

Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*) – an update*

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Abstract. Based on more than 1100 samples of *Emys orbicularis* and *E. trinacris*, data on mtDNA diversity and distribution of haplotypes are provided, including for the first time data for Armenia, Georgia, Iran, and the Volga, Ural and Turgay River Basins of Russia and Kazakhstan. Eight mitochondrial lineages comprising 51 individual haplotypes occur in *E. orbicularis*, a ninth lineage with five haplotypes corresponds to *E. trinacris*. A high diversity of distinct mtDNA lineages and haplotypes occurs in the south, in the regions where putative glacial refuges were located. More northerly parts of Europe and adjacent Asia, which were recolonized by *E. orbicularis* in the Holocene, display distinctly less variation; most refuges did not contribute to northern recolonizations. Also in certain southern European lineages a decrease of haplotype diversity is observed with increasing latitude, suggestive of Holocene range expansions on a smaller scale.

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The mitochondrial cytochrome *b* gene (*cyt b*) became a frequently used marker for inferring phylogeography in reptiles (e.g. Brown and Pestano, 1998; Burbrink et al., 2000; Carranza et al., 2000, 2002; Surget-Groba et al., 2001; Harris et al., 2002; Austin et al., 2003; Podnar et al., 2005; Poulakakis et al., 2005; Fritz et al., 2006a) and several studies on European pond turtles (*Emys orbicularis*, *Emys trinacris*) were based on this marker gene. While Lenk et al. (1999) provided a nearly range-wide phylogeography, their study suffered from a patchy sampling for many parts of the range

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*This study is dedicated to the late Peter Lenk

(North Africa, Iberian and Apennine Peninsulas, France, East Europe, Kazakhstan, Turkey, Caucasus, Iran and Turkmenistan). Subsequent papers used a much denser sampling but covered only small regions (Fritz et al., 2004, 2005a, b, 2006b; Kotenko et al., 2005). Here we provide a range-wide update on haplotype diversity and distribution, and present for the first time data on pond turtles from Armenia, Georgia, Iran, and the Volga, Ural and Turgay River Basins of Russia and Kazakhstan. This note is based on more than 1100 cyt *b* sequences of known-locality samples and specimens of unknown geographic origin and is likely to represent the largest data set ever published for western Palearctic vertebrates. Our paper is aimed as a starting-point for further research, indicating still badly-sampled regions and suggesting future research directions.

Sampling techniques, PCR and sequencing are described in Lenk et al. (1999) and Fritz et al. (2005a). Besides samples from native wild-caught turtles or known-locality specimens from captive breeding projects, samples of confiscated, pet trade, or wild-caught allochthonous turtles were studied. Sequences were approximately 970-1125 bp long and aligned manually for haplotype determination. Haplotype nomenclature follows Lenk et al. (1999) and Fritz et al. (2004, 2005a, b, 2006b): Roman numerals designate major clades of haplotypes as revealed by phylogenetic analyses (=mtDNA lineages); within each lineage individual haplotypes are distinguished by consecutive letters.

In addition to the previously identified 48 haplotypes in nine lineages (I-IX; Lenk et al., 1999; Fritz et al., 2004, 2005a, b, 2006b) we found eight new haplotypes, belonging to already known lineages. For many haplotypes described in earlier studies, distributional data are provided here for the first time. The geographic origin of lineage IX (with haplotype IXa only), discovered in a pet trade turtle (Fritz et al., 2004), is still unknown however (table 1).

Based on an alignment of 1031 bp, relationships of haplotypes are illustrated using a

TCS 1.21 parsimony network (Clement et al., 2000) and a MP strict consensus tree rooted with the closely related Nearctic taxa *Actinemys marmorata* and *Emydoidea blandingii* (PAUP* 4.0b10, TBR branch-swapping algorithm, all characters with equal weight, stepwise random addition of 10 sequences; Swofford, 2002). For the ingroup taxa 957 characters were constant, 30 were variable but parsimony-uninformative and 44 were parsimony-informative; for all taxa 877 characters were constant, 33 variable characters were parsimony-uninformative and 121 were parsimony-informative. Maximum likelihood estimates for genetic differences of all nine lineages and most haplotypes were depicted in a recently published ML phylogram (Fritz et al., 2005a); uncorrected *p* distances were reported in the same study.

The network (fig. 1) is flock-like and haplotypes cluster into eight major branches. One branch splits into lineages I and II (*E. o. orbicularis* and related subspecies); the other eight branches correspond with lineages III (*E. trinacris*) and lineages IV to IX (remaining *E. orbicularis* subspecies). Lineages I and II are interconnected over two loops; a further loop occurs within lineage IV. Under MP phylogenetic analysis (fig. 2), monophyly of haplotypes of lineages II, III, IV, V and VII is well-supported, while monophyly for lineage I and VI haplotypes is only weakly supported. Lineages VIII and IX are represented by only one haplotype each that is located outside of all other lineages. Lineage III (*E. trinacris*) constitutes the sister group of a weakly supported clade containing lineages I-II and IV-IX (*E. orbicularis*); a well-supported clade of lineages I and II is the sister group of the remaining lineages IV-IX within *E. orbicularis*. Bootstrap support for monophyly of the clade comprising lineages IV-IX is below 50% however. Within lineage VI, North African haplotypes (VIc, VIe) are paraphyletic with respect to European haplotypes (VIa, VIb, VIe, VIe).

Regarding geographic distribution and phylogeography, our new data confirm the findings of

Table 1. Frequency, geographic distribution and accession numbers of mitochondrial haplotypes of *Emys orbicularis* (lineages I and II, IV to IX) and *Emys trinacris* (lineage III). Only native turtles were considered for distribution while all sequenced samples were considered for frequency of haplotypes (bracketed, number of allochthonous and pet trade turtles of unknown geographic origin). Asterisked distribution data previously unpublished.

Haplotype	Frequency	Geographic Distribution	Accession Number	Reference
Ia	159 (61)	Central and eastern Poland, Lithuania, Ukraine (not Crimea), Don, *Volga, *Ural and *Turgay River Basins in Russia and Kazakhstan, Black Sea regions of Romania, Bulgaria, Turkey and *Georgia, *regions of Gori and Tbilisi (Georgia), *Kladovo (Serbia)	AJ131407	Lenk et al. (1999), Fritz et al. (2004)
Ib	36 (16)	Eastern Greece, Bulgaria	AJ131408	Lenk et al. (1999), Fritz et al. (2004)
Ic	39 (3)	Central Anatolia, Turkish Black Sea region, southern Crimea (Ukraine), Dagestan and Kalmykia (Russia)	AJ131409	Lenk et al. (1999), Fritz et al. (2004), Kotenko et al. (2005)
Id	2 (1)	Central Anatolia	AJ131410	Lenk et al. (1999), Fritz et al. (2004)
Ie	1	Northern Crimea (Ukraine)	AY652865	Fritz et al. (2005a), Kotenko et al. (2005)
If	3	*Izmir Province (Turkey)	AY652866	Fritz et al. (2005a)
Ig	10	*Central Anatolia, *western Turkish Black Sea region	AY652867	Fritz et al. (2005a)
Ih	1	North-eastern Ukraine	AY652868	Fritz et al. (2005a), Kotenko et al. (2005)
Ii	3	South-eastern Crimea (Ukraine)	AY652879	Fritz et al. (2005a), Kotenko et al. (2005)
Ij	1 (1)	*Unknown	AY652880	Fritz et al. (2005a)
Ila	270 (108)	*Navarra, Huesca and northern Mediterranean coast of Spain, western, southern and central France, Danube Basin, south-eastern Balkan Peninsula	AJ131411	Lenk et al. (1999), Fritz et al. (2005b)
Ilb	31 (1)	North-eastern Germany, western Poland (mainly Oder Basin)	AJ131412	Lenk et al. (1999), Fritz et al. (2005b)
Ilc	3	*Balaton and Velence Lakes (Hungary)	AJ131413	Lenk et al. (1999)
Ild	1 (1)	*Unknown	AJ131414	Lenk et al. (1999)
Ile	1 (1)	*Unknown	AY652869	Fritz et al. (2005a)
Ilf	2 (1)	*Platamonas (Macedonia, Greece)	AY652881	Fritz et al. (2005a)
Ilg	1 (1?)	Département Ain (France)?	AY652870	Fritz et al. (2005a, b)
Ilh	2	Département Pyrénées-Atlantiques (France)	AY652888	Fritz et al. (2005a, b)
Ili	1	Département Gironde (France)	AY652882	Fritz et al. (2005a, b)
Ilj	1 (1)	*Unknown	AY652889	Fritz et al. (2005a)
Ilk	1 (1)	*Unknown	AY652883	Fritz et al. (2005a)

Table 1. (Continued).

Haplotype	Frequency	Geographic Distribution	Accession Number	Reference
IIIa	12 (1 or 2)	Sicily, perhaps Calabria (Italy)	AJ131415	Lenk et al. (1999), Fritz et al. (2005a)
IIIb	1 (1)	Unknown	AJ131416	Lenk et al. (1999)
IIIc	34 (2)	Sicily	AY652890	Fritz et al. (2005a)
IIId	1	Sicily	AM230632	Fritz et al. (2006b)
IIIe	1	Sicily	AM230633	Fritz et al. (2006b)
IVa	149 (88)	Apulia and Adriatic coast of Italy, west coast of Balkan Peninsula (not Cephalonia and Peloponnesus), Corfu, Evvia (Greece)	AJ131417	Lenk et al. (1999), Fritz et al. (2005a)
IVb	3	Cephalonia (Greece)	AJ131418	Lenk et al. (1999)
IVc	7 (2)	Peloponnesus (Greece)	AJ131419	Lenk et al. (1999)
IVd	22 (8)	Southern Apulia (Italy), *Tivat (Montenegro)	AY652871	Fritz et al. (2005a)
IVe	1 (1)	Unknown	AY652884	Fritz et al. (2005a)
IVf	1 (1)	Unknown	AY652872	Fritz et al. (2005a)
IVg	1	Peloponnesus (Greece)	AY652873	Fritz et al. (2005a)
IVh	8	Calabria, southern Apulia (Italy)	AY652874	Fritz et al. (2005a)
IVi	2	Southern Apulia (Italy)	AY652885	Fritz et al. (2005a)
IVj	2	Southern Apulia (Italy)	AY652886	Fritz et al. (2005a)
IVk	1 (1)	*Unknown	AM117937	This study
Va	181 (52)	Northern Mediterranean coast of Spain, southern France, west coast of Apennine Peninsula, Calabria, Basilicata, southern Apulia (Italy), Corsica, Sardinia	AJ131420	Lenk et al. (1999), Fritz et al. (2005a)
Vb	3	Calabria, southern Apulia (Italy)	AY652875	Fritz et al. (2005a)
Vc	7	*Calabria, Basilicata (Italy)	AY652876	Fritz et al. (2005a)
Vd	5	*Calabria (Italy)	AM269887	This study
Vla	46 (1 or 2)	*Alto Trás-os-Montes (Portugal), *Galicia, Huelva, *Madrid and Ciudad Real (Spain), Mediterranean coast of Spain, perhaps Département Pyrénées-Atlantiques (France)	AJ131421	Lenk et al. (1999), Fritz et al. (2005b)
Vlb	1	Algarve (Portugal)	AJ131422	Lenk et al. (1999)
Vlc	1	Middle Atlas (Morocco)	AJ131423	Lenk et al. (1999)
Vld	5	Alentejo (Portugal), *León, Huesca and Ebro Delta (Spain)	AJ131424	Lenk et al. (1999)
Vle	2	*Galicia (Spain)	AY652877	Fritz et al. (2005a)
Vlf	3	*Rif Mts. (Morocco)	AM269888	This study

Table 1. (Continued).

Haplotype	Frequency	Geographic Distribution	Accession Number	Reference
VIIa	27 (2 or 3)	*Armenia (Araxes River), Azerbaijan, *Gilan, *Mazandaran and *Golestan (Iran); a doubtful record exists for the region between Volga and Ural Rivers (northern Caspian Sea, Kazakhstan), see text	AJ131425	Lenk et al. (1999)
VIIb	1	Unknown	AJ131426	Lenk et al. (1999)
VIIc	2	*Azerbaijan, *Mazandaran (Iran)	AM269889	This study
VIIId	1	*Mazandaran (Iran)	AM269890	This study
VIIe	1	*Azerbaijan	AM269891	This study
VIIIf	2	*Azerbaijan, *Golestan (Iran)	AM269892	This study
VIIIf	1	*Gilan (Iran)	AM269893	This study
VIIIa	2	Anamur (southern Turkey)	AY652878	Fritz et al. (2004, 2005a)
IXa	1 (1)	Unknown	AY652887	Fritz et al. (2004, 2005a)
Total	1107 (357 to 361)			

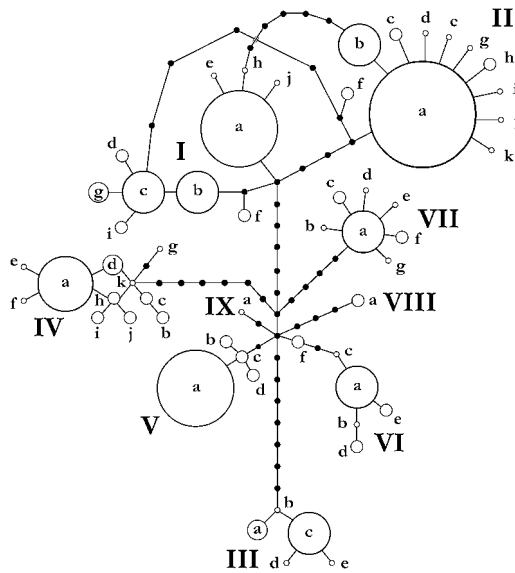


Figure 1. Parsimony network (TCS, spring tree) of all 56 known mtDNA haplotypes of *Emys orbicularis* (haplotypes of lineages I-II, IV-IX) and *Emys trinacris* (haplotypes of lineage III). Branch lengths correspond to inferred number of nucleotide changes along each branch; open circles and dots, identified or hypothesized haplotypes, respectively. Each line between circles or dots indicates one substitution; circle size corresponds to approximate frequency of haplotypes (table 1); size classes: 1, 2-9, 10-25, 26-50, 51-150, 151-200, >200 recorded haplotypes.

Lenk et al. (1999) and Fritz et al. (2005a) in that a high diversity of distinct mtDNA lineages and haplotypes occurs in southern regions where putative glacial refuges were located (fig. 3, table 1). With the advent of Holocene warming, more northerly parts of Europe and adjacent Asia were rapidly recolonized by *E. orbicularis* from few refuges in the Balkans and the northern Black Sea/northern Caucasus region, resulting in the respective mtDNA lineages in decreasing haplotype diversity with increasing distance to the refuge (long distance dispersal model of Hewitt, 1996); most refuges did not contribute to northern recolonizations.

With exception of a doubtful record of haplotype VIIa, the northernmost parts of the range of *E. orbicularis* are occupied only by three haplotypes (Ia, IIa and IIb), while lineages I and II exhibit a much higher diversity in the south. The same pattern, suggestive of Holocene range

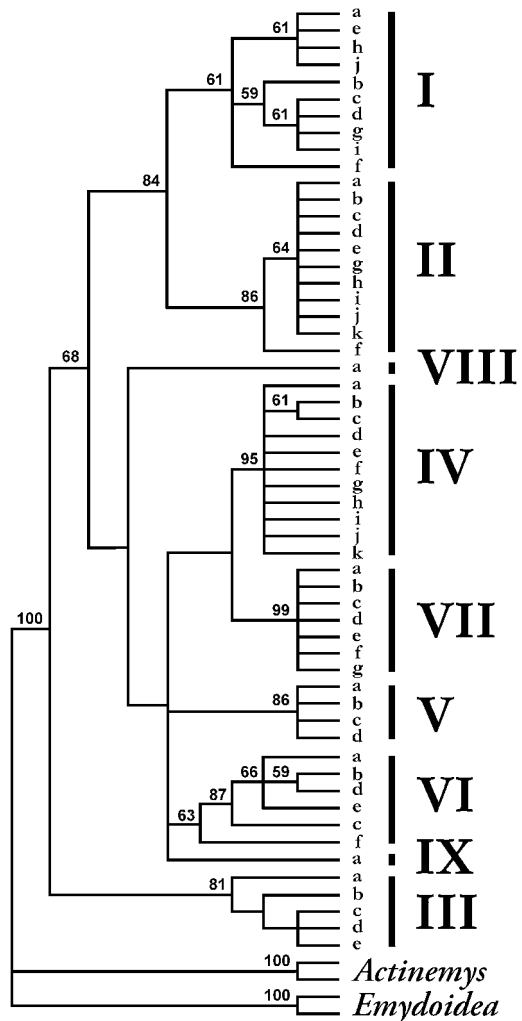


Figure 2. Strict consensus of six equally parsimonious trees of all *Emys* haplotypes using sequences of *Actinemys marmorata* (accession numbers AJ131430, U81344) and *Emydoidea blandingii* (AF258869, AJ131432) as outgroup (CI = 0.7585, RI = 0.9120, RC = 0.6917; 207 steps). Numbers above nodes are bootstrap values (1000 resamplings) greater than 50. The six equally parsimonious trees differ only in the branching pattern of lineage IV haplotypes.

expansions on a smaller scale, is observed in lineages IV and V with several endemic haplotypes in the southernmost parts of their ranges; northern portions harbour only haplotypes IVa or Va. A previous record of haplotype VIIa for the northern Caspian Sea region (Lenk et al., 1999) should be treated with care. It was based on a turtle kept in the zoological garden of Almaty (Kazakhstan), and we cannot exclude lo-

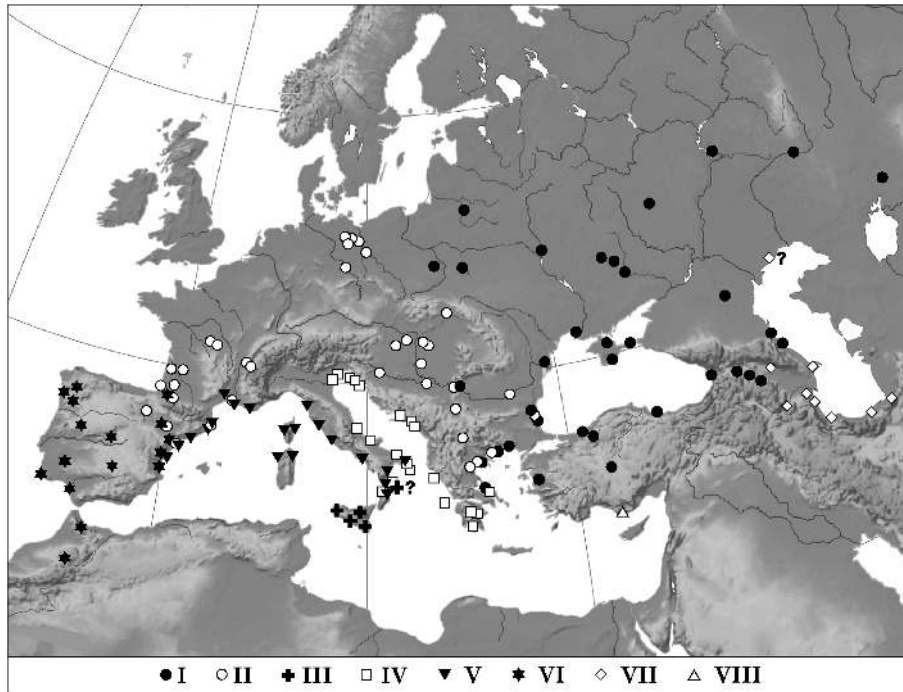


Figure 3. Geographic distribution of mtDNA lineages in *Emys orbicularis* (I-II, IV-IX) and *Emys trinacris* (III). Neighbouring localities combined; overlapping symbols indicate syntopic occurrence of distinct lineages; question marks, doubtful records for lineage III in Calabria (southern Italy) and lineage VII in the northern Caspian region. Evidently allochthonous specimens not considered.

cality confusion. Otherwise, lineage VII occurs far away, in the eastern central Caucasus and along the south coast of the Caspian Sea (fig. 3, table 1).

The wide distribution of lineage I haplotypes around the Black Sea suggests that the entire Black Sea region served as glacial refuge. On the other hand, the localized distribution of endemic lineage I haplotypes (table 1) indicates that distinct microrefuges existed there. An unexpected finding was haplotype Ia in eastern Georgia (regions of Gori and Tbilisi: one record each from Kareli, Gldanula River and Udabno). These sites are situated in the Kura River system that drains into the Caspian Sea. In the same river system occurs another mitochondrial lineage (VII) farther east, suggesting that lineages I and VII meet somewhere in the central Caucasus. This situation is echoed in the phylogeography of *Testudo graeca*. Also in this species two distinct mitochondrial lineages meet or occur in

closest neighbourhood in the central Caucasus (Fritz et al., 2007).

Besides the wide-spread haplotype IIa, the most differentiated haplotype of lineage II (IIb) occurs on the south-eastern Balkan Peninsula (table 1), suggesting the refuge for lineage II was located there. From the south-eastern Balkans, lineage II most probably spread along the valleys of the Vardar and Južna Morava Rivers northward to the Danube catchment basin from where central and western Europe (Fritz et al., 2005b) and, via the Moravian Gate, the Oder River Basin (Germany, Poland) were reached.

As for lineage IV, displaying a classic circum-Adriatic distribution, two distinct refuges existed in southern Italy and the southernmost Balkan Peninsula. The lack of the common haplotype IVa, distributed in Italy, Istria, Dalmatia, Corfu and Evvia, and the occurrence of endemic haplotypes in the Peloponnesus and on

Cephalonia (IVb, IVc, IVg) provide evidence for the colonization of the west coast of the Balkans, Corfu and Evvia from southern Italy and not from the geographically closer southern Balkanic refuge (Fritz et al., 2005a).

Like in *Mauremys leprosa* (Fritz et al., 2006a), mountain chains constitute major biogeographic barriers for mtDNA lineages in *E. orbicularis* (fig. 3). The Pyrenees separate the Ibero-Maghrebinian lineage VI from lineages II and V in the north, and the Apennines separate lineages IV and V in Italy. In the Balkan Peninsula, the Dinarid and Pindos Mts. are a barrier between lineage IV along the west coast and lineages I and II in the east, and south of the Alps occur lineages IV and V while in the north lineage II is distributed. In East Europe the Carpathians act as barrier between lineage II in the south and lineage I in the north; farther eastward the Greater Caucasus separates lineages I and VII and the Taurus Mts. (Turkey) divide lineages I and VIII. Syntopic occurrences of distinct mtDNA lineages, most probably as result of Holocene range expansions, are confined to narrow contact zones in close proximity of these mountain chains in the northern Iberian Peninsula, France and the southern Apennine and Balkan Peninsulas. Obviously, marshlands along sea coasts and river courses were used as colonization corridors during range expansions, enabling development of contact zones.

The basal position of the North African haplotypes VIc and VI f in the MP tree and in the network branch of lineage VI (figs 1, 2) suggests that the Iberian haplotypes VIa, VIb, VI d and VI e are derived from North African haplotypes. This implies a complicated colonization history, from Europe to North Africa and back to Europe, as recently proposed for the ribbed newt *Pleurodeles waltl* (Veith et al., 2004). Denser sampling is still needed in North Africa however, especially in Algeria and Tunisia. Some Moroccan amphibians and reptiles that occur in habitats resembling those of *E. orbicularis* (*Pleurodeles waltl*, *Mauremys l. leprosa*) are replaced in eastern Algeria and Tunisia

by other taxa (*P. nebulosus*, *P. poireti*, *M. l. saharica*, Carranza and Arnold, 2003; Carranza and Wade, 2004; Veith et al., 2004; Fritz et al., 2006a; distinct mitochondrial lineages of *Natrix maura*, Guicking et al., 2006), suggestive of a similar pattern in *E. orbicularis*. Further sampling is also needed in Turkey, Turkmenistan, in the Caucasus and in the northern Black Sea region to reveal haplotype diversity and potential contact zones there; one of these regions must harbour lineage IX.

Besides mtDNA haplotyping, future research should focus on gene flow along contact zones of distinct mitochondrial lineages. A promising approach will be using microsatellites as markers.

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References

- Austin, J.J., Arnold, E.N., Bour, R. (2003): Was there a second adaptive radiation of giant tortoises in the Indian Ocean? Using mitochondrial DNA to investigate speciation and biogeography of *Aldabrachelys*. *Molecular Ecology* **12**: 1415-1424.
- Brown, R.P., Pestano, J. (1998): Phylogeography of skinks (*Chalcides*) in the Canary Islands inferred from mitochondrial DNA sequences. *Molecular Ecology* **7**: 1183-1191.
- Burbrink, F.T., Lawson, R., Slowinski, J.B. (2000): Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* **54**: 2107-2118.
- Carranza, S., Arnold, E.N. (2003): History of West Mediterranean newts, *Pleurodeles* (Amphibia, Salamandridae) inferred from old and recent DNA sequences. *Systematics and Biodiversity* **1**: 327-337.

- Carranza, S., Wade, E. (2004): Taxonomic revision of Algero-Tunisian *Pleurodeles* (Caudata: Salamandridae) using molecular and morphological data. Revalidation of the taxon *Pleurodeles nebulosus* (Guichenot, 1850). *Zootaxa* **488**: 1-24.
- Carranza, S., Arnold, E.N., Mateo, J.A., López-Jurado, L.F. (2000): Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society B* **267**: 637-649.
- Carranza, S., Arnold, E.N., Mateo, J.A., Geniez, P. (2002): Relationships and evolution of the North African geckos, *Gehyra* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* **23**: 244-256.
- Clement, M., Posada, D., Crandall, K.A. (2000): TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657-1660.
- Fritz, U., Guicking, D., Lenk, P., Joger, U., Wink, M. (2004): When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain. *Biologia* **59** (Supplement 14): 19-25.
- Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M.G., Lenk, P., Joger, U., Wink, M. (2005a): A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys*. *Zoologica Scripta* **34**: 351-371.
- Fritz, U., Cadi, A., Cheylan, M., Coïc, C., Détaint, M., Olivier, A., Rosecchi, E., Guicking, D., Lenk, P., Joger, U., Wink, M. (2005b): Distribution of mtDNA haplotypes (cyt *b*) of *Emys orbicularis* in France and implications for postglacial recolonization. *Amphibia-Reptilia* **26**: 231-238 [Erratum: *Amphibia-Reptilia* **27** (2006): 157].
- Fritz, U., Barata, M., Busack, S.D., Fritzsche, G., Castilho, R. (2006a): Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa*. *Zoologica Scripta* **35**: 97-108.
- Fritz, U., d'Angelo, S., Pennisi, M.G., Lo Valvo, M. (2006b): Variation of Sicilian pond turtles, *Emys trinacris* – What makes a species cryptic? *Amphibia-Reptilia* **27**: 513-529.
- Fritz, U., Hundsdoerfer, A.K., Široký, P., Auer, M., Kami, H., Lehmann, J., Mazanaeva, L.F., Türkozan, O., Wink, M. (2007): Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia* **28**: 97-121.
- Guicking, D., Lawson, R., Joger, U., Wink, M. (2006): Evolution and phylogeny of the genus *Natrix*. *Biological Journal of the Linnean Society* **87**: 127-143.
- Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. *Journal of Biogeography* **29**: 1257-1262.
- Hewitt, G.M. (1996): Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247-276.
- Kotenko, T., Zinenko, O., Guicking, D., Sauer-Gürth, H., Wink, M., Fritz, U. (2005): First data on the geographic variation of *Emys orbicularis* in Ukraine: mtDNA haplotypes, coloration, and size. In: *Herpetologica Petropolitana. Proceedings of the 12th Ordinary General Meeting of the Societas Herpetologica Europaea*, August 12-16, 2003, St. Petersburg. Ananjeva, N., Tsinenko, O., Eds. *Russian Journal of Herpetology* **12** (Supplement): 43-46.
- Lenk, P., Fritz, U., Joger, U., Wink, M. (1999): Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758). *Molecular Ecology* **8**: 1911-1922.
- Podnar, M., Mayer, W., Tvrtković, N. (2005): Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology* **14**: 575-588.
- Poulakakis, N., Lymberakis, P., Valakos, E., Pafilis, P., Zouros, E., Mylonas, M. (2005): Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Molecular Ecology* **14**: 2433-2443.
- Surget-Groba, Y., Heulin, B., Guillaume, C.-P., Thorpe, R.S., Kupriyanova, L., Vogrin, N., Maslak, R., Mazzotti, S., Venczel, M., Ghira, I., Odierna, G., Leontyeva, O., Monney, J.C., Smith, N. (2001): Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution* **18**: 449-459.
- Swofford, D.L. (2002): PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sunderland, Massachusetts, Sinauer Associates.
- Veith, M., Mayer, C., Samraoui, B., Donaire Barroso, B., Bogaerts, S. (2004): From Europe to Africa and vice versa: evidence for multiple intercontinental dispersal in ribbed salamanders (genus *Pleurodeles*). *Journal of Biogeography* **31**: 159-171.

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