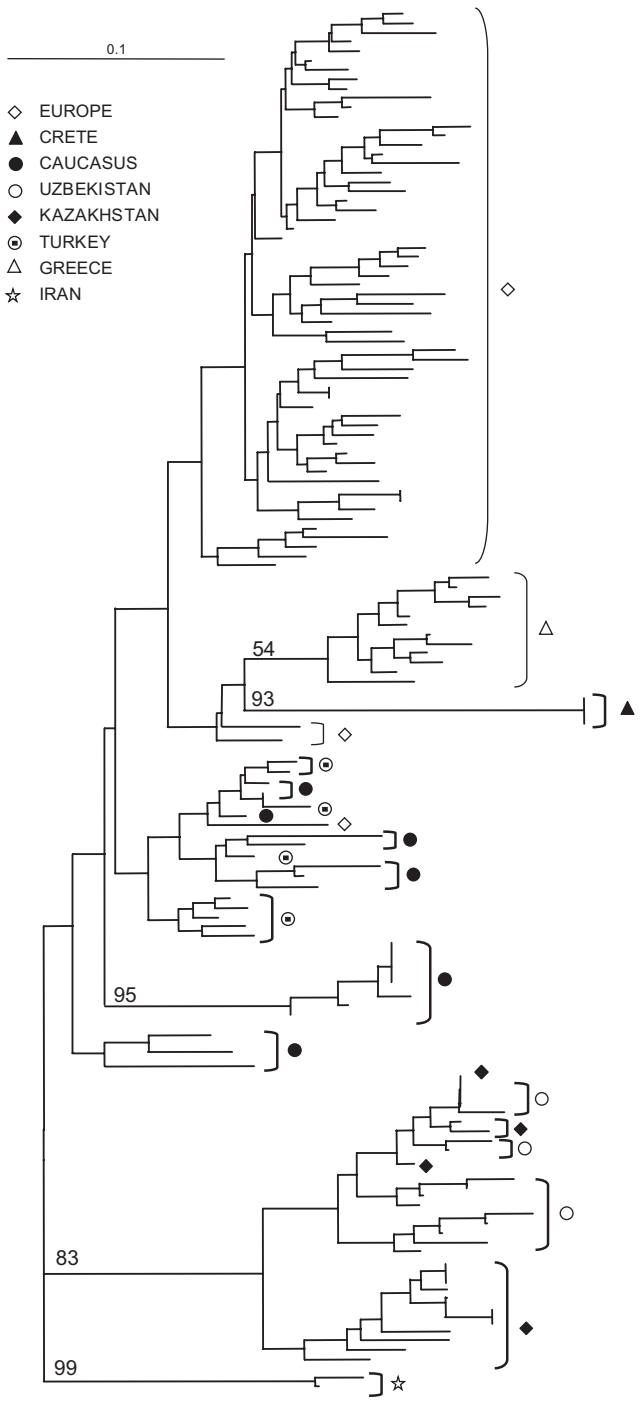

Fig. 19. Neighbor-joining tree of *Natrix tessellata* populations, calculated from ISSR banding patterns. Symbols denote cytochrome *b* haplotype groups (as in Figs. 17 and 18), numbers are bootstrap values greater than 50. As ISSR data reflect recombinant alleles, mixed groups indicate gene flow between haplotypes (modified from Joger et al. 2006).

arvalis: Babik et al. 2004; *Capreolus capreolus*, *Cervus elaphus*, *Sus scrofa*, *Vulpes vulpes*: Sommer and Nadachowski 2006; *Microtus agrestis*: Jaarola and Searle 2002).

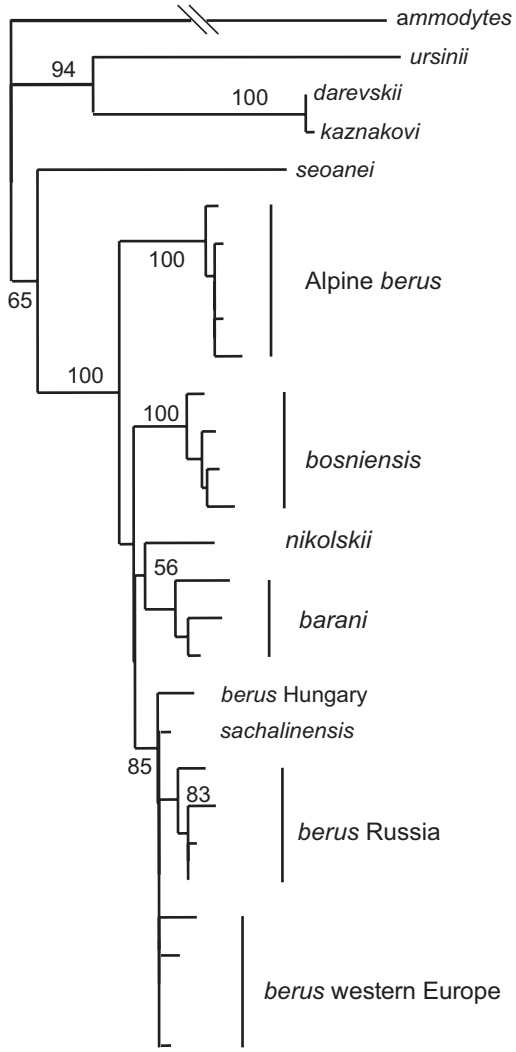

Fig. 20. Neighbor-joining tree of *Vipera berus* and relatives derived from cytochrome *b* haplotypes, rooted with *V. ammodytes*. Bootstrap values greater than 50 indicated. For localities see Kalyabina-Hauf et al. (2004b).

In genera surviving the last glacial in refugia located in all three southern European peninsulas (*Emys*, *Natrix*), the eastern refugia acted as radiation centres for the colonization of northern regions such as Germany. The reasons may be both geographical and ecophysiological. The lack of west–east-oriented mountain barriers allowed a quick northward dispersal once the climatic situation became favourable. On the other hand, in these eastern refugia a continental climate prevailed, with more severe winters than in western refugia. Populations adapted to such climate could start their northward expansion earlier than their warm-adapted counterparts from the Iberian and Italian Peninsulas. In *E. orbicularis*, lineage II even spread from the Balkan refuge to north-eastern Spain after crossing Central Europe north of the Alps. By contrast, the western Mediterranean lineages remained more or

Table 2. Speciation, sequence differences and approximate time estimates

Group	Percentage of sequence difference between sister species (cyt. *b*)	Time estimate for species split (myr)	Time estimate for split between major groups within species (myr)	Reference
Emys	1.0–1.7[a]	2.9–4.9	1.4–5.2	Fritz et al. (2005)
Lacerta agilis complex	6.5–7.3[b]	2.6–2.9	0.7–2.4	Kalyabina et al. (2001)
Lacerta viridis complex	6.6–8.4[c]	2.6–3.4	0.5–2.5	Böhme et al. (2007)
Hierophis	10.5[d]	8–9	0.9–2.7	Nagy et al. (2002, 2004)
Zamenis (*Elaphe*)	7[e]	5–7	0.5–1	Lenk et al. (2001)
Natrix	18[f]	13–22	2–8	Guicking et al. (2006b)
Vipera berus group	5[g]	4	1.1–1.6	Kalyabina-Hauf et al. (2004b)

[a]Distance between *E. orbicularis* and *E. trinacris*.
[b]Distance between *L. agilis* ssp. and *L.* (*a.*) *boemica* (assuming species status).
[c]Distance between *L. viridis* and *L. bilineata*.
[d]Distance between *H. viridiflavus* and *H. gemonensis*.
[e]Distance between *Z. longissimus* and *Z. lineatus*.
[f]Distance between *N. natrix* and *N. tessellata*.
[g]Distance between *V. berus* and *V. seoanei*.

Table 3. Pleistocene refugia inferred from cytochrome *b* haplotype partitions

Group	Iberian	Apennine/Sicilian	Balkans	Pontic	Caspian	Other
Emys orbicularis complex	1	3	Several	Several	1	North Africa, Turkey
Lacerta agilis complex	1	–	2	1	Several	Carpathian Basin
Lacerta viridis complex	–	2	Several	1	–	
Hierophis viridiflavus	–	3	–	–	–	
Zamenis longissimus complex	–	2	2	?	?	
Natrix maura/*N. tessellata*	Several	?	2	Several	Several	North Africa, West Asia
Vipera berus group	1 (*V. seoanei*)	1	1	2	–	Carpathian Basin, France

less confined to the southern peninsulas. This is paralleled by the eastern shrew *Sorex araneus*, which reached north-eastern Spain post-glacially, but had there to retreat into mountain refuges while the warmer climate favoured the western species *S. coronatus* (López-Fuster and Ventura 1996).

4.4. Migration routes and barriers

In half of the studied groups (*Emys*, *Lacerta agilis* complex, *Zamenis*, *Vipera berus* complex) only immigration from the east to Central Europe occurred. This is to be explained by the barrier status of the Pyrenees and Alps, blocking northwards directed range expansions from the Iberian and Italian Peninsulas. Sudhaus et al. (1997) emphasized particularly the barrier status of the Alps, while they considered the Iberian Peninsula important for the Holocene recolonization of Central Europe (examples: the shrew *Sorex coronatus*, the hedgehog *Erinaceus europaeus*, several lizards [e.g., *Timon lepidus*] and the frog *Pelobates cultripes*). However, according to genetic data, just the hedgehog surmounted the Alps and colonized Central Europe and southern Scandinavia from a refuge on the Italian Peninsula (Hewitt 1999). It must be noted, moreover, that only the mammals mentioned in Sudhaus et al. (1997) reached Central Europe from the south while the ectothermic species are restricted to southern France and Italy (resembling the distributional situation of the sole species in our data set with Iberian origin, *Natrix maura*).

Internal mountain barriers in the Iberian, Apennine and Balkan Peninsulas complicate the picture, leading to haplotype diversification within the respective peninsula (north–south by Iberian mountain ridges, west–east by the Apennines, both by the Balkan Mts). Iberian relationships with North Africa (*Emys*, *N. maura*) tell us that the barrier function of the Strait of Gibraltar is present, but not to the same degree for different species (see also Busack 1986; Fritz et al. 2006a). Much less pronounced is the barrier function of the Bosporus, which lost its land connection only 10,000 years ago. Certain haplotypes of *Emys orbicularis*, *N. tessellata* and

L. viridis occur on both sides of the Bosporus, while others are restricted to more isolated areas of Anatolia.

The Balkans, particularly the Danube valley, served as pathway to penetrate Central Europe from the Black Sea Region. It is obvious that many of the species groups treated here, in particular *N. tessellata* (European group), *Z. longissimus* (northern group), and *E. orbicularis* (lineages I and II), reached Central Europe from the southeast. This is paralleled by the sister species fire-bellied toad (*Bombina bombina*) and yellow-bellied toad (*B. variegata*), appearing as typical example of an east–west disjunction when only the Central European distribution is concerned. However *B. variegata*, the western species, also occurs in Greece and Hungary, and the most likely explanation for this distribution type is to assume that both species, adapted to different environments (plains versus mountains/hills), invaded Central Europe from the Balkans (Szymura 1993).

In post-glacial times, the vast plains of the northern Black Sea Region and Central Asia, devoid of any mountain barriers, were apparently invaded rapidly from the west by *L. agilis*, *N. tessellata* and *V. berus*, as evidenced by rather homogeneous populations.

4.5. West–east species pairs: How many ways to conquer Central Europe?

The classical concept of western and eastern European sister species which, after retraction to different glacial refugia, expand and meet in Central Europe (e.g., Sudhaus et al. 1997), is not fully applicable to most of the treated reptile groups. At the first glance, the *Lacerta viridis* complex seems to match this paradigm; *L. viridis* being the eastern, *L. bilineata* the western species, with contact zones in north-eastern Italy and Slovenia. However, the discovery of a third important haplotype group in the Adriatic Region (Böhme et al. 2007) complicates the picture. This three-species pattern is paralleled in spiders of the genus *Atypus* (Kraus and Baur 1974), with the main difference that the Adriatic spider species, *A. piceus*, managed to cross the Alps, while the lizards did not. A similar differentiation pattern with respect to the Balkans also occurs in the pond turtle (Fritz et al. 2007): In all of these taxa, the Dinarid and Pindos Mts serve as divide between a western Balkan, basically Mediterranean, lineage and an eastern Balkan lineage that colonized in the cases of the turtle and the lizard Central Europe, while their Adriatic counterpart remained confined to the Mediterranean Region.

Another example for a three-species pattern is represented by the snake genus *Natrix* – *N. tessellata* being the eastern, *N. maura* the western, and *N. natrix* the "central" species. Yet in this case, *N. natrix* is distributed over most of the combined ranges of the two other species, and in this vast region, *N. natrix* is differentiated, too, into three main clades: an Iberian, a west-central European and an eastern clade (Guicking et al. 2006b). Disregarding the Iberian clade, the two others, *N. n. natrix* and *N. n. helvetica*, display the classical east–west disjunction (though the pattern is more complicated in eastern Europe). *Hierophis viridiflavus* also shows an east–west disjunction at a smaller scale – caused by separate glacial refugia in Italy east and west of the Apennines. Only the western clade expanded its range to the north to Central Europe (Nagy et al. 2002), but this was true for the eastern clade in the Plio-Pleistocene, too (Ivanov 1997).

Acknowledgments

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References

Amann, T., Rykena, S., Joger, U., Nettmann, H.K., Veith, M., 1997. Zur artlichen Trennung von *Lacerta bilineata* Daudin, 1802, und *L. viridis* (Laurenti, 1768). Salamandra 33, 255–268.

Amann, T., Razzetti, E., Joger, U., 2001. La zona di contatto tra *Lacerta bilineata* (Daudin, 1802) e *Lacerta viridis* (Laurenti, 1768) in Italia. Atti della 3° Congresso Nazionale, Societas Herpetologica Italica. Pianura 13, 261–264.

Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Avise, J.C., 2004. Molecular Markers, Natural History and Evolution, second ed. Sinauer Associates, Sunderland, MA.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489–522.

Babik, W., Branicki, W., Sandera, M., Litvinchuk, S., Borkin, L.J., Irwin, J.T., Rafiński, J., 2004. Mitochondrial phylogeography of the moor frog, *Rana arvalis*. Mol. Ecol. 13, 1469–1480.

Barton, N.H., Hewitt, G.M., 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16, 113–148.

Bischoff, W., 1988. Zur Verbreitung und Systematik der Zauneidechse, *Lacerta agilis* Linnaeus 1758. Mertensiella 1, 11–30.

Böhme, M.U., Fritz, U., Kotenko, T., Džukić, G., Ljubisavljević, K., Tzankov, N., Berendonk, T.U., 2007. Phylogeography and cryptic variation within the *Lacerta viridis* complex. Zool. Scr. 36, 119–131.

Brückner, M., Klein, B., Düring, A., Mentel, T., Rabus, S., Soller, J.T., 2001. Phylogeographic analysis of the *Lacerta viridis/bilineata* complex: Molecular patterns and distribution. Mertensiella 13, 45–51.

Busack, S.D., 1986. Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. Nat. Geogr. Res. 2, 17–36.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.

de Lattin, G., 1967. Grundriß der Zoogeographie. Gustav Fischer Verlag, Stuttgart.

Fritz, U., 1995. Kritische Übersicht der Fossilgeschichte der Sumpfschildkröten-Gattung *Emys* A. Duméril, 1806. Zool. Abh. Dresden 48, 243–264.

Fritz, U., 1996. Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 5b. Innerartliche Hierarchie und Zoogeographie. Zool. Abh. Dresden 49, 31–71.

Fritz, U., Guicking, D., Lenk, P., Joger, U., Wink, M., 2004. When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain. Biologia 59 (Suppl. 14), 19–25.

Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M.G., Lenk, P., Joger, U., Wink, M., 2005. A new cryptic species of pond turtle from South Italy, the hottest spot in the range of the genus *Emys* (Reptilia: Testudines: Emydidae). Zool. Scr. 34, 1–21.

Fritz, U., Barata, M., Busack, S.D., Fritzsch, G., Castilho, R., 2006a. Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geoemydidae). Zool. Scr. 35, 97–108.

Fritz, U., d'Angelo, S., Pennisi, M.G., LoValvo, M., 2006b. Variation of Sicilian pond turtles, *Emys trinacris* – what makes a species cryptic? Amphibia-Reptilia 27, 513–529.

Fritz, U., Guicking, D., Kami, H., Arakelyan, M., Auer, M., Ayaz, D., Ayres Fernández, D., Bakiev, A.G., Celani, A., Džukić, G., Fahd, S., Havaš, P., Joger, U., Khabibullin, V.F., Mazanaeva, L.F., Široký, P., Tripepi, S., Valdeón Vélez, A., Velo Antón, G., Wink, M., 2007. Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*) – an update. Amphibia-Reptilia 28, 418–426.

Godinho, R., Crespo, E.G., Ferrand, N., Harris, D.J., 2005. Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphibia-Reptilia 26, 271–285.

Guicking, D., 2004. Phylogeography of the Dice Snake (*Natrix tessellata*) and the Viperine Snake (*Natrix maura*). Dissertation, University of Heidelberg.

Guicking, D., Griffiths, R.A., Moore, R.D., Joger, U., Wink, M., 2006a. Introduced alien or persecuted native? Resolving the origin of the viperine snake (*Natrix maura*) on the island of Mallorca. Biodiv. Conserv. 15, 3045–3054.

Guicking, D., Lawson, R., Joger, U., Wink, M., 2006b. Evolution and phylogeny of the genus *Natrix* (Serpentes: Colubridae). Biol. J. Linn. Soc. 87, 127–143.

Guicking, D., Joger, U., Wink, M., in press. Mitochondrial and nuclear phylogeography of the viperine snake (*Natrix maura*): evidence for strong intraspecific differentiation. Organisms, Diversity & Evolution.

Guillaume, C.O., Heulin, B., Attayago, M.J., Bea, A., Brana, F., 2000. Refuge areas and suture zones in the Pyrenean and Cantabrian regions: geographic variation of the female MPI sex-linked alleles among oviparous populations of the lizard *Lacerta* (*Zootoca*) *vivipara*. Ecography 23, 3–10.

Habel, J.C., Schmitt, T., Müller, P., 2005. The fourth paradigm pattern of post-glacial range expansions of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera). J. Biogeogr. 32, 1489–1497.

Habel, J.C., Meyer, M., El Mousadik, A., Schmitt, T., in press. Africa goes Europe: the complete phylogeography of the Marbled White butterfly species complex. Organisms, Diversity & Evolution.

Hewitt, G.M., 1999. Post-glacial recolonization of European biota. Biol. J. Linn. Soc. 68, 87–112.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Ivanov, M., 1997. Vývoj kenozické hadí fauny evropy [The evolution of European Cenozoic snake fauna]. In: Hladilová, S. (Ed.), Dynamics of Interaction between Marine and Continental Environments. Masaryk University, Brno, pp. 59–91.

Ivanov, M., 2001. Changes in the composition of the European snake fauna during the early Miocene and at the Early/Middle Miocene transition. Paläontol. Zeitschr. 74, 563–573.

Jaarola, M., Searle, J.B., 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. Mol. Ecol. 11, 2613–2621.

Joger, U., Steinfartz, S., 1994. Electrophoretic investigations in the evolutionary history of the West Mediterranean *Salamandra*. Mertensiella 4, 241–254.

Joger, U., Amann, T., Lenk, P., Willand, U., 1998. Molekulare Merkmale und das phylogenetische Artkonzept. Zool. Abh. Dresden 50 (Suppl. "100 Jahre Artkonzepte in der Zoologie"), 109–123.

Joger, U., Amann, T., Veith, M., 2001. Phylogeographie und genetische Differenzierung im *Lacerta viridis-bilineata*-Komplex. Mertensiella 13, 60–68.

Joger, U., Trutnau, L., Böhme, W., 2004. *Vipera aspis* (Linnaeus, 1758) – Aspisviper. In: Joger, U., Stümpel, N. (Eds.), Handbuch der Amphibien und Reptilien Europas, 3/IIB, Schlangen (Serpentes) III. Viperidae. Aula, Wiesbaden, pp. 151–185.

Joger, U., Guicking, D., Kalyabina-Hauf, S., Lenk, P., Nagy, Z.T., Wink, M., 2006. Phylogeographie, Artbildung und postpleistozäne Einwanderung mitteleuropäischer Reptilien. Zeitschr. Feldherpetol. (Suppl. 10), 29–59.

Kalyabina, S.A., Milto, K.D., Ananjeva, N.B., Legal, L., Joger, U., Wink, M., 2001. Phylogeography and systematics of *Lacerta agilis* based on mitochondrial cytochrome *b* gene sequences: first results. Russ. J. Herpetol. 8, 149–159.

Kalyabina-Hauf, S.A., Milto, K.D., Ananjeva, N.B., Joger, U., Kotenko, T.I., Wink, M., 2004a. Reevaluation of the status of *Lacerta agilis tauridica* Suchov, 1926. Russ. J. Herpetol. 11, 65–72.

Kalyabina-Hauf, S., Schweiger, S., Joger, U., Mayer, W., Wink, M., 2004b. Phylogeny and systematics of adders (*Vipera berus* complex). Mertensiella 15, 7–16.

Kraus, O., Baur, H., 1974. Die Atypidae der West-Paläarktis. Systematik, Verbreitung und Biologie (Arach.: Araneae). Abh. Verh. Naturwiss. Ver. Hamburg (NF) 17, 85–116.

Lenk, P., 1997. Molekularbiologische Untersuchungen zur Mikroevolution der Europäischen Sumpfschildkröte *Emys orbicularis* (Linnaeus, 1758). Diploma Thesis, Technical University of Darmstadt.

Lenk, P., Joger, U., 1994. Genetic relationships between populations and intraspecific subdivision of *Elaphe longissima* (Laurenti, 1768). Amphibia-Reptilia 15, 363–373.

Lenk, P., Wüster, W., 1991. A multivariate approach to the systematics of Italian ratsnakes of the *Elaphe longissima* complex (Reptilia: Colubridae): revalidation of Camerano's *Callopeltis longissimus* var. *lineata*. Herpetol. J. 9, 153–162.

Lenk, P., Fritz, U., Joger, U., Wink, M., 1999. Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). Mol. Ecol. 8, 1911–1912.

Lenk, P., Joger, P., Wink, M., 2001. Phylogenetic relationships among European ratsnakes of the genus *Elaphe* Fitzinger based on mitochondrial DNA sequence comparisons. Amphibia-Reptilia 22, 329–339.

López-Fuster, M.J., Ventura, J., 1996. A morphometrical review of the *Sorex araneus-arcticus* species group from the Iberian Peninsula (Insectivora, Soricidae). Bonn. Zool. Beitr. 46, 327–337.

Mayer, W., Beyerlein, P., 2001. Genetische Differenzierung von *Lacerta viridis*/*bilineata* und *L. trilineata* in Griechenland. Mertensiella 13, 52–59.

Murphy, R.W., Sites, J.W., Buth, D.G., Haufler, C.H., 1991. Proteins I: isoenzyme electrophoresis. In: Hillis, D.M., Moritz, C. (Eds.), Molecular Systematics. Sinauer Associates, Sunderland, MA, pp. 45–126.

Nagy, Z.T., 2004. Zur molekularen Systematik und Phylogeographie altweltlicher Nattern (Squamata: Serpentes: Colubridae sensu lato). Dissertation, University of Heidelberg.

Nagy, Z.T., Joger, U., Guicking, D., Amann, T., Wink, M., 2002. Phylogeography of the European whip snake, *Coluber (Hierophis) viridiflavus* Lacépède, 1789, inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene and ISSR genomic fingerprinting. Biota 3, 109–118.

Nagy, Z.T., Joger, U., Wink, M., Glaw, F., Vences, M., 2003. Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies. Proc. R. Soc. London B 270, 2613–2621.

Nagy, Z.T., Lawson, R., Joger, U., Wink, M., 2004. Molecular systematics of racers, whipsnakes and relatives (Reptilia: Colubridae) using mitochondrial and nuclear markers. J. Zool. Syst. Evol. Res. 42, 223–233.

Rykena, S., 1991. Kreuzungsexperimente zur Prüfung der Artgrenzen im Genus *Lacerta* sensu stricto. Mitt. Zool. Mus. Berlin 67, 55–68.

Rykena, S., 1996. Experimental interspecific hybridization in the genus *Lacerta*. Isr. J. Zool. 42, 171–184.

Rykena, S., 2001. Experimental hybridization in green lizards (*Lacerta* s. str.), a tool to study species boundaries. Mertensiella 13, 78–88.

Santucci, F., Emerson, B.C., Hewitt, G.M., 1998. Mitochondrial DNA phylogeography of European hedgehogs. Mol. Ecol. 7, 1163–1172.

Scali, S, Gentilli, A., 1999. Morphometric analysis and sexual dimorphism of extinct adders (*Vipera berus*) of the Po plane (Northern Italy). In: Miaud, C., Guyetant, R., (Eds.), Current Studies in Herpetology. Proceedings of the Ninth Ordinary General Meeting of the SEH. Societas Herpetologica Europaea, Le Bourget du Lac, pp. 391–396.

Schätti, B., Vanni, S., 1986. Intraspecific variation in *Coluber viridiflavus* Lacépède, 1789, and validity of its subspecies (Reptilia, Serpentes, Colubridae). Rev. Suisse Zool. 93, 219–232.

Seddon, J.M., Santucci, F., Reeve, N.J., Hewitt, G.M., 2001. DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonisation routes. Mol. Ecol. 10, 2187–2198.

Seddon, J.M., Santucci, F., Reeve, N.J., Hewitt, G.M., 2002. Caucasus Mountains divide postulated postglacial colonization routes in the white-breasted hedgehog, *Erinaceus concolor*. J. Evol. Biol. 15, 463–467.

Sommer, R.S., Nadachowski, A., 2006. Glacial refugia of mammals in Europe: evidence from fossil records. Mammal Rev. 36, 251–265.

Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebayeva, T., Eremchenko, V., Macey, J.R., Papenfuss, T.J., Wake, D.B., 2006. Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Mol. Phylogenet. Evol. 41, 663–689.

Sudhaus, W., Kiontke, K., Fürst von Lieven, A., Manegold, A., Seitz, V., 1997. Speziation in Mitteleuropa im Gefolge der Eiszeiten. Sitzungsber. Ges. Naturforsch. Freunde Berlin (NF) 36, 143–175.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.

Szymura, J.M., 1993. Analysis of hybrid zones within *Bombina*. In: Harrison, R.G. (Ed.), Hybrid Zones and the Evolutionary Process. Oxford University Press, New York, Oxford, pp. 261–289.

Szyndlar, Z., Böhme, W., 1993. Die fossilen Schlangen Deutschlands: Geschichte der Faunen und ihrer Erforschung. Mertensiella 3, 381–431.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G., Cosson, J.-F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7, 453–464.

Ursenbacher, S., Carlsson, M., Helfer, V., Tegelström, H., Fumagalli, L., 2006a. Phylogeography and Pleistocene refugia of the adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data. Mol. Ecol. 15, 3425–3437.

Ursenbacher, S., Conelli, A., Golay, P., Monney, J.-C., Zuffi, M.A.L., Thiery, G., Durand, T., Fumagalli, L., 2006b. Phylogeography of the asp viper (*Vipera aspis*) inferred from mitochondrial DNA sequence data: evidence for multiple Mediterranean refugial areas. Mol. Phylogenet. Evol. 38, 546–552.

Wink, M., Sauer-Gürth, H., Martinez, F., Doval, G., Blanco, G., Hatzofe, O., 1998. The use of $(GACA)_4$-PCR to sex Old World vultures (Aves: Accipitridae). Mol. Ecol. 7, 779–782.

Wink, M., Guicking, D., Fritz, U., 2001. Molecular evidence for hybrid origin of *Mauremys iversoni* Pritchard et McCord, 1991, and *Mauremys pritchardi* McCord, 1997 (Reptilia: Testudines: Bataguridae). Zool. Abh. Dresden 51, 41–50.

Zuffi, M.A.L., 2002. A critique of the systematic position of asp viper subspecies *Vipera aspis aspis* (Linnaeus, 1758), *Vipera aspis atra* Meisner, 1820, *Vipera aspis hugyi* Schinz, 1833 and *Vipera aspis zinnikeri* Kramer, 1958. Amphibia-Reptilia 24, 238–241.