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Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix, N. megalocephala*)

Carolin Kindler, Wolfgang Böhme, Claudia Corti, Václav Gvoždík, Daniel Jablonski, David Jandzik, Margarita Metallinou, Pavel Široký & Uwe Fritz

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Grass snakes (*Natrix natrix*) represent one of the most widely distributed snake species of the Palaearctic region, ranging from the North African Maghreb region and the Iberian Peninsula through most of Europe and western Asia eastward to the region of Lake Baikal in Central Asia. Within *N. natrix*, up to 14 distinct subspecies are regarded as valid. In addition, some authors recognize big-headed grass snakes from western Transcaucasia as a distinct species, *N. megalocephala*. Based on phylogenetic analyses of a 1984-bp-long alignment of mtDNA sequences (ND4+tRNAs, cyt *b*) of 410 grass snakes, a nearly range-wide phylogeography is presented for both species. Within *N. natrix*, 16 terminal mitochondrial clades were identified, most of which conflict with morphologically defined subspecies. These 16 clades correspond to three more inclusive clades from (i) the Iberian Peninsula plus North Africa, (ii) East Europe and Asia and (iii) West Europe including Corso-Sardinia, the Apennine Peninsula and Sicily. Hypotheses regarding glacial refugia and postglacial range expansions are presented. Refugia were most likely located in each of the southern European peninsulas, Corso-Sardinia, North Africa, Anatolia and the neighbouring Near and Middle East, where the greatest extant genetic diversity occurs. Multiple distinct microrefugia are inferred for continental Italy plus Sicily, the Balkan Peninsula, Anatolia and the Near and Middle East. Holocene range expansions led to the colonization of more northerly regions and the formation of secondary contact zones. Western Europe was invaded from a refuge within southern France, while Central Europe was reached by two distinct range expansions from the Balkan Peninsula. In Central Europe, there are two contact zones of three distinct mitochondrial clades, and one of these contact zones was theretofore completely unknown. Another contact zone is hypothesized for Eastern Europe, which was colonized, like northwestern Asia, from the Caucasus region. Further contact zones were identified for southern Italy, the Balkans and Transcaucasia. In agreement with previous studies using morphological characters and allozymes, there is no evidence for the distinctiveness of *N. megalocephala*. Therefore, *N. megalocephala* is synonymized with *N. natrix*.

Corresponding author: *Uwe Fritz, Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, A. B. Meyer Building, D-01109 Dresden, Germany. E-mail: uwe.fritz@senckenberg.de*
Carolin Kindler, Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, A. B. Meyer Building, D-01109 Dresden, Germany. E-mail: carolin.kindler@senckenberg.de
Wolfgang Böhme, Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany. E-mail: w.boehme.zfmk@uni-bonn.de
Claudia Corti, Sezione di Zoologia "La Specola", Museo di Storia Naturale dell'Università di Firenze, Via Romana, 17, I-50125 Firenze, Italy. E-mail: claudia.corti@unifi.it
Václav Gvoždík, Department of Zoology, National Museum, Cirkusová 1740, CZ-193 00 Prague, Czech Republic and Laboratory of Molecular Ecology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, CZ-277 21 Liběchov, Czech Republic. E-mail: vgvozdik@email.cz
Daniel Jablonski, Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina B-1, SK-842 15 Bratislava, Slovakia. E-mail: daniel.jablonski@balcanica.cz

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David Jandzik, Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina B-1, SK-842 15 Bratislava, Slovakia and Department of Ecology and Evolutionary Biology (EBIO), University of Colorado, Ramaley N122, Boulder, CO, 80309-0334, USA. E-mail: jandzik@fns.uniba.sk
Margarita Metallinou, Animal Phylogeny and Systematics, Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta, 37-49, E-08003 Barcelona, Spain. E-mail: margarita.metallinou@ibe.upf-csic.es
Pavel Široký, Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, CZ-612 42 Brno, Czech Republic. E-mail: sirokyp@vfu.cz
Uwe Fritz, Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, A. B. Meyer Building, D-01109 Dresden, Germany. E-mail: uwe.fritz@senckenberg.de

Introduction

While the knowledge of the phylogeography of many European taxa has made great progress over the past 20 years (Hewitt 1996, 2000, 2011; Taberlet *et al.* 1998; Joger *et al.* 2007; Schmitt 2007; Lymberakis & Poulakakis 2010; Schmitt & Varga 2012), there still remain some wide-ranging iconic taxa understudied. One of these species is the grass snake (*Natrix natrix*). It is one of the most widely distributed snake species of the Palaearctic region, ranging from the North African Maghreb region and the Iberian Peninsula through most of Europe and western Asia eastward to the region of Lake Baikal in Central Asia (Bannikov *et al.* 1977; Kabisch 1999). Grass snakes are more or less associated with aquatic habitats, but they are not true water snakes like the related species *N. maura* and *N. tessellata*. Grass snakes usually reach a total length of up to 120 cm, with maximum sizes of some 200 cm, and feed on amphibians, fish and small mammals or nestling birds (Bannikov *et al.* 1977; Engelmann *et al.* 1986; Gruber 1989; Kabisch 1999; Arnold & Ovenden 2002; Kreiner 2007).

Traditionally, many morphologically defined grass snake subspecies have been distinguished, which differ mainly in body proportions, colouration and size (Hecht 1930; Mertens 1947, 1957, 1966; Mertens & Wermuth 1960; Kramer 1970; Bannikov *et al.* 1977). However, in a sweeping revision using multivariate statistics, Thorpe (1979) reduced the number of subspecies to four (Table 1). Within this four subspecies model, *N. n. natrix* is distributed in the east of the species' range, and *N. n. helvetica* occupies most of the western range, except Corsica and Sardinia. Thorpe (1979) confirmed the earlier view (Mertens & Wermuth 1960) that the two subspecies *natrix* and *helvetica* meet and hybridize in the Rhine region. In addition to these two subspecies, Thorpe (1979) recognized only the morphologically highly distinctive grass snakes from Corsica and Sardinia as further subspecies (*N. n. corsa*, *N. n. cetti*). Nevertheless, other authors continued to treat up to 14 subspecies as valid (Fig. 1; Table 1; Nilson & Andrén 1981; Engelmann *et al.* 1986; Gruber 1989; Kabisch 1999; Arnold & Ovenden 2002; Kreiner 2007; Baier *et al.* 2009). Furthermore, two additional species, *N. cetti* (Corsica, Sardinia) and *N. megalocephala* (western Transcaucasia), are sometimes recognized (Orlov & Tuniyev 1987, 1999; Vanni & Cimmaruta 2010). However, the validity of *N. megalocephala* has been repeatedly doubted (Hille 1997; Böhme 1999; Jandzík 2005; Frotzler *et al.* 2011; Göçmen *et al.* 2011), and the status of *N. c. cetti* and *N. c. corsa* as subspecies of *N. natrix* was recently reinstated (Fritz *et al.* 2012).

Until today, there are only few studies tackling genetic differentiation of grass snakes. In a pioneering study, Hille (1997) examined some grass snake subspecies and *N. megalocephala* using allozyme data. Although he confirmed an east–west differentiation within *N. natrix* and found no evidence for the validity of *N. megalocephala*, his results were largely inconclusive. Using mitochondrial DNA sequences of some 25 specimens, Guicking *et al.* (2006, 2008a) provided a first preliminary phylogeography and compared their findings with morphologically defined subspecies. Fritz *et al.* (2012) added to the data set of Guicking *et al.* (2006, 2008a) sequences of Corsican, Sardinian and continental Italian grass snakes and concluded that Corso-Sardinian snakes do not represent a distinct species owing to their close phylogenetic relationship with *N. n. helvetica*. Furthermore, in the light of the observed mtDNA variation, Guicking *et al.* (2008a) and Fritz *et al.* (2012) agreed that Thorpe's (1979) four subspecies model evidently underestimates genetic and taxonomic variation within *N. natrix*. However, obvious mismatches between preliminary mtDNA data and any subspecies delineation also underscored the need for further investigations using an expanded sampling to achieve a better understanding of the phylogeography and taxonomy of grass snakes (Guicking *et al.* 2008a; Fritz *et al.* 2012).

Table 1 Morphologically defined taxa (Natrix natrix subspecies, N. megalocephala) compared with mitochondrial clades

Thorpe (1979)	Kabisch (1999), Orlov & Tuniyev (1999)	Kreiner (2007)	Mitochondrial clades (this study)
Natrix natrix natrix	*Natrix natrix natrix*	*Natrix natrix natrix*	3, 4, 8
Natrix natrix natrix	*Natrix natrix persa*	*Natrix natrix cypriaca*	7
Natrix natrix natrix	*Natrix natrix fusca*	*Natrix natrix fusca*	?
Natrix natrix natrix	*Natrix natrix gotlandica*	*Natrix natrix gotlandica*	3, 4
Natrix natrix natrix	*Natrix natrix persa*	*Natrix natrix persa*	1, 2, 3, 4, 5, 7, 8
Natrix natrix natrix	*Natrix natrix schweizeri*	*Natrix natrix schweizeri*	3
Natrix natrix natrix	*Natrix natrix scutata*	*Natrix natrix scutata*	8
Natrix natrix natrix	*Natrix natrix syriaca*	*Natrix natrix syriaca*	6
Natrix natrix helvetica	*Natrix natrix astreptophora*	*Natrix natrix astreptophora*	Tu, Eu
Natrix natrix helvetica	*Natrix natrix helvetica*	*Natrix natrix helvetica*	4, C, E
Natrix natrix helvetica	*Natrix natrix lanzai*	*Natrix natrix lanzai*	D, F
Natrix natrix helvetica	*Natrix natrix sicula*	*Natrix natrix sicula*	A, F
Natrix natrix cetti	*Natrix natrix cetti*	*Natrix natrix cetti*	B
Natrix natrix corsa	*Natrix natrix corsa*	*Natrix natrix corsa*	B
—	*Natrix megalocephala*	—	8

Different names on the same line indicate synonymy of the respective taxa according to the different authors.

The present study aims to fulfil this task. Here we use a nearly range-wide sampling of 410 grass snakes to generate a comprehensive phylogeography based on two mitochondrial markers, the NADH dehydrogenase subunit 4 gene (ND4) and the cytochrome *b* gene (cyt *b*). Our samples represent all but one of the 14 nominal *N. natrix* subspecies recognized by some recent authors (Kabisch 1999; Arnold & Ovenden 2002; Kreiner 2007; Baier *et al.* 2009) and *N. megalocephala* (Table 1), and allow comparing mitochondrial differentiation and morphologically defined taxa. Only the subspecies *N. n. fusca*, endemic to the island of Kea, Cyclades, is missing in our sampling.

Materials and methods

Sampling, gene selection and laboratory procedures

Three hundred and eighty tissue, blood and saliva samples of grass snakes were studied. Besides fresh material, the samples included tissues from museum specimens that had been, as a rule, ethanol-preserved for no more than 30 years (Table S1). Two mitochondrial genes were sequenced that were previously successfully used for phylogeographic purposes in *Natrix natrix* and the allied species *N. maura* and *N. tessellata* (Guicking *et al.* 2006, 2008a, b, 2009; Fritz *et al.* 2012), viz. the partial ND4 gene and the cyt *b* gene. The DNA sequence containing the partial ND4 gene embraced also the flanking DNA coding for tRNA-His, tRNA-Ser and tRNA-Leu. The obtained sequences varied in length between 437 and 696 bp (ND4), between 0 and 117 bp (adjacent tRNAs), and between 311 and 1117 bp (cyt *b*). For some samples, sequences of only one of the two genes could be generated (Table S1), due to bad DNA quality or small quantity. Remaining samples and DNA are stored at −80 °C in the tissue collection of the Museum of Zoology, Senckenberg Dresden.

Total genomic DNA was extracted using either the DTAB method (Gustincich *et al.* 1991), the innuPREP DNA Mini Kit or the innuPREP Blood DNA Mini Kit (both Analytik Jena AG, Jena, Germany). DNA fragments were amplified using the primers given in Table S2. When the primers of Guicking *et al.* (2006) did not yield PCR products, newly designed primers were applied to amplify up to three shorter overlapping PCR products for the DNA fragment embracing ND4+tRNAs and up to four PCR products for cyt *b*. For primer combinations and PCR conditions, see Table S3.

PCR was carried out in a total volume of 25 μL containing 1 unit *Taq* polymerase (Bioron, Ludwigshafen, Germany), 1x buffer as recommended by the supplier, 0.4 μM of each primer (Biomers, Ulm, Germany) and 0.2 mM of each dNTP (Thermo-Scientific, St. Leon-Rot, Germany). Challenging samples were additionally treated with 10 μg BSA (Thermo-Scientific). PCR products were purified using the ExoSAP-IT enzymatic clean-up (USB Europe GmbH, Staufen, Germany; modified protocol: 30 min at 37 °C, 15 min at 80 °C) and sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the PCR primers and the BigDye Terminator version 3.1 Cycle Sequencing Kit (Life Technologies, Darmstadt, Germany). Cycle sequencing reactions were purified by ethanol/sodium acetate precipitation or using Sephadex (GE Healthcare, München, Germany).

Alignment, partitioning and phylogenetic analyses

All sequences were aligned and inspected using BIOEDIT 7.0.9.0 (Hall 1999) and MEGA 5.1 (Tamura *et al.* 2011). Thirty homologous GenBank sequences of known-locality grass snakes corresponding to the data set of Fritz *et al.* (2012) were aligned with our newly generated sequences,

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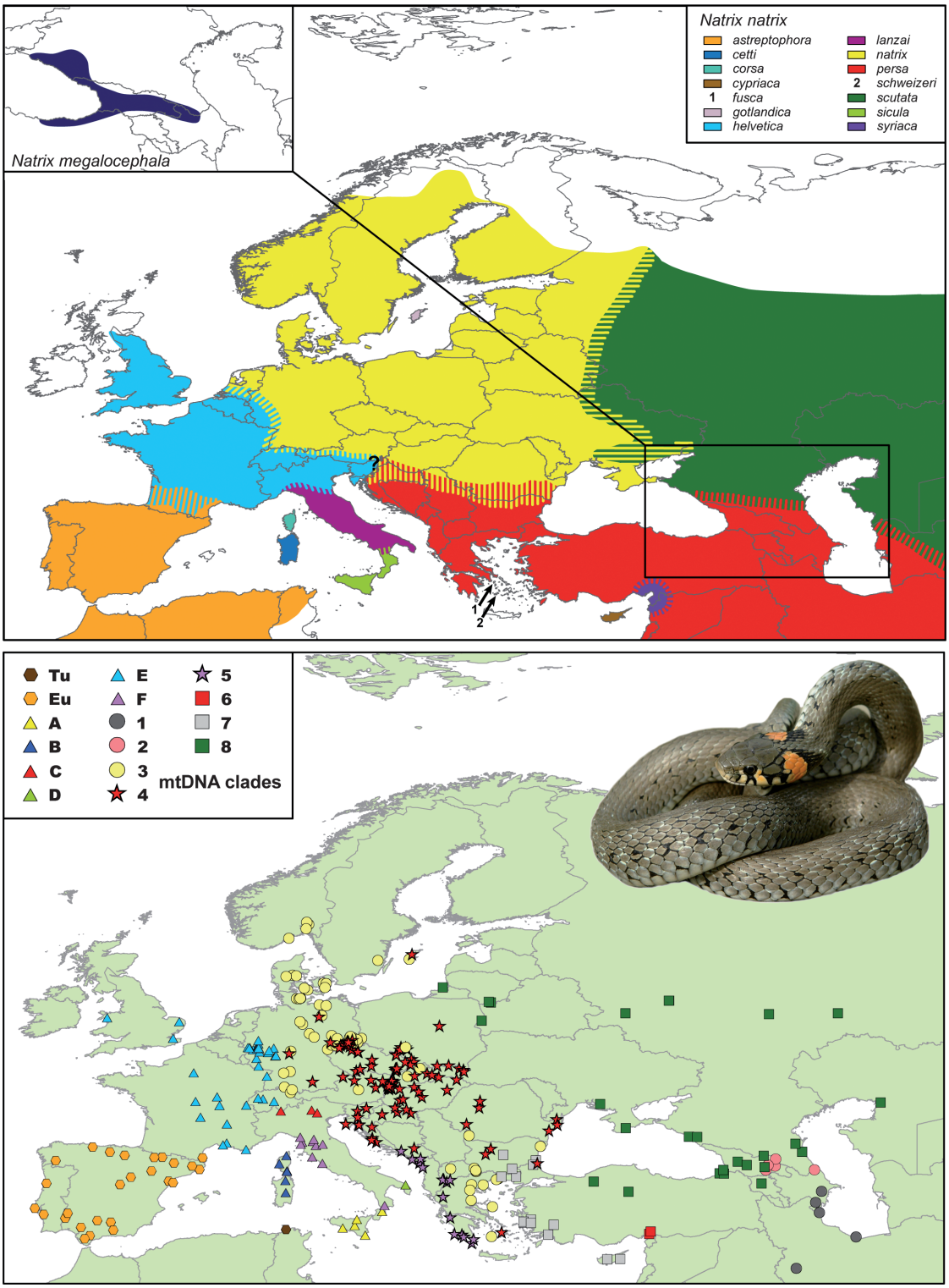

Fig. 1 Distribution of subspecies of *Natrix natrix* and *N. megalocephala* (top) and mitochondrial clades (bottom). Hatching along range borders of *N. natrix* subspecies indicates putative contact or hybrid zones. Distribution ranges combined from Kabisch (1999), Orlov & Tuniyev (1999), Kreiner (2007), Baier *et al.* (2009) and Göçmen *et al.* (2011). Inset (bottom): *N. natrix* from Mtskheta, Georgia (photograph: M. Auer).

resulting in a total of 410 sequences. *Natrix maura*, *N. tessellata* and the more distantly related North American water snake *Nerodia sipedon* were added as outgroups; *N. sipedon* was used for tree rooting. GenBank accession numbers of all sequences are listed in Table S1.

Phylogenetic relationships were inferred for sequences of all 410 grass snakes (Table S1) using three data sets, that is, for each mtDNA fragment alone (ND4+tRNAs vs. cyt *b*) and for the two fragments concatenated. For the combined analyses and for the mtDNA fragment embracing the partial ND4 gene plus adjacent DNA coding for tRNAs, the data were partitioned by gene; the DNA coding for tRNAs was treated as a single partition. In the alignment of concatenated sequences (1984 bp total length), 696 bp corresponded to the partial ND4 gene, 171 bp to the DNA coding for tRNA-His, tRNA-Ser and tRNA-Leu, and 1117 bp to the cyt *b* gene. The 867-bp-long ND4+tRNA sequences contained 175 variable sites, and the 1117-bp-long cyt *b* sequences, 270 variable sites (ingroup sequences only). The best evolutionary model was determined for each partition using the Akaike information criterion of MRMODELTEST 2.3 (Nylander 2004), resulting in the GTR+I+G model for ND4 and cyt *b* and the HKY+I model for the combined tRNAs. Phylogenetic trees were calculated using MRBAYES 3.2.1 (Ronquist *et al.* 2012) and the implemented Metropolis-coupled Markov chain Monte Carlo algorithm. Two parallel runs, each with one cold and three heated chains, were conducted. The chains ran for 10^7 generations, with every 100th generation sampled. However, using the default settings of MRBAYES the two runs for cyt *b* and the concatenated data set did not converge on a stationary level. Therefore, analyses were rerun setting the heating parameter λ to 0.05. For generating the final 50% majority rule consensus, a burn-in of 25% was used to sample only the most likely trees. In addition, phylogenetic relationships were inferred using the Maximum Likelihood (ML) approach as implemented in RAxML 7.2.8 (Stamatakis 2006). Using the GTR+G model across all partitions, five independent ML searches were run with different starting conditions and the fast bootstrap algorithm. The robustness of the branching patterns was examined by comparing the best trees. Subsequently, 1000 nonparametric thorough bootstrap replicates were calculated and the values plotted against the tree with the highest likelihood value. In addition, uncorrected *p* distances were computed using MEGA and the 'pairwise deletion' option.

Results

For each data set (ND4+tRNAs and cyt *b* alone or concatenated), there are no significant differences in the topologies of the Maximum Likelihood and Bayesian trees, and the trees for the combined data set have completely identical topologies. Differences between the trees based on each mtDNA fragment alone and the trees based on the concatenated sequence data occur only with respect to the weakly resolved deeper nodes, while the placement of individual sequences in terminal clades is consistent. In the trees based on the combined sequences, some of the more basal branching patterns are better supported than in the trees based on ND4+tRNAs and cyt *b* alone. However, some deeper nodes remain also then weakly supported (Fig. 2). In agreement with previous results (Guicking *et al.* 2006), *Natrix tessellata* and *N. maura* constitute the successive sister taxa of a well-supported clade corresponding to *N. natrix* (Fig. S1). Our three sequences of *N. megalocephala* are consistently embedded within *N. natrix*, and there are 16 major subordinated clades within this paraphyletic group (clades Tu, Eu, 1–8 and A–F; Fig. 2). With respect to ND4+tRNAs, uncorrected average *p* distances among these 16 clades amount to 0.29–7.29%, while divergences of 0–0.63% are observed within the clades. For cyt *b* sequences, the respective values are 0.28–7.51% and 0–0.62% (Table S4).

The sequences of *N. megalocephala* cluster in a well-supported subordinated clade containing also sequences of *N. n. natrix*, *N. n. persa* and *N. n. scutata* (clade 8 in Fig. 2). A closer inspection of clade 8 reveals that the average divergence between the three sequences of *N. megalocephala* and the 35 sequences of *N. natrix* subspecies is only 0.26% (ND4+tRNAs and cyt *b* concatenated). The sequences of *N. megalocephala* represent three haplotypes, and the same haplotypes also occur among the sequences of *N. natrix* within clade 8.

While mismatches between taxonomy and phylogenetic placement within *N. natrix* are the rule and not the exception (Table 1), there are a few monophyletic taxa. The well-supported most basal clade (Fig. 2) corresponds to sequences of *N. n. astreptophora* from North Africa and the Iberian Peninsula plus adjacent France. Within this clade, a sequence of a Tunisian grass snake (Tu) is deeply divergent from all European *N. n. astreptophora* (Eu). Also most sequences of *N. n. sicula* (with one exception) represent a deeply divergent clade (clade A), and the sequences of Corso-Sardinian grass snakes constitute another well-supported clade (clade B). However, the four sequences from Corsica (subspecies *corsa*) and the three sequences from Sardinia (subspecies *cetti*) are not reciprocally monophyletic (Fig. S1). Furthermore, our only two sequences of *N. n. syriaca* represent a deeply divergent clade (clade 6).

The sister group of *N. n. astreptophora* is a major clade whose monophyly is well supported only in Bayesian analyses (Fig. 2). This clade comprises grass snake sequences from most of the species' range (except North Africa,

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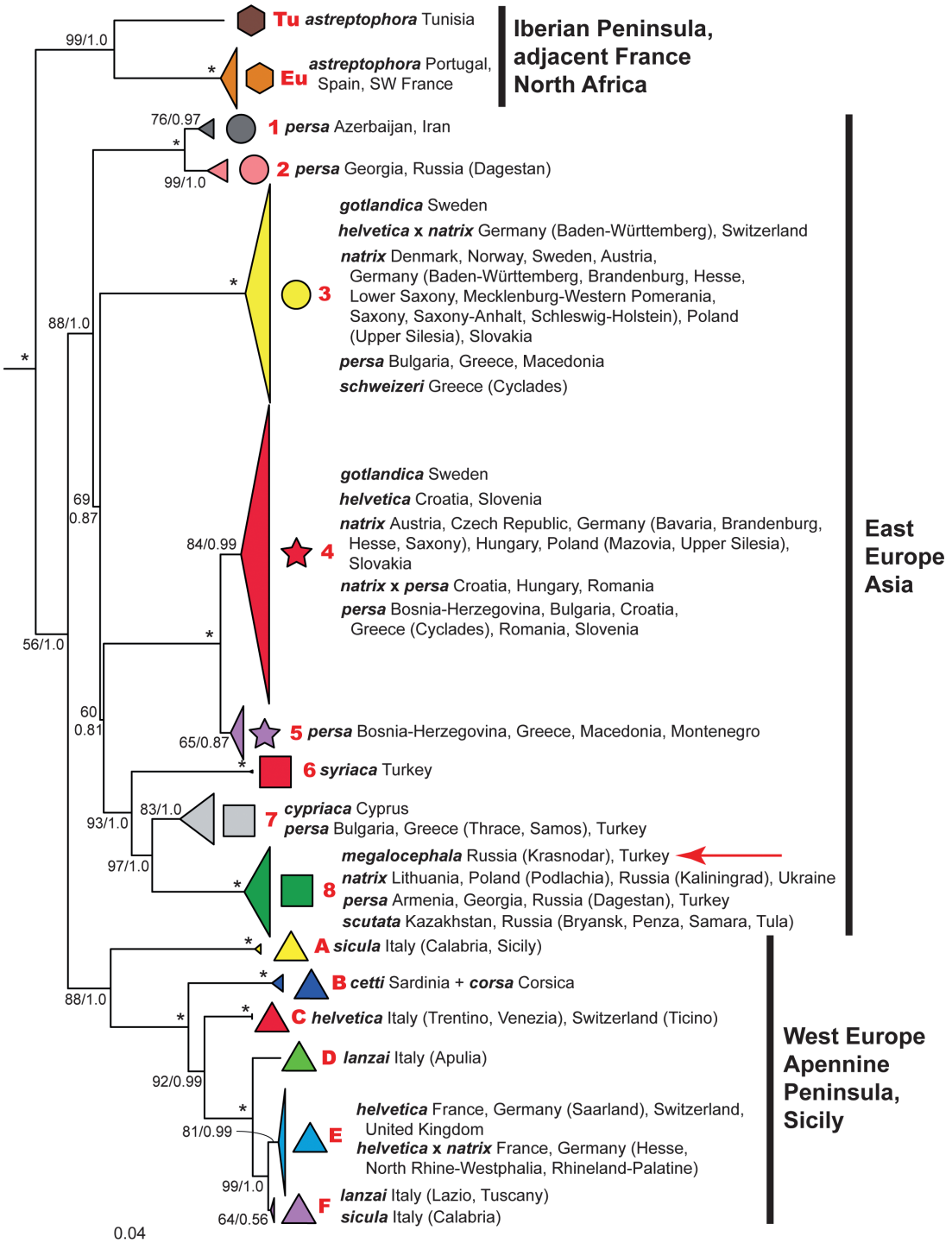

Fig. 2 Mitochondrial phylogeny of grass snakes inferred from Maximum Likelihood analyses using 1984-bp mtDNA (ND4+tRNAs, cyt *b*) of 407 samples of *Natrix natrix* and three samples of *N. megalocephala*. Terminal clades collapsed to cartoons. Outgroups (*N. maura*, *N. tessellata*, *Nerodia sipedon*) removed for clarity; for a complete tree, see Fig. S1. Numbers along nodes indicate branch support under Maximum Likelihood (1000 bootstrap replicates) and Bayesian analyses (posterior probabilities). Asterisks indicate maximum support under both tree-building methods. Clade symbols correspond to Fig. 1; red letters and numbers preceding taxon names refer to the text and Table 1. Red arrow highlights placement of *Natrix megalocephala* among *N. natrix*.

Iberian Peninsula and adjacent France = *N. n. astreptophora*). Within this clade, there are two other subordinated major clades, one well-supported clade from the eastern part and another well-supported clade from the western part of the range (including Apennine Peninsula and Sicily). The clade from the east contains the eight subordinated clades 1–8, and the clade from the west, the six subordinated clades A–F. Most of these 14 clades conflict with subspecies delineations (Table 1).

Some deeper nodes of the eastern clade are weakly resolved and have very short branch lengths. By contrast, seven of the eight terminal clades (1–4, 6–8) are well supported (Fig. 2). However, clade 5, corresponding to sequences of *N. n. persa* from Bosnia and Herzegovina, Greece, Macedonia and Montenegro, receives only weak support, and mean uncorrected *p* distances between clade 5 and its sister group (clade 4) amount only to 0.55% (ND4+tRNAs) and 1.38% (cyt *b*; Table S4). Within the eastern clade, a similarly weak divergence occurs only between the well-supported sister clades 1 and 2 (ND4+tRNAs: 0.72%, cyt *b*: 1.43%); among the other terminal clades, much higher values of 2.37–5.56% (ND4+tRNAs) and 3.23–5.74% (cyt *b*) are observed (Table S4).

Sequences of *N. n. persa* and of grass snakes from the putative hybrid zone between *N. n. natrix* and *N. n. persa* occur in not less than seven distinct, in part deeply divergent, clades (clades 1–5 and 7–8). Sequences of the nominotypical subspecies *N. n. natrix* occur together with sequences of *N. n. persa* and other taxa in the well-supported clades 3, 4 and 8 (Fig. 2).

The deeper branching patterns within the western clade are distinctly better resolved than in the eastern clade, and branch lengths of the respective deeper nodes are longer than in most of the deeper nodes of the eastern clade. Within the western clade, sequences of Calabrian and Sicilian grass snakes of the subspecies *N. n. sicula* (clade A) constitute the sister group of the remaining sequences. The successive clades are B (Corso-Sardinian grass snakes, *N. n. corsa* and *N. n. cetti*), C (*N. n. helvetica* from Trentino and Venezia in north-eastern Italy and Ticino, Switzerland) and D (corresponding to a single sequence of a grass snake from Apulia, Italy). The terminal crown group is formed by clade E (*N. n. helvetica* and individuals from the hybrid zone of *N. n. helvetica* and *N. n. natrix*) and clade F (sequences of *N. n. lanzai* from Lazio and Tuscany, Italy, and a sequence of *N. n. sicula* from Calabria). However, compared with the basal clades A, B and C, the divergences among clades D, E and F are distinctly less pronounced and also weaker than between most other clades (Table S4). This is mirrored by shorter branch lengths (Fig. 2) and uncorrected *p* distances of only 0.29–0.80% (ND4+tRNAs) and 0.28–1.16% (cyt *b*) for clades D, E and F, opposed to values of 2.12–6.12% (ND4+tRNAs) and 2.06–5.36% (cyt *b*) when clades A, B and C are compared with one another and with clades D, E and F (Table S4).

Discussion

Phylogeography

Our results, based on a nearly range-wide sampling, demonstrate considerable phylogeographic differentiation of grass snakes. Uncorrected *p* distances for the two studied mitochondrial markers (Table S4) resemble or clearly exceed divergences observed within the other two western Palaearctic *Natrix* species, both having a pronounced phylogeographic structure (Guicking *et al.* 2008b, 2009). With respect to the cyt *b* gene, mean divergences of 0.28–7.51% were observed among grass snake clades (Table S4). The maximum uncorrected *p* distance described for cyt *b* sequences of *Natrix maura* is 4.74% (European vs. Moroccan snakes; Guicking *et al.* 2008b), and a maximum value of 8.42% was observed when *N. tessellata* from the Caucasus and Greece were compared (Guicking *et al.* 2009). However, these values are maxima, and not mean values, as reported in the present study.

Based on mtDNA sequences of 410 grass snakes, our phylogenetic analyses revealed 16 distinct terminal clades (Figs 1 and 2). One of these clades includes the three sequences of *N. megalocephala* together with *N. natrix* sequences. These 16 clades correspond to three more inclusive clades from (i) the Iberian Peninsula plus North Africa, (ii) East Europe and Asia and (iii) West Europe including Corso-Sardinia, the Apennine Peninsula and Sicily (Fig. 2). These three major clades had already been identified in previous studies (Guicking *et al.* 2006, 2008a; Fritz *et al.* 2012).

Guicking *et al.* (2006, 2008a) and Fritz *et al.* (2012) had distinguished within clades (i), (ii) and (iii) a maximum of eight terminal clades. However, these studies had used a quite restricted sampling of 23–30 individuals and therefore had lumped for phylogeographic purposes some distinct lineages together, which were represented by single sequences only. If the terminal clades and lineages of Guicking *et al.* (2006, 2008a) and Fritz *et al.* (2012) are compared with our 16 clades, it is clear that just the following four clades are missing: clade Tu (*N. n. astreptophora* from Tunisia), clade 2 (*N. n. persa* from Georgia and Dagestan), clade 6 (*N. n. syriaca*) and clade A (*N. n. sicula* from Sicily and Calabria).

Based on molecular clock calculations, Fritz *et al.* (2012) had inferred that the basal clades (i), (ii) and (iii) diverged some 7–11 million years ago, while the then known 12 terminal lineages were dated to the Late Miocene to the Early Pleistocene, a time frame matching also well with the

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branch lengths of the four newly discovered terminal clades (compare Fig. 2 of this study with Figs 1 and 3 in Fritz *et al.* 2012). It is obvious that the branch lengths of some deeper nodes of the East European–Asian clade (ii) are distinctly shorter than in the West European clade (iii), suggestive of more rapid radiation. In the West European clade, however, the terminal subordinated clades D, E and F also have rather short branches, indicating a later divergence compared with the remaining clades A, B and C.

The deep divergence between the Tunisian clade Tu and European samples of *N. n. astreptophora* (clade Eu; Fig. 2; Table S4) suggests an old separation of these two lineages. However, without studying grass snakes from Morocco and Algeria, any interpretation of this pattern has to remain highly speculative. It is well known that Algerian and Tunisian populations are often highly distinct from Moroccan ones (e.g. *Pleurodeles* spp., Carranza & Arnold 2004; Veith *et al.* 2004; *Hyla meridionalis*, Recuero *et al.* 2007; *Mauremys leprosa*, Fritz *et al.* 2006; *Testudo graeca*, Fritz *et al.* 2009a; *Chamaeleo chamaeleon*, Dimaki *et al.* 2008; *Timon* spp., Paulo *et al.* 2008; *Coronella girondica*, Santos *et al.* 2012; *Macroprotodon* spp., Carranza *et al.* 2004; *Malpolon* spp., Carranza *et al.* 2006; *Natrix maura*, Guicking *et al.* 2008b; *Vipera latastei* complex, Velo-Antón *et al.* 2012), while the differentiation between Moroccan and Iberian populations can be sometimes quite shallow (e.g. *Pleurodeles waltl*, Carranza & Arnold 2004; Veith *et al.* 2004; *Emys orbicularis*, Fritz *et al.* 2007a; *Mauremys leprosa*, Fritz *et al.* 2006; *Macroprotodon brevis*, Carranza *et al.* 2004; *Malpolon monspessulanus*, Carranza *et al.* 2006).

Due to a patchy sampling in south-western France, we were unable to confirm the putative contact zone between clade Eu (*N. n. astreptophora*) and the neighbouring clade E (*N. n. helvetica*). However, thanks to our much denser sampling in other regions, not only some new clades were discovered. Also the distribution ranges of previously identified clades became much clearer (Fig. 1), allowing to refine and to expand the preliminary phylogeographic considerations of Fritz *et al.* (2012). These authors concluded that, in agreement with general phylogeographic patterns of the western Palaearctic (Hewitt 1996, 2000, 2011; Taberlet *et al.* 1998; Joger *et al.* 2007; Schmitt 2007; Schmitt & Varga 2012), at least one glacial refuge was located in each of the southern European peninsulas and in Anatolia. Fritz *et al.* (2012) suggested that multiple refugia existed south of the Alps and in the Balkan Peninsula. This is confirmed by our present study. However, the situation is even more complex than thought before.

Our additional samples indicate the existence of not less than four distinct refugia in the Apennine Peninsula and Sicily: (i) in western Italy (corresponding to clade F; Figs 1 and 2), (ii) in north-eastern Italy plus adjacent Switzerland (clade C), (iii) in south-eastern Italy (clade D), and (iv) a newly identified refuge in Sicily and Calabria (clade A).

This fine-scale pattern of 'refugia-within-refugia' (Gómez & Lunt 2007) or 'microrefugia' (Joger *et al.* 2007) in Italy and adjacent regions is at first glance unexpected because Schmitt (2007) argued that the phylogeographic patterns within the Apennine Peninsula are simpler than in the other, larger, southern European peninsulas. Pedall *et al.* (2011) contradicted this view and listed many plants and animals for which more than one refuge has to be postulated in the Italian peninsula. If the putatively four continental Italian and Sicilian refugia of *N. natrix* are compared with other taxa, it is obvious that this is by far not an idiosyncratic pattern. A phylogeographic differentiation in lineages east and west of the Apennine chain, paralleling clades C and F, occurs in some other reptiles, for instance, in European pond turtles (*E. orbicularis*, Pedall *et al.* 2011), wall lizards (*Podarcis siculus*, Podnar *et al.* 2005) and whip snakes (*Hierophis viridiflavus*, Rato *et al.* 2009). A distinct 'Padano-Venetian refuge', suggested by the distribution of our clade C, is further supported by the occurrence of the endemic frog species *Rana latastei* (Grossenbacher 2004) and endemic mitochondrial clades of the Italian crested newt *Triturus carnifex* (Canestrelli *et al.* 2012a), the pool frog *Pelophylax lessonae* (Canestrelli & Nascetti 2008), the spadefood toad *Pelobates fuscus* (Crottini *et al.* 2007), the tree frog *Hyla intermedia* (Canestrelli *et al.* 2007) and the Italian wall lizard *Podarcis siculus* (Podnar *et al.* 2005). Finally, the distinct refuge in Sicily and adjacent southernmost continental Italy (Calabria), harbouring the most divergent clade A within the western group of *N. natrix* (Figs 1 and 2), is corroborated by quite a number of other taxa. Besides the endemic Sicilian species *Emys trinacris* (Fritz *et al.* 2005; Pedall *et al.* 2011), *Podarcis waglerianus* (Böhme 1986) and *Bufotes siculus* (Stöck *et al.* 2008), there are deeply divergent mitochondrial clades in Sicily and Calabria in species such as hedgehogs (*Erinaceus europaeus*, Seddon *et al.* 2001), Italian hares (*Lepus corsicanus*, Pierpaoli *et al.* 1999), bank voles (*Myodes glareolus*, Colangelo *et al.* 2012), red squirrels (*Sciurus vulgaris*, Grill *et al.* 2009), green and wall lizards (*Lacerta bilineata*, Godinho *et al.* 2005; Böhme *et al.* 2007 and *P. siculus*, Podnar *et al.* 2005), newts (*Lissotriton italicus* and *T. carnifex*, Canestrelli *et al.* 2012a,b) and frogs (*H. intermedia*, Canestrelli *et al.* 2007; *Pelophylax lessonae*, Canestrelli & Nascetti 2008; *Rana italica*, Canestrelli *et al.* 2008).

Fritz *et al.* (2012) concluded that western Central Europe was colonized in the postglacial by grass snakes originating from the western Apennine refuge. However, our expanded sampling rather suggests that the endemic clade F, distributed in the north-western Italian peninsula (Fig. 1), did not expand into western Central Europe. The closely related

clade E from West Europe (including southern France) is clearly distinct (Fig. 2), and it seems more likely that its extant range results from a range expansion from within the south of its present distribution range (southern France).

Based on morphological evidence, it is generally accepted that the western subspecies *N. n. helvetica* meets and intergrades with the eastern subspecies *N. n. natrix* in the Rhine region (e.g. Thorpe 1979; Kabisch 1999; Kreiner 2007). Our mitochondrial data generally confirm this pattern in that the geographical distribution of the western clade E abuts the range of the eastern clade 3 there. However, what was completely unexpected is the finding that another eastern clade (clade 4) encroaches deeply into Central Europe (Fig. 1). Haplotypes of clades 3 and 4 occur in broad sympatry throughout southern and eastern Germany, on Gotland (Sweden), in southern Poland, Austria and Slovakia, indicating another Central European contact zone. Yet, to the south, the range of clade 4 seems to interrupt the distribution of clade 3. Records of clade 3 are lacking for the western central Balkan Peninsula, where clade 4 is widely distributed. In the south-eastern Balkans, there are again many records for clade 3, which occurs there in part in close proximity to haplotypes of clade 4 and another clade (clade 7). Moreover, in the south-western Balkans, clade 4 is replaced by the allied clade 5. We cannot exclude that the disjunct range of clade 3 is an artefact and that we simply missed to sample clade 3 in some regions. Moreover, the new records for clade 3 in the south-eastern Balkan Peninsula contradict the hypothesis (Fritz *et al.* 2012) that grass snakes harbouring haplotypes of this clade survived the last glaciation north of the Alps and suggest rather a northward range expansion from a Balkanic refuge.

In summary, this complicated phylogeographic pattern indicates that four, and not only two (Fritz *et al.* 2012), distinct glacial refugia were located in the Balkan Peninsula, which harboured grass snakes of clades 3, 4, 5 and 7. The exact location of these refugia is difficult to determine because the extant distribution ranges seem to be blurred by extensive Holocene range expansions and, perhaps, range shifts. The relatively restricted distribution range of clade 5 suggests that its refuge was in the south-west of the Balkan Peninsula. The refugia of the other three clades (3, 4, 7) were most probably further east, somewhere south of the boundary of permafrost or deep seasonal freezing (Frenzel *et al.* 1992), that is, south of the Danube Basin. Therefore, it is possible that the refugial range of clade 7 differed not too much from its extant range, embracing the northern Aegean region, western and southern Anatolia and Cyprus. The relatively pronounced differentiation within clade 7 (Fig. S1; Table S4) could indicate several microrefugia in its extant range, being richly structured by sea straits and mountain chains. The disjunct range of clade 3 suggests that it originated in the south-eastern Balkan Peninsula. It resembles the distribution range of a certain mitochondrial lineage of the European pond turtle (*Emys orbicularis* lineage II), which is thought to have spread northward via the Axios/Vardar and Danube Rivers and the Moravian Gate (Fritz *et al.* 2007a; Sommer *et al.* 2009). It is plausible that *N. natrix*, living in similar habitats as *E. orbicularis*, used the same pathway.

Another parallel to *E. orbicularis* is the distribution of clade 8, which is largely congruent with the distribution range of another mitochondrial lineage of the pond turtle (lineage I; Fritz *et al.* 2007a, 2009b). The glacial refuge of both was most probably located somewhere close to the Caucasus Mountains. Also the localized range of clade 6 agrees perfectly with an endemic lineage of *E. orbicularis* (lineage X; Fritz *et al.* 2009b). However, unlike *E. orbicularis*, there are two distinct mitochondrial clades of *N. natrix* present in eastern Transcaucasia and northern Iran (clades 1 and 2), whereas only one lineage is present in the turtle (lineage VII; Fritz *et al.* 2007a, 2009b).

Taxonomy

It has been repeatedly argued that mtDNA sequences alone are by far no perfect proxy for phylogeography and taxonomic differentiation, among others due to their strictly maternal inheritance, the lack of recombination, their reduced effective population size and resulting bias caused by genetic drift, sex-specific dispersal and sometimes massive mitochondrial introgression, even across species borders (e.g. Ballard & Whitlock 2004; Edwards *et al.* 2005; Mallet 2005; Bazin *et al.* 2006; Currat *et al.* 2008). However, with regard to western Palaearctic amphibians and reptiles, there is a good agreement between taxonomic units (species, subspecies) and mitochondrial differentiation (Joger *et al.* 2007), with mismatches typically indicating bad taxonomy (e.g. Podnar *et al.* 2005; Ursenbacher *et al.* 2006, 2008; Fritz *et al.* 2007b, 2009b; Gvoždík *et al.* 2010a,b; Pedall *et al.* 2011). Other causes for mismatches are either hybridization and intraspecific gene flow (e.g. in the pond turtle *Emys orbicularis*, Pedall *et al.* 2011), interspecific mitochondrial introgression (e.g. in crested newts of the *Triturus cristatus* complex, Wielstra *et al.* 2013) or, rarely, complete mitochondrial replacement, like in the Carpathian newt *Lissotriton montandoni* (Zieliński *et al.* 2013), so that the application of mitochondrial markers, at least as a first step, is still well justified. This is in line with a careful review of recent literature on birds (Zink & Barrowclough 2008), showing that mtDNA delivers robust results for phylogeography and species limits in the vast majority of cases.

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In grass snakes, most previous assessments of geographical variation were based on morphological investigations that used, with the notable exception of Thorpe (1979), small or heavily biased samples with respect to geographical coverage and age or sex classes (e.g. Hecht 1930; Mertens 1947, 1957, 1966; Kramer 1970; Nilson & Andrén 1981). Putatively diagnostic characters for subspecies of *Natrix natrix*, and for *N. megalocephala*, concern differences in total size, body proportions, colouration and pattern, while meristic characters typically broadly overlap (Kabisch 1999; Orlov & Tuniyev 1999). Apart from the publications by Hille (1997), Guicking *et al.* (2006, 2008a) and Fritz *et al.* (2012), no attempt has been undertaken yet to analyse geographically correlated genetic variation in *N. natrix*. Hille (1997) presented preliminary allozyme data for *N. megalocephala* and some subspecies of *N. natrix*, largely without novel insights. Based on a quite limited sampling of 23–30 snakes, Guicking *et al.* (2006, 2008a) and Fritz *et al.* (2012) presented phylogenetic analyses and molecular clock calculations using mtDNA sequences and found deeply divergent clades within *N. natrix* that often conflicted with traditionally recognized subspecies. However, our study provides for the first time a comprehensive and nearly range-wide examination of mitochondrial variation of grass snakes, based on sequences of 410 snakes. We included in our investigations samples of *N. megalocephala* and of 13 of the 14 subspecies of *N. natrix* recognized by some authors (Table 1).

If each taxon represents an evolutionarily distinct lineage, it should be expected that there is a general agreement between the geographical distribution of the mitochondrial clades and the 15 taxa, with the possible exception of the range borders of the subspecies of *N. natrix*, where the exchange of mitochondria could cause mismatches. In other words, it should be expected that the mitochondrial haplotypes in the core regions of the distribution ranges of each taxon are reciprocally monophyletic and constitute clearly distinct mitochondrial clades.

However, we found 16, and not 15, mitochondrial clades, and only few of them correspond well to any taxon (Figs 1 and 2; Table 1). Most notably, *N. megalocephala*, a taxon described as a distinct species (Orlov & Tuniyev 1987), does not represent a distinct clade. Rather, the three sequences of *N. megalocephala* are nested in the paraphyletic clade 8 containing also sequences of three subspecies of *N. natrix* (*N. n. natrix*, *N. n. persa*, *N. n. scutata*). Moreover, none of the three haplotypes of the *N. megalocephala* is unique; each haplotype is also represented by *N. natrix* sequences of clade 8. Using 32 allozyme loci, Hille (1997) found *N. megalocephala* undifferentiated from samples of *N. n. natrix* from Central Europe and *N. n. persa* from the Peloponnesus, Greece, and the morphological distinctiveness of *N. megalocephala* was repeatedly questioned (Jandzík 2005; Frotzler *et al.* 2011; Göçmen *et al.* 2011). According to the original description (Orlov & Tuniyev 1987), *N. megalocephala* differs from *N. natrix* by its wider head, less distinct frontal and parietal scutes, and the more massive body (see also Orlov & Tuniyev 1999). However, there are intermediate specimens known (Jandzík 2005), and big-headed grass snakes also occur in other parts of the range of *N. natrix* (Göçmen *et al.* 2011), implying that the putatively diagnostic characters of *N. megalocephala* represent merely ontogenetic variation.

The only taxa being represented by reciprocally monophyletic mitochondrial clades are *N. n. astreptophora* and *N. n. syriaca*. However, the clade of *N. n. astreptophora* contains two deeply divergent lineages, one corresponding to the only studied North African grass snake and the other to all European samples of the subspecies *astreptophora*. This suggests that North African representatives of *N. n. astreptophora* could be taxonomically distinct and a morphological reassessment is warranted, as is the inclusion of Moroccan and Algerian samples in genetic investigations (see 'Phylogeography').

Also the putative range of *N. n. sicula* (Sicily, Calabria) agrees quite well with the distribution of clade A. The occurrence of clade A and clade C haplotypes in close proximity in Calabria indicates a contact zone in this region, which is paralleled by some other taxa (*Podarcis siculus*, Podnar *et al.* 2005; *Lissotriton italicus* and *Triturus carnifex*, Canestrelli *et al.* 2012a,b; *Hyla intermedia*, Canestrelli *et al.* 2007; *Pelophylax lessonae*, Canestrelli & Nascetti 2008; *Rana italica*, Canestrelli *et al.* 2008; perhaps *Emys orbicularis* and *E. trinacris*, Fritz *et al.* 2005).

Sequences of the Corsican and Sardinian subspecies *N. n. corsa* and *N. n. cetti* together constitute the well-supported clade B (Fig. 2); however, sequences of the two subspecies are not reciprocally monophyletic (Fig. S1). These two subspecies differ significantly in colouration and pattern (Engelmann *et al.* 1986; Gruber 1989; Kabisch 1999; Arnold & Ovenden 2002; Kreiner 2007; Vanni & Cimmaruta 2010), suggesting that such characters may reflect rather population-specific characters.

All other subspecies of *N. natrix* conflict with mitochondrial clades, supporting the view (Guicking *et al.* 2008a; Fritz *et al.* 2012) that a taxonomic revision of grass snakes is required. Mitochondrial DNA sequences of Cypriot grass snakes (*N. n. cypriaca*), characterized by distinctive colouration and pattern (Baier *et al.* 2009), are not reciprocally monophyletic with respect to sequences of grass snakes from neighbouring eastern Mediterranean regions (clade 7; Figs 1 and S1). Also samples of the island-endemic subspecies *N. n. gotlandica* (Gotland, Sweden) and *N. n. schweizeri* (Cyclades, Greece) yielded no unique

haplotypes (Fig. 2; Table 1). The haplotypes detected in both subspecies belong to clades 3 and 4, which are also present in neighbouring mainland populations. This does not corroborate the validity of *N. n. gotlandica* and *N. n. schweizeri* and argues again rather in favour of population specificity of their distinctive colouration and pattern characters (Engelmann *et al.* 1986; Gruber 1989; Kabisch 1999; Arnold & Ovenden 2002; Kreiner 2007). The island of Gotland was completely inundated by the Baltic Ice Lake 11 000 years ago. It did not emerge before 10 300 years ago and was later not connected by land bridges to the surrounding Baltic coasts (Björck 1995). This implies that the Gotland population of grass snakes was founded only in the Holocene by oversea dispersal from what is now Poland, northern Germany, Denmark or Sweden, regions where grass snakes of clades 3 and 4 are currently widely distributed (Fig. 1), and that the distinctive colouration and pattern characters of the Gotland snakes evolved quite rapidly.

We cannot exclude that some mismatches between morphological taxon delimitation and clade assignment (Figs 1 and 2; Table 1) result from mitochondrial introgression. This could be, for instance, the case with respect to the conflicting sequences assigned to *N. n. helvetica* from westernmost Croatia and Slovenia, a region where several subspecies are thought to meet (*N. n. helvetica*, *N. n. natrix*, *N. n. persa*; Fig. 1). However, introgression is unlikely when large parts of a subspecies' range are occupied by endemic mitochondrial clades, as in the northern Italian part of the range of *N. n. helvetica* (Fig. 1: clade C). This suggests rather the occurrence of cryptic or overlooked distinct taxa there (see 'Phylogeography' for other endemic genetic lineages in the Padano-Venetian region). A similar, but more complex, situation refers to the striped subspecies *N. n. persa*, in whose range several endemic and locally restricted clades are distributed. Four of these clades are confined to the putative range of *N. n. persa* (clades 1, 2, 5, 7), while three further clades (clades 3, 4, 8) deeply encroach into the ranges of the two unstriped subspecies *N. n. natrix* and *N. n. scutata* (Fig. 1), rendering the situation even more complicated.

Striped grass snakes identified with *N. n. persa* are distributed over a vast range, from the Balkan Peninsula through Turkey and Transcaucasia to the south-eastern range border in Iran (Fig. 1). The considerable mitochondrial diversity within this vast territory implies that back stripes occur in several distinct taxa, which were historically lumped together due to similar morphology.

In the north of the distribution range of grass snakes (corresponding to the distribution ranges of the subspecies *N. n. helvetica*, *N. n. natrix* and *N. n. scutata*), back stripes are rare, but there are some records of striped snakes from Central Europe (Austria: Grillitsch & Cabela 2001; Czech Republic: Werner 1929; Germany: Günther & Völkl 1996; Slovakia: Lác 1968; Rehák 1992). This situation suggests that clinal variation or selection could play a role.

For the time being, it is impossible to disentangle the conflicting patterns of morphological and mitochondrial variation. Further research employing nuclear markers, like nuclear genes and rapidly evolving microsatellite loci, may help to elucidate this confusing situation, also with respect to the newly discovered Central and South-east European contact zone between the mitochondrial clades 3 and 4 (Fig. 1). Also a morphological re-examination of striped grass snakes seems promising to unravel possibly overlooked morphological differences between representatives of the distinct mitochondrial clades.

Conclusions

Several distinct lines of evidence (morphology, allozymes, mtDNA) indicate that *Natrix megalocephala* Orlov & Tuniyev, 1987 is invalid. Therefore, this species is synonymized here with *N. natrix* (Linnaeus, 1758). From within the range of *N. natrix*, 16 terminal mitochondrial clades were identified, most of which conflict with morphologically defined subspecies. These 16 clades correspond to three more inclusive clades from (i) the Iberian Peninsula plus North Africa, (ii) East Europe and Asia and (iii) West Europe including Corso-Sardinia, the Apennine Peninsula and Sicily. The highest mitochondrial diversity is found in the south of the range, where the putative glacial refugia were located. Endemic mitochondrial clades occur in each of the southern peninsulas of Europe, on Corso-Sardinia, in northern Africa, Anatolia and the neighbouring Near and Middle East, suggesting that the respective glacial refugia were located there. While there is evidence for only one refuge in the Iberian Peninsula, the Apennine Peninsula and Sicily harboured most likely four distinct microrefugia. Also for the Balkan Peninsula, there is evidence for four microrefugia. Further multiple microrefugia were located in Anatolia and the neighbouring Near and Middle East. According to the extant distributions of mitochondrial clades, the north-west of the species' range was colonized from southern France, while Central Europe was reached by two distinct range expansions from within the southern Balkan Peninsula, resulting in two distinct secondary contact zones across Central Europe. One of these contact zones was theretofore unknown. Eastern Europe and north-western Asia were invaded from another refuge close to the Caucasus Mountains, and the existence of a third northern secondary contact zone in eastern Poland and Ukraine seems likely. Further, in part newly identified, secondary contact zones are in southern Italy, the Balkans and Transcaucasia. To disentangle the manifold conflicts

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between morphologically defined subspecies and mitochondrial differentiation, further research is needed, including the application of nuclear genomic markers and a morphological re-examination of morphologically similar populations representing deeply divergent mitochondrial clades.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Maximum Likelihood tree, depicting relationships of all 410 samples of *Natrix natrix* and *N. megalocephala*. Sample codes are GenBank accession numbers (Table S1). Colours correspond to Fig. 2 except for clade 3, which is shown in black. For further explanations, see Fig. 2.

Table S1. Grass snake samples studied and GenBank accession numbers of their DNA sequences.

Table S2. Primers used for PCR and sequencing. For primer combinations and PCR conditions, see Table S3.

Table S3. PCR protocols.

Table S4. Mean uncorrected *p* distances (percentages) between and within mtDNA clades of grass snakes (top: ND4+tRNAs, bottom: cyt *b*). Below the diagonal, divergences between groups; on the diagonal, within group divergences in boldface.

Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*)

CAROLIN KINDLER, WOLFGANG BÖHME, CLAUDIA CORTI, VÁCLAV GVOŽDÍK, DANIEL JABLONSKI, DAVID JANDZIK, MARGARITA METALLINOU, PAVEL ŠIROKÝ & UWE FRITZ

Zoologica Scripta

Supporting Information

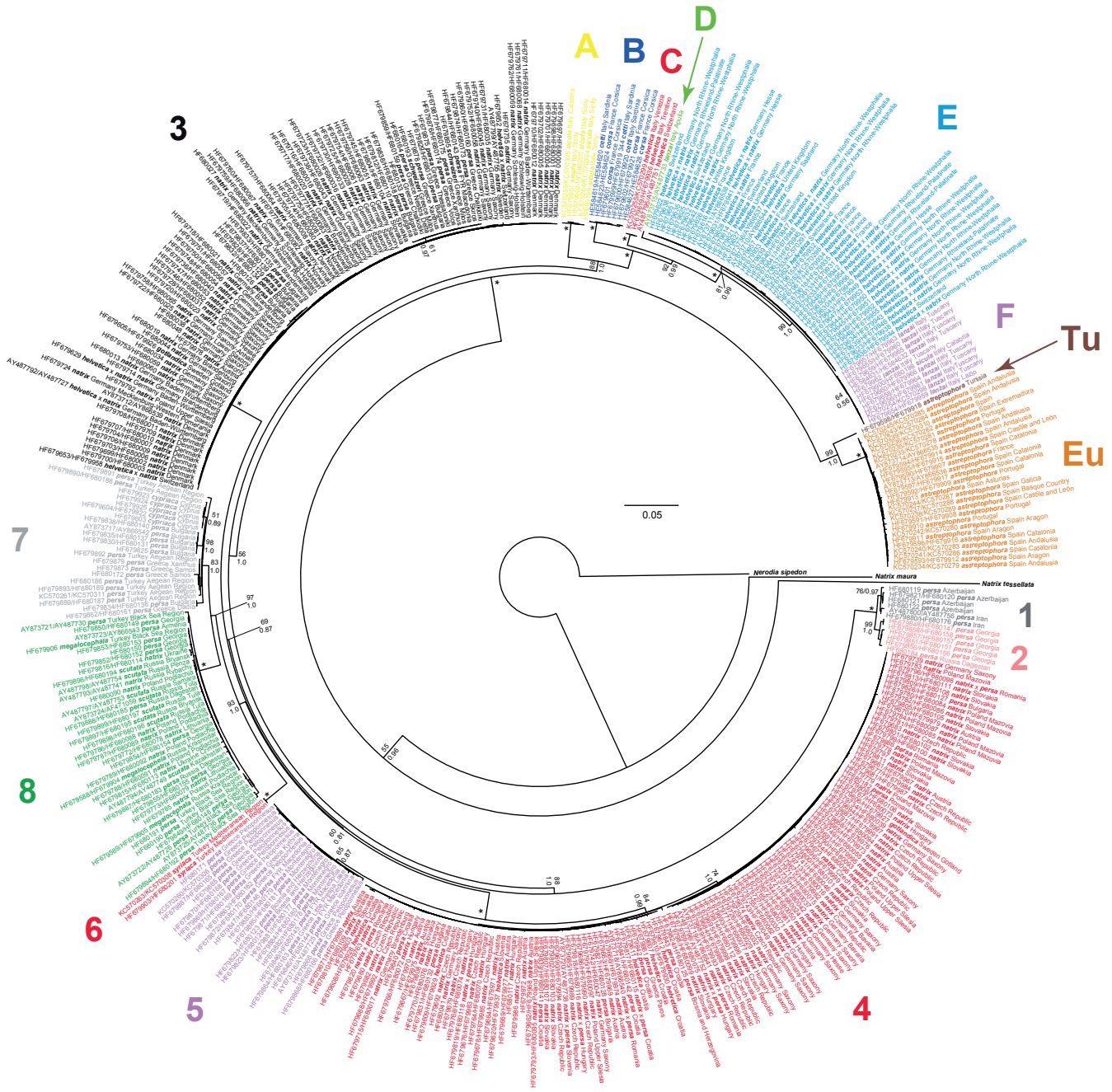

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Table S1 Grass snake samples studied and GenBank accession numbers of their DNA sequences

Taxon	Collecting site	Clade	Genbank accession numbers			
			Voucher	ND4+tRNAs	Cyt *b*	Reference
Natrix megalocephala Orlov & Tuniyev, 1987	Russia: Krasnodar: Otdalyonnyy	8	ZFMK 60732	HF679588	HF679904	This study
Natrix megalocephala Orlov & Tuniyev, 1987	Russia: Krasnodar: Tuapse	8	ZFMK 58029	HF679589	HF679905	This study
Natrix megalocephala Orlov & Tuniyev, 1987	Turkey: Black Sea Region: Hopa	8	MTD D 32025	----------	HF679906	This study
Natrix natrix astreptophora (Seoane, 1884)	France: Argelés-sur-Mer	Eu	ZFMK 89090	HF679590	HF679907	This study
Natrix natrix astreptophora (Seoane, 1884)	Portugal: S Setúbal: Torre	Eu	ZFMK 87516	HF679591	HF679908	This study
Natrix natrix astreptophora (Seoane, 1884)	Portugal: Santo Aleixo Restauracao	Eu	----------	KC570235	KC570281	This study
Natrix natrix astreptophora (Seoane, 1884)	Portugal: Serra de Sintra	Eu	ZFMK 91113	HF679592	HF679909	This study
Natrix natrix astreptophora (Seoane, 1884)	Portugal: W Loure	Eu	ZFMK 91112	----------	HF679910	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain	Eu	----------	KC570239	KC570310	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Cádiz Province	Eu	MNCN 13796	AY873714	AY866535	Guicking *et al.* (2006)
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Granada: Orijiva	Eu	----------	KC570240	KC570283	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Jaén: Fresnedilla wood	Eu	----------	KC570243	KC570284	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Río Guadalmez near Córdoba	Eu	----------	KC570245	KC570285	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Sevilla	Eu	----------	KC570233	KC570278	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Andalusia: Sierra de Grazalema: Río Guadalete	Eu	----------	KC570234	KC570279	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Aragon	Eu	ZFMK 65694	----------	HF679911	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Aragon: Jaca: San Juan de la Peña	Eu	----------	KC570242	KC570280	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Aragon: Nerín	Eu	MTD T 9650	HF679593	HF679912	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Asturias: Canges de Onís	Eu	MTD D 35537	----------	HF679913	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Basque Country: Treviño	Eu	----------	KC570237	KC570288	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Castile and León: Cilleruelo de Bezanac	Eu	----------	KC570238	KC570289	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Castile and León: Layna	Eu	ZFMK 79781	HF679594	HF679914	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Catalonia: Blanes	Eu	ZFMK 90573	HF679595	HF679915	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Catalonia: Ebro Delta	Eu	ZFMK 60734	HF679596	HF679916	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Catalonia: Osona	Eu	MVZ 200534	AY873713	AY866536	Guicking *et al.* (2006)
Natrix natrix astreptophora (Seoane, 1884)	Spain: Catalonia: Tortosa: Port de Comte	Eu	ZFMK 64913	HF679597	HF679917	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Catalonia: Tremp	Eu	----------	KC570241	KC570286	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Extremadura: Higuera de Vargas	Eu	----------	KC570236	KC570282	This study
Natrix natrix astreptophora (Seoane, 1884)	Spain: Galicia: Ourense: Reparade	Eu	----------	KC570244	KC570287	This study
Natrix natrix astreptophora (Seoane, 1884)	Tunisia: Cap Serrat	Tu	ZFMK 67196	HF679598	HF679918	This study
Natrix natrix cetti Gené, 1883	Italy: Sardinia: Belvì (Nuoro)	B	MZUF 40268	HE584619	HE584620	Fritz *et al.* (2012)

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix cetti Gené, 1883	Italy: Sardinia: Limbara Mountains	B	ZFMK 60736	HF679599	HF679919	This study
Natrix natrix cetti Gené, 1883	Italy: Sardinia: Limbara Mountains	B	ZFMK 60737	HF679600	HF679920	This study
Natrix natrix corsa (Hecht, 1930)	France: Corsica: Étang de Loto	B	MTD T 9902	HF679601	----------	This study
Natrix natrix corsa (Hecht, 1930)	France: Corsica: Gulf of Sagone	B	MTD D 39091	HF679602	HF679921	This study
Natrix natrix corsa (Hecht, 1930)	France: Corsica: Porto-Vecchio	B	MTD D 35388	HE584623	HE584624	Fritz *et al.* (2012)
Natrix natrix corsa (Hecht, 1930)	France: Corsica: Santa Giulia	B	MTD D 42489	HE584627	HE584628	Fritz *et al.* (2012)
Natrix natrix cypriaca (Hecht, 1930)	Cyprus: Larnaka: Paralimni	7	ZFMK 76296	HF679603	----------	This study
Natrix natrix cypriaca (Hecht, 1930)	Cyprus: Larnaka: Paralimni	7	ZFMK 76753	HF679604	HF679922	This study
Natrix natrix cypriaca (Hecht, 1930)	Cyprus: Xyliatos Dam	7	ZFMK 54318	----------	HF679923	This study
Natrix natrix cypriaca (Hecht, 1930)	Cyprus: Xyliatos Dam	7	ZFMK 61144	----------	HF679924	This study
Natrix natrix cypriaca (Hecht, 1930)	Cyprus: Xyliatos Dam	7	ZFMK 66524	----------	HF679925	This study
Natrix natrix gotlandica Nilson & Andrén, 1981	Sweden: Gotland	3	ZFMK 36114	HF679605	HF679926	This study
Natrix natrix gotlandica Nilson & Andrén, 1981	Sweden: Gotland	4	ZFMK 38356	HF679606	HF679927	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: 25 km N Rijeka: Gomance	4	ZFMK 54705	HF679607	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: Istria: Rovinj	4	MTD D 32031	HF679608	HF679928	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: Istria: Rovinj	4	MTD D 32032	HF679609	HF679929	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: Krk	4	MTD D 20790	HF679610	HF679930	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: Krk: N Krk	4	ZFMK 49094	HF679611	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	Croatia: Krk: Punat	4	ZFMK 54706	HF679612	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	France: Anost	E	ZFMK 61095	HF679613	HF679931	This study
Natrix natrix helvetica (Lacepède, 1789)	France: Camargue	E	ZFMK 54712	HF679614	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	France: Charolles	E	ZFMK 64930	HF679615	HF679932	This study
Natrix natrix helvetica (Lacepède, 1789)	France: Monaux (Massif Central)	E	MTD T 10092	HF679616	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	France: N La Londe les Maures	E	MTD D 40712	----------	HF679933	This study
Natrix natrix helvetica (Lacepède, 1789)	France: N Tournon	E	ZFMK 54711	HF679617	HF679934	This study
Natrix natrix helvetica (Lacepède, 1789)	France: St. Amand-Montront	E	----------	KC570252	KC570296	This study
Natrix natrix helvetica (Lacepède, 1789)	France: St. Martin de Crau	E	ZFMK 54710	HF679618	HF679935	This study
Natrix natrix helvetica (Lacepède, 1789)	France: vicinity of Paris	E	----------	AY873736	AY866537	Guicking *et al.* (2006)
Natrix natrix helvetica (Lacepède, 1789)	Italy: Trentino: Vela	C	MTD T 9656	HF679619	HF679936	This study
Natrix natrix helvetica (Lacepède, 1789)	Italy: Venezia: NW Asiago	C	----------	KC570255	KC570299	This study
Natrix natrix helvetica (Lacepède, 1789)	Slovenia: Ljubljana	4	ZFMK 65382	HF679620	HF679937	This study
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: between Neuchâtel, Bern and Biel: Grosses Moos	E	MTD T 10084	HF679621	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: between Neuchâtel, Bern and Biel: Grosses Moos	E	MTD T 10095	HF679622	----------	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: near Bern	E	MTD T 10083	HF679623	HF679938	This study
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: near Meirigen: Gadmental	E	MTD T 10085	----------	HF679939	This study
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: near Meirigen: Gadmental	E	MTD T 10086	HF679624	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: Astano (Ticino)	C	----------	AY487795	AY487751	Guicking *et al.* (2006)
Natrix natrix helvetica (Lacepède, 1789)	Switzerland: Vinzel	E	MTD T 10079	HF679625	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	United Kingdom: Kent: Isle of Sheppey	E	LSUMZ 41506	AY873710	AY866544	Guicking *et al.* (2006)
Natrix natrix helvetica (Lacepède, 1789)	United Kingdom: Norfolk: Norwich: Brandon	E	----------	KC570253	KC570297	This study
Natrix natrix helvetica (Lacepède, 1789)	United Kingdom: North Wales: Gwaith Powder	E	MTD T 9982	HF679626	----------	This study
Natrix natrix helvetica (Lacepède, 1789)	United Kingdom: North Wales: Gwaith Powder	E	MTD T 9983	HF679627	----------	This study
Natrix natrix helvetica x *natrix*	France: Strasbourg	E	MTD T 10091	HF679628	----------	This study
Natrix natrix helvetica x *natrix*	Germany: Baden-Württemberg: Böhringer See	3	MTD D 40907	HF679629	----------	This study
Natrix natrix helvetica x *natrix*	Germany: Baden-Württemberg: Lake Constance: Radolfzell	3	----------	AY487792	AY487727	Guicking *et al.* (2006)
Natrix natrix helvetica x *natrix*	Germany: Hesse: Herborn	E	ZFMK 89403	HF679630	HF679940	This study
Natrix natrix helvetica x *natrix*	Germany: Hesse: Hofheim/Lorsbuch	E	MTD D 35776	HF679631	HF679941	This study
Natrix natrix helvetica x *natrix*	Germany: Hesse: Kelkheim/Taunus	E	----------	AY873711	AY866538	Guicking *et al.* (2006)
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bad Honnef	E	ZFMK 68431	HF679632	----------	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bad Honnef	E	ZFMK 68432	HF679633	HF679942	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bad Münstereifel	E	ZFMK 92193	HF679634	HF679943	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: between Bergisch-Gladbach and Herrenstrunden	E	ZFMK 75088	HF679635	----------	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: between Hürtgenwald and Gey	E	ZFMK 70420	HF679636	HF679944	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bonn	E	ZFMK 68433	HF679637	HF679945	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bonn	E	ZFMK 92228	HF679638	----------	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bonn	E	ZFMK 92536	HF679639	HF679946	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bonn	E	ZFMK 92537	HF679640	HF679947	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Bonn (Kottenforst)	E	ZFMK 89393	HF679641	HF679948	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Drove	E	ZFMK 86786	HF679642	----------	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Königswinter, Ittenbach	E	ZFMK 82773	HF679643	HF679949	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Nideggen/Eifel	E	ZFMK 83771	HF679644	HF679950	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Sankt Augustin-Hangelar	E	ZFMK 89086	HF679645	HF679951	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Wuppertal: Morsbachtal	E	ZFMK 73645	HF679646	HF679952	This study
Natrix natrix helvetica x *natrix*	Germany: North Rhine-Westphalia: Wuppertal: Vorwerk	E	ZFMK 73646	HF679647	HF679953	This study
Natrix natrix helvetica x *natrix*	Germany: Rhineland-Palatinate: Horhausen	E	ZFMK 80876	HF679648	HF679954	This study
Natrix natrix helvetica x *natrix*	Germany: Rhineland-Palatinate: Lieser	E	MTD D 35933	HF679649	HF679955	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix helvetica x *natrix*	Germany: Rhineland-Palatinate: Maria Laach	E	ZFMK 83703	HF679650	HF679956	This study
Natrix natrix helvetica x *natrix*	Germany: Rhineland-Palatinate: Weltersburg	E	ZFMK 80875	HF679651	----------	This study
Natrix natrix helvetica x *natrix*	Germany: Saarland: Hirschberg/Neunkirchen	E	ZFMK 54321	----------	HF679957	This study
Natrix natrix helvetica x *natrix*	Switzerland: Lake Constance: near Thal	3	MTD T 10088	HF679652	----------	This study
Natrix natrix helvetica x *natrix*	Switzerland: St. Gallen: Altenrhein	3	MTD D 30567	HF679653	HF679958	This study
Natrix natrix lanzai Kramer, 1970	Italy: Apulia: Torre San Gennaro (Brindisi)	D	----------	AY873715	AY487733	Guicking *et al.* (2006)
Natrix natrix lanzai Kramer, 1970	Italy: Lazio: Monti della Tolfa	F	MZUF 31620	HF679654	HF679959	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Chiaveretto	F	ZFMK 64931	HF679655	HF679960	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: E Campagrina	F	----------	KC570254	KC570298	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Florence	F	MZUF 39870	HF679656	HF679961	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Florence: Scandicci	F	MZUF 38124	----------	HF679962	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Florence: Vaglia	F	MZUF 40267	HE584631	HE584632	Fritz *et al.* (2012)
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Livorno	F	MTD T 9981	HF679657	HF679963	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Pisa	F	MTD D 33721	HF679658	HF679964	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Sienna: Gaiole	F	MZUF 40189	HF679659	HF679965	This study
Natrix natrix lanzai Kramer, 1970	Italy: Tuscany: Uccellina	F	MZUF 7388	HF679660	HF679966	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Andau	4	ZFMK 65105	HF679661	HF679967	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Apetlon	4	ZFMK 68656	HF679662	HF679968	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Breitenbrunn	4	ZFMK 55995	HF679663	HF679969	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Hölle	4	ZFMK 51746	----------	HF679970	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Illmitz	4	ZFMK 74926	HF679664	HF679971	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Illmitz	4	ZFMK 83775	----------	HF679972	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Jois	4	ZFMK 65687	HF679665	HF679973	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Weiden am See	4	ZFMK 51670	HF679666	HF679974	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Burgenland: Zitzmannsdorfer Wiesen	4	ZFMK 91242	HF679667	HF679975	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Lower Austria: Bergern	4	MTD T 9912	HF679668	HF679976	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Lower Austria: Losenheim	4	ZFMK 83922	HF679669	HF679977	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Styria: Admont/Gesäuse	3	ZFMK 73648	----------	HF679978	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Vienna: Donauinsel	4	MTD T 9903	HF679670	HF679979	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Vienna: Donauinsel	4	MTD T 9904	HF679671	HF679980	This study
Natrix natrix natrix (Linnaeus, 1758)	Austria: Vienna: Donauinsel	4	MTD T 9905	HF679672	HF679981	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Bartošovice	4	MTD T 9888	HF679673	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Běstvina	4	MTD T 9878	HF679674	----------	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: between Horušice and Ruda	4	MTD T 9579	HF679675	HF679982	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: between Horušice and Ruda	4	MTD T 9580	HF679676	HF679983	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: České Budějovice	4	MTD T 8963	HF679677	HF679984	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Halenkovice	4	MTD T 9886	HF679678	HF679985	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Jankovice	4	MTD T 9880	HF679679	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Jankovice	4	MTD T 9885	----------	HF679986	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Kudlovice	4	MTD T 9882	HF679680	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Kudlovická dolina	4	MTD T 8974	HF679681	HF679987	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Chřiby Mountains: Salaš	4	MTD T 9879	HF679682	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Dolní Lištná	4	MTD T 8636	HF679683	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Dolní Lištná	4	MTD T 8642	HF679684	HF679988	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Heřmanovice	4	MTD T 8962	HF679685	HF679989	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Heřmanovice	4	MTD T 8964	HF679686	HF679990	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Kamýk: Litoměřice	4	MTD T 8972	HF679687	HF679991	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Karviná: Olšiny	4	MTD T 8634	HF679688	HF679992	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Kokořín	4	MTD T 8966	HF679689	HF679993	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Kokořínský Důl	4	MTD T 8965	HF679690	HF679994	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Kundratice: Litoměřice	4	MTD T 8971	HF679691	HF679995	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Moravský Písek	4	MTD T 9881	HF679692	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Staré Hamry	4	MTD T 8643	HF679693	HF679996	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Vysoká: Jihlava	4	MTD T 8969	HF679694	HF679997	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Vysoká: Jihlava	4	MTD T 8970	HF679695	HF679998	This study
Natrix natrix natrix (Linnaeus, 1758)	Czech Republic: Zlaté Hory	4	MTD T 8961	HF679696	HF679999	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Funen: NNW Svendborg	3	MTD T 9652	HF679697	HF680000	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Funen: NNW Svendborg	3	MTD T 9653	HF679698	HF680001	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Funen: NNW Svendborg	3	MTD T 9654	HF679699	HF680002	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Funen: NNW Svendborg	3	MTD T 9655	HF679700	HF680003	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Jutland	3	----------	AY873712	AY866539	Guicking *et al.* (2006)
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Jutland: SE Gammel Rye	3	MTD T 9913	HF679701	HF680004	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Jutland: SE Gammel Rye	3	MTD T 9914	HF679702	HF680005	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Jutland: SE Gammel Rye	3	MTD T 9915	HF679703	HF680006	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Jutland: SE Skanderborg	3	MTD T 9916	HF679704	HF680007	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Langeland: S Tranekær	3	MTD T 9651	HF679705	HF680008	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Zealand: N Præstø: Feddet	3	MTD T 9649	HF679706	HF680009	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Zealand: S Borup	3	MTD T 9269	HF679707	HF680010	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Zealand: S Borup	3	MTD T 9270	HF679708	HF680011	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Zealand: S Borup	3	MTD T 9271	HF679709	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Denmark: Zealand: W Køge	3	MTD T 9648	HF679710	HF680012	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Baden-Württemberg: between Leonberg and Gerlingen	3	ZFMK 78777	----------	HF680013	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Baden-Württemberg: Oberweissach	3	MTD D 39068	HF679711	HF680014	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Bavaria: Mammendorf	4	ZFMK 71166	HF679712	HF680015	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Bavaria: Passau	4	ZFMK 56016	HF679713	HF680016	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Brieske	3	MTD D 47570	HF679714	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Cumlosen	4	MTD D 29503	HF679715	HF680017	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Guteborn	3	MTD D 47197	HF679716	HF680018	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Guteborn	3	MTD T 3813	----------	HF680019	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Hosena	3	MTD D 45304	HF679717	HF680020	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Perleberg	3	MTD D 29504	HF679718	HF680021	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Brandenburg: Senftenberg	3	ZFMK 76358	HF679719	HF680022	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Hesse: Flieden	3	ZFMK 82929	HF679720	HF680023	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Hesse: Steinau	4	ZFMK 82930	HF679721	HF680024	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Lower Saxony: Lachendorf	3	ZFMK 86134	HF679722	HF680025	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Lower Saxony: Leiferde	3	ZFMK 89088	HF679723	HF680026	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Mecklenburg-Western Pomerania: Güstrow	3	ZFMK 61035	----------	HF680027	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Mecklenburg-Western Pomerania: Neukloster	3	ZFMK 47435	HF679724	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Bad Düben	3	MTD D 39712	HF679725	HF680028	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Bad Gottleuba	4	MTD D 39331	HF679726	HF680029	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Bärnsdorf	4	MTD D 47231	HF679727	HF680030	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Biehla	3	MTD T 3183	HF679728	HF680031	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Borna	4	MTD D 31066	HF679729	HF680032	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Chemnitz	3	MTD D 47317	HF679730	HF680033	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Chemnitz	3	MTD D 47318	----------	HF680034	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Cunnersdorf-Biehla	3	MWLK 626/05	HF679731	HF680035	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dahlen	3	MTD D 46238	HF679732	HF680036	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dorfhain	4	MTD D 47638	HF679733	HF680037	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dresden	3	MTD D 45527	----------	HF680038	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dresden	4	MTD D 47639	HF679734	HF680039	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dresden	3	MTD D 47728	HF679735	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Dubringer Moor	3	MWLK 51/04	HF679736	HF680040	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Großdittmannsdorf	4	MTD D 38701	----------	HF680041	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Großdittmannsdorf	4	MWLK 370/04	HF679737	HF680042	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Guttau	3	MWLK 776/05	HF679738	HF680043	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Hartmannsgrün	3	MTD D 47002	----------	HF680044	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Kamenz	4	MWLK 466/05	HF679739	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Kamenz	3	MWLK 988/05	HF679740	HF680045	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Kreischa-Lockmittel	4	MTD D 40611	HF679741	HF680046	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Langebrück	4	MTD D 48178	HF679742	HF680047	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Lauterbach	3	MTD D 45996	----------	HF680048	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Lengefeld/Neunzehnhain	4	MTD T 9528	HF679743	HF680049	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Linz	4	MWLK 76/01	HF679744	HF680050	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Michalken	3	MWLK 371/01	HF679745	HF680051	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Michalken	3	MWLK 372/01	HF679746	HF680052	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Milkel	3	MTD D 45006	HF679747	HF680053	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Neukirch	3	MWLK 311/05	HF679748	HF680054	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Oberschöna	4	MTD D 47429	HF679749	HF680055	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Oppach	3	MTD D 32501	HF679750	HF680056	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Ottendorf-Okrilla	3	MTD D 42679	HF679751	HF680057	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Rehnsdorf	3	MWLK 284/99	HF679752	HF680058	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Schwepnitz	3	MWLK 53/04	HF679753	HF680059	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Teicha	3	MWLK 795/05	----------	HF680060	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Waldenburg	3	MTD D 42680	HF679754	HF680061	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Weixdorf	4	MTD D 47258	HF679755	HF680062	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony: Wittichenau	3	MWLK 144/01	HF679756	HF680063	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony-Anhalt: Altenbrak	3	MTD D 41418	HF679757	HF680064	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Saxony-Anhalt: Sangerhausen	3	ZFMK 89087	HF679758	HF680065	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Schleswig-Holstein: Kiel: Landwehr	3	ZFMK 62405	HF679759	HF680066	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Schleswig-Holstein: Kiel-Elmschenhagen	3	ZFMK 85184	HF679760	HF680067	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Schleswig-Holstein: Probstei: Hagener Moor	3	ZFMK 92535	HF679761	HF680068	This study
Natrix natrix natrix (Linnaeus, 1758)	Germany: Schleswig-Holstein: Probsteierhagen	3	ZFMK 73639	HF679762	HF680069	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Balatonszepezd	4	ZFMK 58024	HF679763	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: between Sarród and Fertóujlak	4	ZFMK 88062	HF679764	HF680070	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Györ	4	ZFMK 61029	HF679765	HF680071	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Hansági-főcsatorna	4	ZFMK 91241	HF679766	HF680072	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Hortobágy	4	MTD T 9906	HF679767	HF680073	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Kunpeszér	4	MTD T 7571	HF679768	HF680074	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Kunpeszér	4	MTD T 7574	HF679769	HF680075	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: S Budapest	4	ZFMK 85905	HF679770	HF680076	This study
Natrix natrix natrix (Linnaeus, 1758)	Hungary: Tihany	4	ZFMK 74927	HF679771	HF680077	This study
Natrix natrix natrix (Linnaeus, 1758)	Lithuania: Marcinkonys	8	MTD T 8967	HF679772	HF680078	This study
Natrix natrix natrix (Linnaeus, 1758)	Lithuania: Trasininkas	8	MTD T 8968	HF679773	HF680079	This study
Natrix natrix natrix (Linnaeus, 1758)	Norway: Akershus: Son	3	ZMUO 54-96	HF679774	HF680080	This study
Natrix natrix natrix (Linnaeus, 1758)	Norway: Oslo: Grønmo: Sølvdobla	3	ZMUO 31-83	HF679775	HF680081	This study
Natrix natrix natrix (Linnaeus, 1758)	Norway: Oslo: Maridalen: Skjervensaga	3	ZMUO 26-85	HF679776	HF680082	This study
Natrix natrix natrix (Linnaeus, 1758)	Norway: Telemark: Levangshalvøya	3	ZMUO 1H 10-2000	HF679777	HF680083	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9965	HF679778	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9966	HF679779	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9968	HF679780	HF680084	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9969	HF679781	HF680085	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9972	HF679782	HF680086	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 9974	HF679783	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Mazovia: Kampinos National Park	4	MTD T 10318	HF679784	HF680087	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 9975	HF679785	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 9976	HF679786	HF680088	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 9977	HF679787	HF680089	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 10314	----------	HF680090	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 10315	HF679788	HF680091	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Podlachia: Białowieża National Park	8	MTD T 10316	HF679789	HF680092	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: Golczowice	4	MTD T 9637	HF679790	HF680093	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: Kotórz Wielki	4	MTD T 10025	HF679791	HF680094	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: Kotórz Wielki	3	MTD T 10026	HF679792	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: Krośnica	4	MTD T 9635	HF679793	HF680095	This study
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: Ligota Tułowicka	4	MTD T 9638	HF679794	HF680096	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix natrix (Linnaeus, 1758)	Poland: Upper Silesia: SE Opole	4	MTD T 9636	HF679795	HF680097	This study
Natrix natrix natrix (Linnaeus, 1758)	Russia: Kaliningrad Oblast: Rybachy	8	----------	AY487793	AY487741	Guicking *et al.* (2006)
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: between Topoľa and Runina	4	MTD T 8975	HF679797	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Bujakovo	4	MTD T 9017	HF679798	HF680099	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Červený Kláštor	4	MTD T 8648	HF679799	HF680100	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Devín	4	MTD T 9014	HF679800	HF680101	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Janova Lehota	4	MTD T 8641	HF679801	HF680102	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Jurský Šúr	4	MTD T 9011	HF679802	HF680103	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Kamenica nad Hronom	4	MTD T 8635	HF679803	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Kraľovany: Kraľovanská dolina	3	MTD T 8632	HF679804	HF680104	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Muránska Lehota	4	MTD T 9019	HF679805	HF680105	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Regetovka	4	MTD T 9018	HF679806	HF680106	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Rusovce	4	MTD T 9016	HF679807	HF680107	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Silica: Farárova jama	4	MTD T 9009	HF679808	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Strážne	4	MTD T 8637	HF679809	HF680108	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Stupava	4	MTD T 9020	HF679810	HF680109	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Svetlice	4	MTD T 8638	HF679811	----------	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Tatra Mountains: Žiar	3	MTD T 9015	HF679812	HF680110	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Ulič	4	MTD T 9022	HF679813	HF680111	This study
Natrix natrix natrix (Linnaeus, 1758)	Slovakia: Weitov lom	4	MTD T 9010	HF679814	HF680112	This study
Natrix natrix natrix (Linnaeus, 1758)	Sweden: Småland: Högsby	3	----------	AY487799	AY487755	Guicking *et al.* (2006)
Natrix natrix natrix (Linnaeus, 1758)	Ukraine: Crimea: Luchyste	8	MTD D 42725	HF679815	HF680113	This study
Natrix natrix natrix (Linnaeus, 1758)	Ukraine: Oblast Herson: Hola Prystan': Herois'ke	8	MTD D 42724	HF679816	HF680114	This study
Natrix natrix natrix x *persa*	Croatia: NE Lupoglav	4	ZFMK 91558	HF679817	HF680115	This study
Natrix natrix natrix x *persa*	Hungary: Barcs	4	ZFMK 65686	HF679818	HF680116	This study
Natrix natrix natrix x *persa*	Hungary: Pécsi-tó	4	----------	AY487796	AY487752	Guicking (2004)
Natrix natrix natrix x *persa*	Hungary: Somogyszob	4	ZFMK 82795	----------	HF680117	This study
Natrix natrix natrix x *persa*	Hungary: Villány	4	MTD T 7594	HF679819	HF680118	This study
Natrix natrix natrix x *persa*	Romania: Cerna Sat	4	MTD T 8640	HF679820	----------	This study
Natrix natrix natrix x *persa*	Romania: Cluj-Napoca	4	----------	AY873719	AY866541	Guicking *et al.* (2006)
Natrix natrix natrix x *persa*	Romania: Geoagiu de Sus	4	MTD T 8650	HF679796	HF680098	This study
Natrix natrix natrix x *persa*	Romania: Tulcea Region	4	----------	AY873718	AY866540	Guicking *et al.* (2006)
Natrix natrix natrix x *persa*	Slovenia: Zalec	4	----------	AY873720	AY487738	Guicking *et al.* (2006)

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix persa (Pallas, 1814)	Armenia: Ankavan	8	ROM 26842	AY873723	AY866543	Guicking *et al.* (2006)
Natrix natrix persa (Pallas, 1814)	Azerbaijan: Calilabad	1	MTD T 8955	----------	HF680119	This study
Natrix natrix persa (Pallas, 1814)	Azerbaijan: Calilabad	1	MTD T 8956	HF679821	HF680120	This study
Natrix natrix persa (Pallas, 1814)	Azerbaijan: Istisu: near Astara	1	MTD T 3680	----------	HF680121	This study
Natrix natrix persa (Pallas, 1814)	Azerbaijan: Qazimammad	1	MTD T 8957	----------	HF680122	This study
Natrix natrix persa (Pallas, 1814)	Bosnia and Herzegovina: Hutovo Blato	5	MTD T 8644	HF679822	HF680123	This study
Natrix natrix persa (Pallas, 1814)	Bosnia and Herzegovina: Hutovo Blato	5	MTD T 8645	HF679823	HF680124	This study
Natrix natrix persa (Pallas, 1814)	Bosnia and Herzegovina: Maglič Mountain: Prijevor	4	MTD T 8976	HF679824	HF680125	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Albena	3	MTD D 40692	----------	HF680126	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Arkutino	7	ZFMK 59679	HF679825	----------	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Bezhanovo	4	MTD T 8653	HF679826	HF680127	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Etropole	4	MTD T 8654	HF679827	HF680128	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Levunovo	3	MTD D 18923	HF679828	HF680129	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Levunovo	3	MTD D 18924	HF679829	HF680130	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Malko Gradište (pass)	7	MTD T 9321	HF679830	HF680131	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Malko Tarnovo District	7	CAS 219930	AY873717	AY866542	Guicking *et al.* (2006)
Natrix natrix persa (Pallas, 1814)	Bulgaria: Mičurin	4	MTD D 29984	HF679831	HF680132	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Novo Selo	3	MTD T 9910	----------	HF680133	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Pejo Javorov	3	MTD T 9012	HF679832	HF680134	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Pejo Javorov	3	MTD T 9013	HF679833	HF680135	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Ropotamo	7	MTD T 9318	HF679834	HF680136	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Ropotamo	7	MTD T 9319	HF679835	HF680137	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Sandanska Bistrica Valley	3	MTD D 20867	HF679836	HF680138	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Sandanski	3	MTD D 19566	HF679837	HF680139	This study
Natrix natrix persa (Pallas, 1814)	Bulgaria: Shipka pass	7	MTD T 8649	HF679838	HF680140	This study
Natrix natrix persa (Pallas, 1814)	Croatia: Benkovac	4	ZFMK 92199	HF679839	HF680141	This study
Natrix natrix persa (Pallas, 1814)	Croatia: near Šibenik: Krka falls	4	ZFMK 54709	HF679840	----------	This study
Natrix natrix persa (Pallas, 1814)	Croatia: Plitvice	4	ZFMK 54707	HF679841	HF680142	This study
Natrix natrix persa (Pallas, 1814)	Croatia: Vransko Jezero	4	MTD T 9873	HF679842	----------	This study
Natrix natrix persa (Pallas, 1814)	Croatia: Vransko Jezero	4	MTD T 9874	HF679843	----------	This study
Natrix natrix persa (Pallas, 1814)	Former Yugoslav Republic of Macedonia: Jakubica Mountains	3	MTD T 9911	HF679844	HF680143	This study
Natrix natrix persa (Pallas, 1814)	Former Yugoslav Republic of Macedonia: National Park Galičica	5	MTD T 9877	HF679845	HF680144	This study
Natrix natrix persa (Pallas, 1814)	Former Yugoslav Republic of Macedonia: Porodin	5	MTD T 9909	HF679846	HF680145	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix persa (Pallas, 1814)	Former Yugoslav Republic of Macedonia: Stenje	5	MTD T 8633	HF679847	HF680146	This study
Natrix natrix persa (Pallas, 1814)	Georgia: approx. 80 km NE Telavi	2	MTD D 26659	HF679848	HF680147	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Batumi	8	ZFMK 76546	HF679849	HF680148	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Batumi	8	----------	AY873725	AY487736	Guicking *et al.* (2006)
Natrix natrix persa (Pallas, 1814)	Georgia: Borshomi	8	ZFMK 73721	HF679850	HF680149	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Borshomi	8	ZFMK 73722	----------	HF680150	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Kumisi	2	MTD T 9337	HF679851	HF680151	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Kutaisi	8	MTD T 8960	HF679852	HF680152	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Mtskheta	8	MTD T 9341	HF679853	HF680153	This study
Natrix natrix persa (Pallas, 1814)	Georgia: N Stepanzminda	8	MTD T 9339	HF679854	HF680154	This study
Natrix natrix persa (Pallas, 1814)	Georgia: N Stepanzminda	8	MTD T 9340	HF679855	HF680155	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Sakdrioni	2	MTD T 9338	HF679856	HF680156	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Telavi	2	MTD T 8958	HF679857	HF680157	This study
Natrix natrix persa (Pallas, 1814)	Georgia: Telavi	2	MTD T 8959	HF679858	HF680158	This study
Natrix natrix persa (Pallas, 1814)	Greece: Chalkidiki: N Stratoni	3	ZFMK 62945	HF679859	HF680159	This study
Natrix natrix persa (Pallas, 1814)	Greece: Chalkidiki: N Stratoni	3	ZFMK 69946	HF679860	HF680160	This study
Natrix natrix persa (Pallas, 1814)	Greece: Cyclades: Paros	4	MTD D 25827	HF679861	----------	This study
Natrix natrix persa (Pallas, 1814)	Greece: Dadía	7	MTD T 8646	HF679862	HF680161	This study
Natrix natrix persa (Pallas, 1814)	Greece: Ioánnina	5	----------	AY873716	AY487725	Guicking *et al.* (2006)
Natrix natrix persa (Pallas, 1814)	Greece: Kythira	5	MTD D 25843	HF679863	HF680162	This study
Natrix natrix persa (Pallas, 1814)	Greece: Little Prespa Lake near Florina	5	ZFMK 54702	HF679864	HF680163	This study
Natrix natrix persa (Pallas, 1814)	Greece: Nestos Delta	3	ZFMK 71679	----------	HF680164	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Itilo	5	ZFMK 62500	HF679865	----------	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Kalogria	5	ZFMK 65179	HF679866	----------	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Kalogria	5	----------	KC570260	KC570306	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Kato Kastanea	5	ZFMK 71680	HF679867	HF680165	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Limni Strofilia near Kalogria	5	ZFMK 92217	HF679868	HF680166	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Pylos	5	ZFMK 82103	HF679869	HF680167	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Pylos	5	ZFMK 83029	HF679870	HF680168	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Pylos	5	ZFMK 84037	HF679871	HF680169	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Pylos	5	ZFMK 86043	HF679872	HF680170	This study
Natrix natrix persa (Pallas, 1814)	Greece: Peloponnesus: Pylos	5	ZFMK 86045	----------	HF680171	This study
Natrix natrix persa (Pallas, 1814)	Greece: Samos	7	ZFMK 89415	HF679873	----------	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix persa (Pallas, 1814)	Greece: Samos: Agios Konstantinos	7	ZFMK 65117	----------	HF680172	This study
Natrix natrix persa (Pallas, 1814)	Greece: Skiros	3	MTD T 9907	HF679874	HF680173	This study
Natrix natrix persa (Pallas, 1814)	Greece: Thessaloniki	3	MTD T 8639	HF679875	----------	This study
Natrix natrix persa (Pallas, 1814)	Greece: Volos	3	MTD D 29269	HF679876	HF680174	This study
Natrix natrix persa (Pallas, 1814)	Greece: Xanthus: Avdira	3	ZFMK 62956	HF679877	HF680175	This study
Natrix natrix persa (Pallas, 1814)	Greece: Xanthus: Avdira	3	ZFMK 92957	HF679878	----------	This study
Natrix natrix persa (Pallas, 1814)	Greece: Xanthus: Jasmos	7	ZFMK 62958	HF679879	----------	This study
Natrix natrix persa (Pallas, 1814)	Iran: Kermanshah Province	1	----------	AY487800	AY487756	Guicking (2004)
Natrix natrix persa (Pallas, 1814)	Iran: Nowshahr	1	MTD T 8954	HF679880	HF680176	This study
Natrix natrix persa (Pallas, 1814)	Montenegro: near island Ada	5	ZFMK 92216	HF679881	HF680177	This study
Natrix natrix persa (Pallas, 1814)	Montenegro: Sasoviči, Zelenica, Boka kotorska	5	MTD T 9875	HF679882	HF680178	This study
Natrix natrix persa (Pallas, 1814)	Montenegro: Skutari Lake: Limljani	5	ZFMK 92205	HF679883	HF680179	This study
Natrix natrix persa (Pallas, 1814)	Montenegro: Skutari Lake: Limljani	5	ZFMK 92206	HF679884	HF680180	This study
Natrix natrix persa (Pallas, 1814)	Montenegro: Spuz	5	MTD T 9908	HF679885	HF680181	This study
Natrix natrix persa (Pallas, 1814)	Romania: Chilia Veche	4	MTD T 9889	HF679886	----------	This study
Natrix natrix persa (Pallas, 1814)	Romania: Constanța: Histria	4	ZFMK 82775	----------	HF680182	This study
Natrix natrix persa (Pallas, 1814)	Russia: Dagestan: Machačkala	8	ZFMK 65692	HF679887	HF680183	This study
Natrix natrix persa (Pallas, 1814)	Russia: Dagestan: Samur	2	ZFMK 62940	----------	HF680184	This study
Natrix natrix persa (Pallas, 1814)	Russia: Dagestan: Tatayurt	8	ZFMK 62936	HF679888	HF680185	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: between Söke and Doğanbey	7	ZFMK 47034	----------	HF680186	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Dalyan	7	ZFMK 82946	HF679889	HF680187	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Selçuk	7	MTD D 25227	HF679890	HF680188	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Selçuk	7	MTD D 25228	HF679891	----------	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Selçuk	7	MTD D 25229	HF679892	----------	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Selçuk	7	MTD D 25230	HF679893	HF680189	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Aegean Region: Tavas	7	----------	KC570261	KC570311	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Black Sea Region: between Hopa and Arhavi	8	ZFMK 71143	----------	HF680190	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Black Sea Region: between Hopa and Arhavi	8	ZFMK 71144	----------	HF680191	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Black Sea Region: Borçka	8	ZFMK 71145	HF679894	HF680192	This study
Natrix natrix persa (Pallas, 1814)	Turkey: Black Sea Region: Hattuşa (Sarıkale)	8	----------	AY873722	AY487726	Guicking *et al.* (2006)
Natrix natrix persa (Pallas, 1814)	Turkey: Black Sea Region: Yeniçaga	8	----------	AY873721	AY487730	Guicking *et al.* (2006)
Natrix natrix schweizeri L. Müller, 1932	Greece: Cyclades: Milos	3	MTD D 19183	----------	HF680193	This study
Natrix natrix schweizeri L. Müller, 1932	Greece: Cyclades: Milos	3	ZFMK 85407	HF679895	----------	This study

■ Table S1 continued ■			Genbank accession numbers			
Taxon	**Collecting site**	**Clade**	**Voucher**	**ND4+tRNAs**	**Cyt *b***	**Reference**
Natrix natrix scutata (Pallas, 1771)	Kazakhstan: Emba River	8	----------	AY487794	AY487749	Guicking *et al.* (2006)
Natrix natrix scutata (Pallas, 1771)	Russia: Bryansk	8	ZFMK 91040	HF679896	HF680194	This study
Natrix natrix scutata (Pallas, 1771)	Russia: Bryansk	8	ZFMK 91041	HF679897	HF680195	This study
Natrix natrix scutata (Pallas, 1771)	Russia: Penza District	8	----------	AY487798	AY487754	Guicking (2004)
Natrix natrix scutata (Pallas, 1771)	Russia: Samara District	8	----------	AY487797	AY487753	Guicking *et al.* (2006)
Natrix natrix scutata (Pallas, 1771)	Russia: Tula District	8	CAS 175878	AY873724	AF471059	Lawson *et al.* (2005), Guicking *et al.* (2006)
Natrix natrix scutata (Pallas, 1771)	Russia: Tula: S Tula: Chopr River	8	ZFMK 70614	HF679898	HF680196	This study
Natrix natrix scutata (Pallas, 1771)	Russia: Tula: S Tula: Chopr River	8	ZFMK 72270	HF679899	HF680197	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Calabria	F	ZFMK 90574	HF679900	HF680198	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Calabria: Serro San Bruno	A	----------	KC570265	KC570305	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Sicily: Agrigento: Siculiana: Biosphere Reserve Torre Salsa	A	MTD T 2116	HF679901	----------	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Sicily: Caronia (Castello di Marescotto)	A	----------	KC570264	KC570304	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Sicily: Lago di Pergusa	A	MZUF 24175	----------	HF680199	This study
Natrix natrix sicula (Cuvier, 1829)	Italy: Sicily: Siracusa: Vendicari	A	MTD T 8464	HF679902	HF680200	This study
Natrix natrix syriaca (Hecht, 1930)	Turkey: Mediterranean Region: Burmaz (near Botaş)	6	ZFMK 71176	HF679903	HF680201	This study
Natrix natrix syriaca (Hecht, 1930)	Turkey: Mediterranean Region: SW Osmaniye	6	----------	KC570263	KC570308	This study
Outgroups:						
Natrix maura (Linnaeus, 1758)	Spain: Southern Spain	—	MNCN 12016	AY873708	AY866530	Guicking *et al.* (2006)
Natrix tessellata (Laurenti, 1768)	Armenia: Geolazar	—	ROM 23418	AY873734	AY866531	Guicking *et al.* (2006)
Nerodia sipedon (Linnaeus, 1758)	USA: Tennessee	—	----------	JF964960	JF964960	GenBank

Museum acronyms of voucher specimens: CAS – California Academy of Sciences, San Francisco, California; LSUMZ – Louisiana Museum of Natural History, Baton Rouge, Louisiana; MNCN – Museo Nacional de Ciencias Naturales, Madrid; MTD D – Museum of Zoology, Senckenberg Dresden (Herpetological Collection); MTD T – Museum of Zoology, Senckenberg Dresden (Tissue Collection); MWLK – Museum der Westlausitz, Kamenz; MZUF – Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola"; ROM – Royal Ontario Museum, Department of Natural History, Toronto, Ontario; ZFMK – Zoologisches Forschungsmuseum Alexander Koenig, Bonn; ZMUO – Zoologisk museum, Universitetet i Oslo.

Table S2 Primers used for PCR and sequencing. For primer combinations and PCR conditions, see Table S3

mtDNA fragment	Primer	Direction	Primer sequence (5' to 3')	Reference
ND4+tRNAs	ND4ab	Forward	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Guicking *et al.* (2006)
ND4	Natrix_ND4_For1	Forward	GGATCAAT(AG)GT(AG)CTAGCAGC	This study
ND4	Natrix_ND4_For2	Forward	CAAACAGACTTAAAATC(AC)CT	This study
ND4+tRNAs	Natrix_ND4_For3	Forward	CCCAGCATTAATTT(CT)AC(AGT)GG	This study
ND4	ND4F_nat	Forward	GGATCAAT(AG)GTACTAGCAGC	This study
ND4+tRNAs	tRNA-leu	Reverse	CATTACTTTTACTTGGATTTGCACCA	Guicking *et al.* (2006)
ND4	Natrix_ND4_Rev1_neu	Reverse	(CT)AG(AG)CTTCA(GT)TGTGTTTGGA	This study
ND4	Natrix_ND4_Rev2	Reverse	AATTGTTGTTGGGCATCA	This study
ND4	ND4R_nat	Reverse	ATTCAGGTTTTAT(CT)GAGATAAG	This study
cyt *b*	L14724NAT	Forward	GACCTGCGGTCCGAAAAACCA	Guicking *et al.* (2006)
cyt *b*	Natrix_Cytb_For2	Forward	CCCTACGGATGAATAATACAAAA(CT)AC	This study
cyt *b*	Natrix_Cytb_For3	Forward	CTAGGAAA(CT)ACCCTCACAACC	This study
cyt *b*	Natrix_Cytb_For4	Forward	TACCACTCACACAAAGA(CT)ATA(CT)TA(CT)	This study
cyt *b*	Natrix_Cytb_For5	Forward	ATAAACT(CGT)GG(AGT)GG(AG)ACAA	This study
cyt *b*	CBF_nat	Forward	GTAGGCCTAAATATTTC(AG)ACCTG	This study
cyt *b*	inCBF_nat	Forward	ACCCTCACAACCTGACTCTG	This study
cyt *b*	Thrsnr2	Reverse	CTTTGGTTTACAAGAACAATGCTTTA	Guicking *et al.* (2006)
cyt *b*	Natrix_Cytb_Rev2	Reverse	AGGGCAAAGAATCGGGTT	This study
cyt *b*	Natrix_Cytb_Rev3_neu	Reverse	TTAATGTGTTG(AG)GGGGTTACTA	This study
cyt *b*	Natrix_Cytb_Rev4_neu	Reverse	AGGTGTGGGTGAA(ACT)GG(CT)A	This study
cyt *b*	inCBR_nat	Reverse	TCAGTGTGAAGAAGTATAATGTG	This study
cyt *b*	CBR_nat	Reverse	GTTGTTATAAAAAATGT(AG)AAGTA	This study

Table S3 PCR protocols

		Thermocycling conditions					
mtDNA fragment	**Primer combinations**	**ID**	**C**	**D**	**A**	**E**	**FE**
ND4+tRNAs	ND4ab, tRNA-leu	94°C, 5 min	35	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min
ND4	ND4ab, Natrix_ND4_Rev2	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 40 s	72°C, 10 min
ND4	Natrix_ND4_For1, Natrix_ND4_Rev1_neu	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
ND4	Natrix_ND4_For2, Natrix_ND4_Rev2	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
ND4+tRNAs	Natrix_ND4_For3, tRNA-leu	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
ND4	ND4F_nat, ND4R_nat	94°C, 5 min	35	94°C, 30 s	50°C, 45 s	72°C, 80 s	72°C, 5 min
cyt *b*	L14724NAT, Thrsnr2	94°C, 5 min	35	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min
cyt *b*	L14724NAT, Natrix_Cytb_Rev2	94°C, 5 min	40	94°C, 45 s	54°C, 45 s	72°C, 40 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For2, Natrix_Cytb_Rev2	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For3, Natrix_Cytb_Rev3_neu	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For3, Thrsnr2	94°C, 5 min	40	94°C, 45 s	54°C, 45 s	72°C, 40 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For4, Natrix_Cytb_Rev4_neu	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 30 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For4, Thrsnr2	94°C, 5 min	40	94°C, 45 s	56°C, 45 s	72°C, 40 s	72°C, 10 min
cyt *b*	Natrix_Cytb_For5, Thrsnr2	94°C, 5 min	40	94°C, 45 s	54°C, 45 s	72°C, 30 s	72°C, 10 min
cyt *b*	CBF_nat/inCBR_nat	94°C, 5 min	35	94°C, 30 s	52°C, 45 s	72°C, 70 s	72°C, 5 min
cyt *b*	inCBF_nat/CBR_nat	94°C, 5 min	35	94°C, 30 s	44°C, 45 s	72°C, 80 s	72°C, 5 min

Abbrevations: ID = initial denaturing, C = number of cycles, D = denaturing, A = annealing, E = extension, FE = final extension.

Table S4 Mean uncorrected *p* distances (percentages) between and within mtDNA clades of grass snakes (top: ND4+tRNAs, bottom: cyt *b*). Below the diagonal, divergences between groups; on the diagonal, within group divergences in boldface

ND4+tRNAs	***n***	**Tu**	**Eu**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**A**	**B**	**C**	**D**	**E**	**F**
Tu	1	—															
Eu	23	3.13	**0.12**														
1	3	5.12	5.78	**0.33**													
2	5	5.17	6.12	0.72	**0.13**												
3	78	5.41	6.13	4.49	4.67	**0.20**											
4	118	5.98	6.73	4.66	4.70	5.33	**0.21**										
5	22	5.60	6.25	4.47	4.53	5.06	0.55	**0.17**									
6	2	5.69	5.79	4.63	4.90	4.32	4.41	4.35	**0**								
7	17	4.95	5.70	3.70	3.97	4.28	3.63	3.41	2.95	**0.63**							
8	33	6.17	6.76	5.01	5.25	5.56	4.93	4.91	3.99	2.37	**0.23**						
A	4	6.29	6.74	6.08	6.55	4.85	7.15	6.61	6.14	5.84	7.16	**0.08**					
B	7	4.74	5.43	4.11	4.41	4.79	5.18	5.01	4.52	3.92	4.91	4.71	**0.06**				
C	3	5.90	5.78	5.44	5.77	4.93	5.95	5.72	5.06	4.82	5.93	5.03	2.12	**0**			
D	1	6.62	6.93	5.92	6.28	5.91	7.29	7.06	6.24	6.00	6.77	6.12	2.98	2.92	—		
E	41	5.49	6.30	5.18	5.33	5.17	6.02	5.84	5.73	5.05	6.02	5.72	2.19	2.32	0.80	**0.03**	
F	10	5.25	6.02	5.18	5.30	5.15	6.27	6.11	5.69	5.05	6.05	5.63	2.18	2.32	0.78	0.29	**0**
cyt *b*																	
Tu	1	—															
Eu	26	4.52	**0.25**														
1	6	6.71	5.83	**0.31**													
2	6	6.69	6.05	1.43	**0.47**												
3	81	6.48	6.62	4.51	4.59	**0.19**											
4	99	6.79	6.64	4.78	5.02	5.26	**0.42**										
5	21	6.34	6.37	4.34	4.57	4.76	1.38	**0.21**									
6	2	6.86	6.82	5.02	5.41	5.74	5.20	4.89	**0.10**								
7	15	6.02	5.38	3.90	3.90	4.27	4.14	3.85	3.81	**0.62**							
8	37	6.49	6.67	5.19	5.26	5.19	4.80	4.96	4.16	3.23	**0.39**						
A	4	6.69	6.27	5.11	5.33	5.87	6.03	5.58	6.13	4.76	5.60	**0.05**					
B	6	7.07	7.51	6.29	6.25	6.73	6.47	5.89	7.10	6.08	6.70	4.86	**0.16**				
C	3	6.80	7.20	5.74	5.80	6.62	5.73	5.20	6.12	5.24	5.88	4.71	3.11	**0**			
D	1	6.78	7.27	5.91	6.15	7.06	5.83	5.52	6.97	5.98	6.58	5.36	3.52	2.06	—		
E	30	6.93	7.11	5.68	5.88	6.63	6.16	5.66	6.94	5.74	6.54	5.07	3.42	2.45	1.16	**0.05**	
F	11	6.81	6.99	5.71	5.89	6.66	6.14	5.63	7.01	5.66	6.52	5.11	3.33	2.33	0.91	0.28	**0.07**

Additional References

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