HERPETOLOGIA PETROPOLITANA

Proceedings of the 12th Ordinary General Meeting of the Societas Europaea Herpetologica

12 – 16 August 2003
Saint-Petersburg, Russia

Edited by
Natalia Ananjeva and Olga Tsinenko

Saint-Petersburg, 2005

FIRST DATA ON THE GEOGRAPHIC VARIATION OF *Emys orbicularis* IN UKRAINE: mtDNA HAPLOTYPES, COLORATION, AND SIZE

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Keywords: *Emys orbicularis*, geographic variation, molecular phylogeography, Ukraine.

INTRODUCTION

Morphological differences between European pond turtles, *Emys orbicularis* (Linnaeus, 1758), from the Crimea and of other parts of Ukraine were noted for the first time by Szczerbak (1966). He stated that Crimean turtles are reaching a maximum carapacial length of only 160.5 mm and are, therefore, distinctly smaller than in other regions. Szczerbak (1966) believed that this low maximum shell length is due to a poorer food supply and the smaller size of Crimean water bodies compared with pond turtle habitats in other parts of the range. However, according to Fritz (1992, 1994), this size difference reflects a taxonomic differentiation. Fritz (1992, 1994) attributed the small and light colored pond turtles from the southern Crimea to the eastern Mediterranean subspecies *Emys orbicularis hellenica* (Valenciennes, 1832), whereas the large, dark specimens from the northern Crimea and the mainland of Ukraine were thought by him to represent *Emys orbicularis orbicularis* (Linnaeus, 1758). Later, investigations on the mitochondrial phylogeography of *E. orbicularis* supported that pond turtles from the Ukrainian mainland represent the nominotypical subspecies. These specimens share their mtDNA haplotype with *E. o. orbicularis* from most other northern parts of the species' range (Lenk et al., 1999). However, until now no mtDNA data have been available for Crimean *E. orbicularis*. Moreover, Fritz's (1992, 1994) taxonomic allocation of Crimean pond turtles was based on only few specimens as Ukrainian *E. orbicularis* are rare in Central European museum collections. Thus, also additional morphological data are in dire need. Here we present new data on size, coloration, and the molecular phylogeography of *E. orbicularis* in Ukraine and focus on the status of Crimean populations.

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MATERIAL AND METHODS

Turtles for this study were collected during our field trips in Ukraine and its Autonomous Republic of Crimea in 1974, 1979, and 2000 – 2003. Additional specimens were studied from the following museum collections: National Museum of Natural History (National Academy of Sciences of Ukraine, Kiev, Ukraine), Museum of Nature (Kharkov National Karazin University, Kharkov, Ukraine), and Museum für Tierkunde (Dresden, Germany). Among these museum specimens are pond turtles from the Dnepr Delta, Luchyste, Sovet'skyi, and the Kerch Peninsula which were collected in the early 1960's, mainly by N. N. Szczerbak and V. A. Sedov. 152 adult and 51 juvenile turtles from 15 localities, representing 10 populations, were measured with a caliper to the nearest 0.1 mm and their color and pattern were recorded (Table 1, Fig. 1). During field work, blood samples for genetic investigations were obtained by coccygeal vein puncture (Haskell and Pokras, 1994). Samples were stored as described in Arctander (1988). Total genomic DNA was extracted following standard proteinase K and phenol-chloroform protocols (Sambrook et al., 1989). PCR and sequencing are explained in detail in Lenk et al. (1999). We define haplotypes according to individual mtDNA sequences (Lenk et al., 1999). Haplotype and haplotype lineage nomenclature follows Lenk et al. (1999) and Fritz et al. (in press).

We sequenced a 1031 bp portion of the mitochondrial cytochrome b gene for 33 specimens as given in Table 1. Of the 1031 aligned sites, 69 are variable. 63 substitutions are transitions, and six are transversions; 41 sites are parsimony informative. 13 sites are variable at the first, eight at the second, and 48 at the third codon position. For each sequence, variable sites were checked individually to prevent errors from wrong sequencer output. We calculated a minimum spanning network with the program Arlequin (Schneider et al., 2000), in which all *Emys orbicularis*

TABLE 1. Studied *Emys orbicularis* Populations, Straight Line Carapacial Lengths (SCL, only adults) and mtDNA Haplotypes

Population	Sex	*n*	SCL, mm			mtDNA haplotypes
			mean	minimum	maximum	
Desna River (***A***, Litochky, 2001)	Females	2	–	176.0	182.5	Ia (*n* = 2)
Merla River (***B***, Kolontayiv, 2001; ***C***, Volodymyrivs'ke Forestry, 1975, and Gorodne, 2001)	Males	2	–	145.5	154.5	Ia (*n* = 2, Kolontayiv); Ia, Ih (*n* = 2, Gorodne)
	Females	7	168.2	155.0	178.0	
Mzha River (***D***, Merefa, 2001, 2003)	Females	9	170.0	156.0	188.5	Ia (*n* = 1)
Siverskii Donets River (***E***, Gaidary, Koropovo, Gomol'sha, and Cherkas'-kyi Byshkyn, 2002; ***F***, Balakleya, 2003, and Sezonna, 1969; ***G***, Izyum, 2001)	Males	1	–	175.0		Ia (*n* = 1, Izyum)
	Females	11	175.9	145.5	195.0	
Siverskii Donets River (***H***, Levkivka, Lysogirka, and Chervonyi Donets', 1990 – 1993) (Lyamzin, 1993)	Males	44	160.0	126.0	171.0	–
	Females	43	175.0	160.0	200.0	
Siverskii Donets River, total (localities ***E – H***)	Males	45	160.3	126.0	175.0	Ia (*n* = 1, Izyum)
	Females	54	175.2	145.5	200.0	
Dnepr Delta (***J***, Gerois'ke, 1960's, 1979, 2000; ***K***, Rybal'che and Vynogradne, 1974, 1979 and 2000; ***L***, Gola Prystan', 1974)	Males	10	145.9	112.9	173.5	Ia (*n* = 15, Gerois'ke); Ia (*n* = 1, Vynogradne)
	Females	33	166.4	143.5	189.5	
Northern Crimea (***M***, Dzhankoi, 2000)	Males	1	–	165.6		Ie (*n* = 1)
East Sivash Region, Crimea (***N***, Sovet'skyi, 1961, 1962)	Males	20	127.3	113.4	137.0	–
	Females	9	138.5	122.5	153.6	
Crimean Mountains (***O***, Luchyste, 1961, 2000, 2001)	Males	16	127.9	112.9	137.1	Ic (*n* = 4)
	Females	15	131.9	113.4	154.2	
Kerch Peninsula, Crimea (***P***, Él'tigen, 2001)	Males	6	138.6	135.3	140.7	Ic (*n* = 1); Ii (*n* = 3)
	Females	6	158.5	147.5	172.5	
Kerch Peninsula, Crimea (***Q***, Kerch, 1961)	Females	4	141.0	134.5	149.3	–
Crimea (mainly Luchyste, Sovet'skyi, and Kerch) (Szczerbak, 1966)	Both sexes	80	–	–	160.5	–

Collection dates (years) are given for all localities; specimens collected prior to 2000 are mainly museum vouchers, others have been released after study. Letters preceding localities refer to Fig. 1. For the Siverskii Donets population (locality *H*) and the Crimea, literature data are added.

mtDNA haplotypes identified until now have been included (Fritz et al., in press) to demonstrate how the Ukrainian haplotypes are related.

RESULTS

Crimean *Emys orbicularis* (East Sivash Region, Crimean Mountains, Kerch Peninsula) are on average smaller than European pond turtles from the Dnepr Delta and more northerly Ukrainian localities. However, a male from Dzhankoi (northern Crimea) and some females from Él'tigen (Kerch Peninsula) are of medium to large size (Table 1). They exceed the previously recorded maximum size of 160.5 mm for Crimean *E. orbicularis* (Szczerbak, 1966). All specimens from Luchyste, Sovet'skyi, and Kerch are distinctly lighter colored than the turtles from all other Ukrainian localities. These light colored turtles possess mainly yellow plastra and throats. Moreover, the primary carapacial color of many specimens from Sovet'skyi and of some turtles from Luchyste and Kerch is yellowish brown or smoky brown instead of black. The iris coloration of an adult male from Luchyste is yellow (the other males from Luchyste, Sovet'skyi, and Kerch are museum specimens so that the iris coloration could not be studied). Similar coloration characters are also known to occur in *E. o. hellenica* from the Balkans, which is also similar in size (Fritz, 2003).

Remarkably, pond turtles from Kerch Peninsula show considerable variation. Specimens from Kerch (Fig. 1, locality *Q*) in the holdings of the National Museum of Natural History (Kiev, Ukraine) and the Museum für Tierkunde (Dresden, Germany), collected in 1961, resemble in coloration and size turtles from Luchyste and Sovet'skyi. However, turtles captured in 2001 near Kerch, in Él'tigen (Fig. 1, locality *P*), are larger and darker colored. Their shell and soft part coloration resembles Dnepr Delta turtles or other specimens of *E. o. orbicularis*; the iris coloration of adult males is brick-red, orange, or red-brown, as characteristic for *E. o. orbicularis*.

At the remote locality Luchyste in the Crimean Mountains we found in 2000 and 2001 light colored, small turtles, which are morphologically in good agreement with the old museum specimens from there. Unfortunately, we are not sure about the current situation at Sovet'skyi. All studied turtles from Sovet'skyi are museum specimens collected in 1961 and 1962. The only male from Dzhankoi, studied in 2000, is very dark colored, has a reddish iris

Fig. 1. Localities and mtDNA haplotypes (n = 33) for *Emys orbicularis* in Ukraine. Localities represented by asterisks were not studied genetically. Letters of localities refer to Table 1, numbers by the letters or symbols refer to the number of specimens bearing this haplotype.

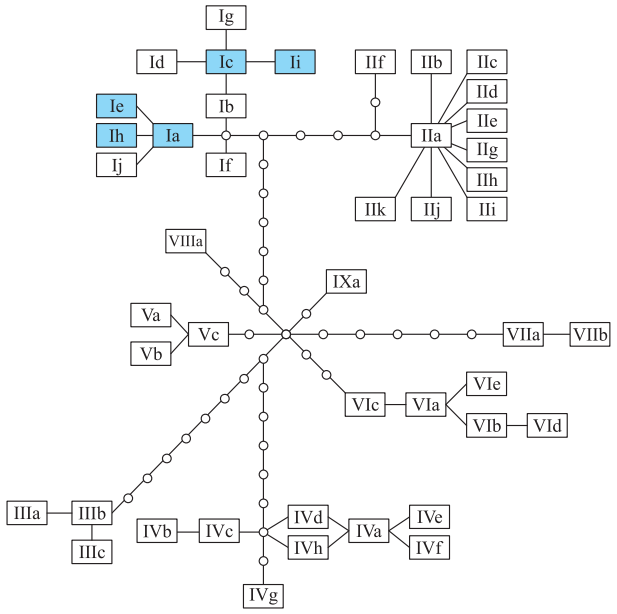

Fig. 2. Minimum spanning network of all 44 known *Emys orbicularis* mtDNA haplotypes (modified from Fritz et al., in press). Each line represents a single mutation step, missing haplotypes are presented by open circles. Gray boxes indicate haplotypes found in Ukrainian samples.

and is indistinguishable from *E. o. orbicularis* from more northerly regions.

Regarding genetics, we identified five different mtDNA haplotypes in Ukraine (Fig. 1). All haplotypes belong to lineage I of Lenk et al. (1999). In the Crimean Mountains and on the Crimean Kerch Peninsula the most differentiated haplotypes were detected (Ic, Ii). They differ by three to five mutation steps from the haplotypes found in the northern steppe part of the Crimea and on the Ukrainian mainland (Fig. 2).

DISCUSSION

In this paper we confirm that turtles from some Crimean localities resemble *Emys orbicularis hellenica* from the Balkans morphologically. However, in no case mtDNA haplotypes of lineage IV have been recorded in the Crimea. This lineage is characteristic for *E. o. hellenica* (Lenk et al., 1999; Fritz, 2001, 2003). Although we cannot exclude that this finding is due to a loss of lineage IV haplotypes during a former genetic bottleneck, the current data set argues rather for an independently acquired morphological similarity of Crimean and Balkanic pond turtles.

In the south of Ukraine a much higher mtDNA haplotype diversity occurs than in the north. This reflects surely a rapid postglacial range expansion of *E. orbicularis*. A similar situation is found in many other taxa displaying the same long distance dispersal pattern (e.g., Hewitt, 1996, 2001; Taberlet et al., 1998; Cruzan and Templeton, 2000).

However, we are not sure that a glacial refugium for haplotype Ia turtles was located on the Crimea. Today, haplotype Ia is known from the Ukrainian mainland, Poland, Lithuania, northern Russia, Kazakhstan, the southeastern Balkans, and Turkey (Fritz et al., in press). It corresponds mainly to large, dark pond turtles. The distribution of haplotype Ia agrees well with the range of the nominotypical subspecies *E. o. orbicularis* (Lenk et al., 1999; Fritz, 2003). The mtDNA haplotypes (Ic, Ii) of the Crimean Mountains and the Kerch Peninsula differ from the haplotypes found in the Dnepr Delta (Ia) and from more northerly localities in Ukraine (Ia, Ih; Fig. 2). This differentiation is also paralleled by a morphological gap in size and coloration of turtles from the Dnepr Delta vs. the Crimean Mountains (Luchyste) plus old museum specimens from the Crimean localities Sovet'skyi and Kerch.

Haplotype Ia and its rare variant Ih have been not recorded in the Crimea until now, but the closely related haplotype Ie. However, the turtle bearing haplotype Ie originated from a locality in the northern steppe zone of the Crimea (Dzhankoi). This area has been connected with the Dnepr by the construction of the North Crimean Canal in the late 1960's. The museum specimens from the Kerch Peninsula, collected in 1961, differ morphologically from the turtles studied by us forty years later there. The North Crimean Canal was extended to the Kerch Peninsula around 1975. Thus, it might be that northern *E. o. orbicularis* bearing haplotype Ie (and probably Ia) are recent invaders, which perhaps already genetically impacted the Kerch Peninsula population. If this hypothesis is correct, the survival of native Crimean turtles is seriously threatened by the current immigration of the Dnepr turtles.

It is obvious that we need further research to understand the diversity of Ukrainian *E. orbicularis* better. This will be the prerequisite for developing any effective conservation measures.

REFERENCES

Arctander P. (1988), "Comparative studies of Avian DNA by restriction fragment polymorphism analysis," *J. Ornithol.*, **129**, 205 – 216.

Cruzan M. B. and Templeton A. R. (2000), "Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record," *TREE*, **15**, 491 – 496.

Fritz U. (1992), "Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 2. Variabilität in Osteuropa und Redefinition von *Emys orbicularis orbicularis* (Linnaeus, 1758) und *E. o. hellenica* (Valenciennes, 1832)," *Zool. Abh. Mus. Tierkd. Dresden*, **47**(5), 37 – 77.

Fritz U. (1994), "Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 4. Variabilität und Zoogeographie im pontokaspischen Gebiet mit Beschreibung von drei neuen Unterarten," *Zool. Abh. Mus. Tierkd. Dresden*, **48**(4), 53 – 93.

Fritz U. (2001), "*Emys orbicularis* (Linnaeus, 1758) — Europäische Sumpfschildkröte," in: U. Fritz (ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/IIIA. Schildkröten* (*Testudines*). *I.* (*Bataguridae*, *Testudinidae*, *Emydidae*), AULA-Verlag, Wiebelsheim, pp. 343 – 515.

Fritz U. (2003), *Die Europäische Sumpfschildkröte*, Laurenti, Bielefeld.

Fritz U., Guicking D., Lenk P., Joger U., and Wink M. (in press), "When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain," *Biologia*.

Haskell A. and Pokras M. A. (1994), "Nonlethal blood and muscle tissue collection from redbelly turtles for genetic studies," *Herpetol. Rev.*, **25**, 11 – 12.

Hewitt G. M. (1996), "Some genetic consequences of ice ages, and their role in divergence and speciation," *Biol. J. Linn. Soc.*, **58**, 247 – 276.

Hewitt G. M. (2001), "Speciation, hybrid zones and phylogeography — or seeing genes in space and time," *Mol. Ecol.*, **10**, 537 – 549.

Lenk P., Fritz U., Joger U., and Wink M. (1999), "Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758)," *Mol. Ecol.*, **8**, 1911 – 1922.

Lyamzin I. I. (1993), *Peculiarities of the Ecology and Morphological Variation of the European Pond Turtle* Emys orbicularis *in the Middle Course of the Siverskii Donets River. Graduate Thesis*, Kharkov University [in Russian].

Sambrook J., Fritsch E. F., and Maniatis T. (1989), *Molecular Cloning*: *a Laboratory Manual. 2nd Edition*, Cold Spring Harbor Lab. Press, Cold Spring Harbor.

Schneider S., Roessli D., and Excoffier L. (2000), *Arlequin ver. 2.000. A Software for Population Genetics Data Analysis*, Genetics and Biometry Laboratory, University of Geneva, Geneva.

Szczerbak N. N. (1966), *Amphibians and Reptiles of the Crimea. Herpetologia Taurica*, Naukova dumka, Kiev [in Russian].

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