

Variation of Sicilian pond turtles, *Emys trinacris* – What makes a species cryptic?

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Abstract. Variation of the Sicilian pond turtle, *Emys trinacris*, is described, based on morphological data of more than 200 adult and immature turtles and mtDNA sequences of 31 new known-locality specimens. *Emys trinacris* is morphologically more variable than thought before. There exist pronounced population-specific differences. Adults are barely distinguishable from *Emys orbicularis galloitalica*. Hatchlings of *E. trinacris* are, however, significantly different patterned, allowing immediate species determination. Moreover, hatchlings of *E. trinacris* seem to be on average smaller and lighter than in *E. orbicularis*. This could be related with different reproductive strategies. Coloration polymorphism of adult Sicilian pond turtles comprises fair part of variation known in *E. orbicularis*. Thus, adult *E. trinacris* might have preserved the whole array of variation of the last common ancestor with *E. orbicularis*. We hypothesize that similarity of certain southern *E. orbicularis* subspecies and *E. trinacris* on one hand and pronounced differences in size, coloration and pattern of northern *E. orbicularis* subspecies on the other result from stabilising selection in the north. While dark coloration and large body-size seem to be beneficial in the north, light coloration and small size could be simply not disadvantageous in the south, allowing coloration polymorphisms in *E. trinacris* and southern *E. orbicularis* subspecies. Further, the term 'cryptic species' and its meaning in regard to species concepts and bar-coding is discussed. It is concluded that species delineation based on mtDNA bar-coding requires application of a Phylogenetic Species Concept. Under the Biological Species Concept, animal mtDNA is not always an ideal tool for delineating species boundaries because taxa with monophyletic mitochondrial gene trees are neither necessarily genetically isolated nor must represent the same Biological Species. Cryptic species are nothing special in nature because difficulties with their identification are due to deficits in cognitive abilities of man. This is illustrated by the fact that distinct live stages of 'cryptic species' may differ to various degrees from similar species, as is the case in hatchlings and adults of *E. trinacris* and *E. o. galloitalica*.

Introduction

Recently, pond turtles occurring on Sicily were described as new species, *Emys trinacris* Fritz et al., 2005, due to lacking gene-flow with the wide-spread mainland species *E. orbicularis* (Linnaeus, 1758). Although a few extant (*Podarcis wagneriana*, Böhme, 1986; *Crocodylus sicula*, Maddalena et al., 1990) and many fossil endemic vertebrate species (Bonfiglio et al.,

2002) are known from Sicily (and the nearby Maltese Islands), the discovery of genetic distinctness of Sicilian pond turtles was unexpected because sea straits constitute no significant biogeographic barriers to western Palearctic freshwater turtles (Fritz et al., 2006) and repeated exchange of Sicilian island and Italian mainland faunas occurred during Pleistocene low sea level stands (Palombo, 1986, 2001; Bonfiglio et al., 1997, 2002). Even more intriguing was that *Emys trinacris* could not be distinguished morphologically from *Emys orbicularis galloitalica*, occurring on the Italian mainland. Therefore, *E. trinacris* was considered a cryptic species (Fritz et al., 2005a).

The original description of *E. trinacris* was based on morphological data of only 15 adults of this species. Therefore, it could not be excluded that diagnostic characters will emerge with larger sample size (Fritz et al., 2005a). During field-work for nature conservation purposes, two authors of the present paper

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(S. d'Angelo, M. Lo Valvo) accumulated a considerable amount of morphological data and made this data set available to the senior author after the description of *E. trinacris*. It documents distinctly more morphological variation than previously known. Here we report this new data and additional mtDNA sequences of Sicilian pond turtles. Further, we compare two *E. trinacris* populations with *E. o. galloitalica* to reveal whether both taxa are really morphologically indistinguishable and describe for the first time hatchlings of *E. trinacris*. Finally, we discuss our findings in the light of the recent renaissance of interest in sibling or cryptic species due to the advent of DNA bar-coding (Hebert et al., 2003a, b, 2004a, b; Lambert et al., 2005).

Materials and Methods

From 2002 to 2005 field-work was conducted mainly in southern and western regions of Sicily. This resulted in records of more than 300 pond turtles. Basic morphological data (shell dimensions, body mass, coloration, pattern) were documented for more than 250 individuals; dorsal and ventral aspects of most turtles were photographed. Representative digital images are filed in the Museum of Zoology Dresden. Not all characters were recorded for all turtles, resulting in part in different sample sizes.

Blood samples of 31 turtles from eleven localities and of three captive individuals kept by local residents were taken by coccygeal vein puncture (table 1). MtDNA haplotypes were determined using the mitochondrial cytochrome *b* gene as marker according to the procedures described in Fritz et al. (2005a). Sequences were aligned with previously identified haplotypes using CLUSTALX v.1.83 (Thompson et al., 1997). Detailed morphological data was obtained for two populations at the 'Lago Preola e Gorgi Tondi' Nature Reserve near Mazara del Vallo (Trapani Province) and the 'Torre Salsa' Biosphere Reserve near Siculiana (Agrigento Province). The distance between both reserves is approximately 80 km.

Turtles from both reserves were assigned to different age classes and sexed, if possible, using external secondary sexual characteristics as defined in Fritz (2003). After reaching sexual maturity, there is significant reduction in growth speed, resulting in formation of very narrow or no new growth lines (annuli) on the shell (Fritz, 2003), and only such individuals were defined as adults. Hatchlings were defined as turtles that did not start growing yet, while turtles showing wide fresh annuli were defined as juveniles. In the 'Lago Preola e Gorgi Tondi' Reserve 79 males, 47 females, six hatchlings plus more than 20 juveniles and in the 'Torre Salsa' Reserve 58 males, 35 females, eight hatchlings plus more than 20 juveniles were studied.

Measurements (all straight line) were taken with a caliper to the nearest 0.1 mm as follow: Maximum carapacial length – CL; maximum carapacial width – CW; maximum plastral length – PL; mid-seam plastral length – PL_(med); maximum shell height – SH; bridge width – BW; maximum tail length – TL. Besides, body mass was recorded to the nearest gram. Data were analyzed using STATISTICA 6.0 software (StatSoft Italia, 2003). Obtained data were compared for males and females within and between populations by a two-tailed *t*-test in order to find out whether statistically significant differences exist between sexes within one population and if so, whether males or females differ significantly between the two populations. In addition, hatchlings and juveniles of each population were measured, but juveniles were excluded from further analyses. Measurements (CL) and body masses of *E. trinacris* hatchlings were compared with hatchlings of *E. orbicularis*, including literature data. The senior author of this paper studied during the past twenty years several hundred *E. orbicularis* hatchlings, among others specimens from all major European museum collections and captive-bred individuals. Captive-bred hatchlings originated either from the breeding station for *E. o. orbicularis* in Linum (Brandenburg, Germany) or the Zoological Garden Stuttgart, where the senior author was employed for several years as reptile curator, and belonged to *E. o. orbicularis*, *E. o. galloitalica* and *E. o. hellenica* (several distinct populations each). This experience served as basis for comparison of coloration and pattern of hatchlings.

To find out whether adult *Emys trinacris* are indeed morphometrically indistinguishable from *Emys orbicularis galloitalica*, as suggested by Fritz et al. (2005a), we compared 11 males and 10 females from the type locality of *E. o. galloitalica* (Département Var, southern France; specimens from the data set of Schulze and Fritz, 2003) with 29 males and nine females of *E. trinacris* (17 males and three females from the Lago Preola population and 12 males and six females from the Torre Salsa population) by using the following straight-line measurements (Schulze and Fritz, 2003): (1) Maximum carapacial length – CL; (2) maximum carapacial width – CW; (3) maximum shell height – SH; (4) maximum plastral length – PL; lengths of median seams between plastral scutes: (5) intergular length – GuL, (6) interhumeral length – HumL, (7) interpectoral length – PecL, (8) inter-abdominal length – AbdL, (9) interfemoral length – FemL, (10) interanal length – AnL; (11) nuchal length – NuL; (12) nuchal width – NuW. For this data set, Linear Discriminant Function Analysis (Discriminant Analysis, DA) as implemented in STATISTICA 6.0 was used to find out whether *E. o. galloitalica* and *E. trinacris* can be discriminated by multivariate methods. As our descriptive statistics revealed, in accordance with previous results (Fritz et al., 2005a), a pronounced sexual dimorphism for *E. trinacris*, data for males and females were assigned to different groupings. By including the two *E. trinacris* populations as separate groupings into DA, we intended to test whether any detected difference between *E. o. galloitalica* and *E. trinacris* reflects (i) a species-specific difference or (ii) only interpopulational variation. The first option should be the case if DA fails to discriminate both *E. trinacris* populations, but discriminates *E. trinacris* from *E. o. galloitalica*, and the second if DA

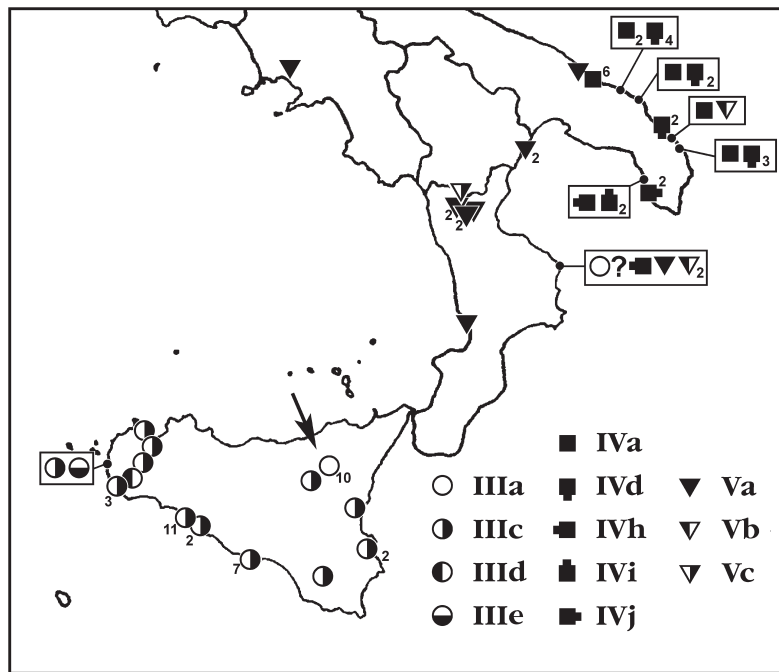


Figure 1. Distribution of *Emys* mtDNA haplotypes in Sicily and adjacent southern Italy (only native turtles; modified from Fritz et al., 2005a). MtDNA lineage III is characteristic for *E. trinacris*; lineages IV and V for Italian mainland subspecies of *E. orbicularis*. The arrow indicates where a probably introduced *E. o. galloitalica* (haplotype Va) was caught together with 10 *E. trinacris* (haplotype IIIa; Lenk et al., 1999; Fritz et al., 2005a).

discriminates all three populations from one another. The correlation matrix of the data set was further tested by Principal Component Analysis (PCA) to reveal possible influence of size on components and to identify linear combinations of variables uncorrelated with size that display maximal variance contributing to group discrimination (Chessel and Auda, 1986; James and McCulloch, 1990; Camussi et al., 1991).

Results

mtDNA variation

We found among our 31 newly sequenced known-locality samples 29 bearing haplotype IIIc that was previously recorded from *Emys trinacris* (Fritz et al., 2005a). Two samples differed in one nucleotide each from this haplotype; these new haplotypes may be retrieved under accession numbers AM230632 (haplotype IIIId) and AM230633 (haplotype IIIe). Together with previously published data, 48 cytochrome *b* sequences are available for Sicilian turtles

(fig. 1, table 1). Forty-six correspond to haplotypes of *E. trinacris* (IIIa, c-e). One wild-caught individual harboured a haplotype (Va) characteristic for *E. o. galloitalica* (Lenk et al., 1999) and was considered representing an introduced turtle (Fritz et al., 2005a). Two of three samples originating from captive turtles correspond to haplotype IIIc of *E. trinacris*, while the third was bearing a haplotype (IVa) characteristic for *Emys orbicularis hellenica*, a taxon occurring in eastern continental Italy and on the Balkan Peninsula (Fritz et al., 2005a).

Morphological variation

Adults

Morphometry. We found in all studied characters a statistically significant sexual dimorphism for both populations. Males have longer tails than females and are smaller and lighter (table 2). If the two populations are compared, it is apparent that both males and females from

Table 1. MtDNA haplotypes of Sicilian pond turtles. Accession numbers: IIIa – AJ131415, IIIc – AY652890, III d – AM230632, IIIe – AM230633, IVa – AJ131417, Va – AJ131420. Allochthonous and probably allochthonous individuals (*Emys orbicularis*) asterisked. MTD T = Museum für Tierkunde (Museum of Zoology) Dresden, Tissue Collection.

Locality	mtDNA haplotype							Reference / Voucher sample + photo
	<i>n</i>	IIIa	IIIc	III d	IIIe	IVa	Va	
<i>Agrigento Province:</i>								
Agrigento	2	–	2	–	–	–	–	Fritz et al. (2005a)
Licata, Salso River Mouth	7	–	7	–	–	–	–	MTD T 2120, 2122, 2131-34, 2136
Siculiana, ‘Torre Salsa’ Biosphere Reserve	11	–	11	–	–	–	–	MTD T 2117-19, 2123-26, 2144-47
<i>Catania Province:</i>								
Simeto River	1	–	1	–	–	–	–	MTD T 1522
<i>Enna Province:</i>								
Nicosia (Nebrodi)	1	–	1	–	–	–	–	Fritz et al. (2005a)
<i>Messina Province:</i>								
Mistretta, Val di Demone	11	10	–	–	–	–	1*	Lenk et al. (1999)
<i>Ragusa Province:</i>								
Ragusa	1	–	1	–	–	–	–	MTD T 2509
<i>Siracusa Province:</i>								
San Leonardo River	2	–	2	–	–	–	–	MTD T 1523-24
<i>Trapani Province:</i>								
Alcamo	1	–	1	–	–	–	–	MTD T 2130
Castellamare del Golfo	1	–	1	–	–	–	–	MTD T 2137
Marsala	2	–	1	1	–	–	–	MTD T 2138-39
Mazara del Vallo, ‘Lago Preola e Gorgi Tondi’ Nature Reserve	3	–	3	–	–	–	–	MTD T 2121, 2128-29
Mazara del Vallo, Mazaro River	1	–	–	–	1	–	–	MTD T 2127
Salemi	1	–	1	–	–	–	–	MTD T 2135
Captive turtles, allegedly from Sicily	3	–	2	–	–	1*	–	MTD T 1043-44, 2510
Total	48	10	34	1	1	1*	1*	

the ‘Torre Salsa’ Biosphere Reserve are significantly larger (fig. 2) and heavier than the respective sex from the population in the ‘Lago Preola e Gorgi Tondi’ Nature Reserve.

DA using variance of the entire character set succeeded in classifying high percentages of males and females of both *E. trinacris* populations and of *E. o. galloitalica* properly (tables 3-4; fig. 3), reflecting significant differences in the first three canonical axes ($r = 0.93$; Wilk’s $\lambda = 0.007$; $F_{(60,200)} = 6.35$; $p < 0.000$). PCA revealed a distinction among the two species and the two sexes in the order of the factorial level (fig. 4). Most variables are linked to one another and with factorial axis PC1, which is the main representative of the turtle’s body size. Only interfemoral seam length (FemL) and nuchal width (NuW) are correlated with factorial axis

PC2. Relative to body size is the interfemoral seam in males of *E. trinacris* slightly longer than in *E. o. galloitalica* ($p < 0.05$), while there is no difference in females. The nuchal scute is in both sexes of *E. trinacris* smaller than in *E. o. galloitalica* in that it is shorter (males: $p < 0.001$, females $p < 0.05$) and narrower (males: $p < 0.001$, females $p < 0.01$) relative to body size. However, the observed differences lie on average within a range of 1-2 mm and are not helpful in identifying single individuals.

Coloration and pattern. The carapaces of all studied adults from Lago Preola were dark coloured, matching the original description of *E. trinacris* (Fritz et al., 2005a). In the Torre Salsa population, however, we discovered a marked polymorphism. Many turtles have a light brownish to chestnut-coloured carapace

Table 2. Basic morphometric data of two *Emys trinacris* populations from western Sicily. For abbreviations see Materials and Methods.

		Lago Preola e Gorgi Tondi				Torre Salsa			
		Males	Females	<i>t</i>	<i>p</i>	Males	Females	<i>t</i>	<i>p</i>
CL (mm)	mean ± SE	112.9 ± 0.8	118.9 ± 1.6	−3.7	0.000	128.6 ± 1.1	134.6 ± 2.1	−2.7	0.008
	min-max	100.7-143.4	102.5-155.2			103.7-152.3	103.3-149.9		
	<i>n</i>	79	47			57	34		
CW (mm)	mean ± SE	84.5 ± 0.7	91.8 ± 1.2	−5.5	0.000	99.5 ± 1.0	107.4 ± 1.7	−4.2	0.000
	min-max	73.4-108.0	79.2-120.6			73.7-122.1	82.0-123.6		
	<i>n</i>	78	47			58	34		
PL (mm)	mean ± SE	100.8 ± 0.8	112.1 ± 1.6	−7.2	0.000	116.5 ± 1.1	124.5 ± 3.8	−2.7	0.008
	min-max	83.9-125.6	93.5-148.5			93.2-134.0	87.5-147.2		
	<i>n</i>	79	47			55	20		
PL _(med) (mm)	mean ± SE	97.7 ± 0.8	108.4 ± 1.6	−6.6	0.000	112.2 ± 1.3	121.2 ± 3.9	−2.8	0.007
	min-max	80.7-123.5	91.2-147.4			76.5-129.3	83.3-144.1		
	<i>n</i>	79	45			54	21		
SH (mm)	mean ± SE	42.1 ± 0.5	48.3 ± 0.8	−7.2	0.000	45.3 ± 0.4	53.4 ± 1.1	−8.1	0.000
	min-max	30.8-54.5	37.7-63.2			40.7-54.2	35.0-64.7		
	<i>n</i>	78	47			58	35		
BW (mm)	mean ± SE	24.4 ± 0.3	30.0 ± 0.5	−10.2	0.000	30.8 ± 0.4	38.7 ± 1.5	−4.1	0.000
	min-max	15.8-29.8	23.0-43.7			24.7-37.9	23.5-81.9		
	<i>n</i>	79	47			58	34		
TL (mm)	mean ± SE	68.8 ± 0.8	64.3 ± 1.2	3.3	0.001	78.7 ± 1.2	69.1 ± 1.9	4.4	0.000
	min-max	49.7-82.4	46.9-90.1			54.0-99.7	47.2-91.6		
	<i>n</i>	74	43			48	32		
Body mass (g)	mean ± SE	223.4 ± 5.2	292.6 ± 13.0	−5.7	0.000	362.0 ± 9.2	474.0 ± 21.3	−5.5	0.000
	min-max	166-435	183-682			224-590	195-678		
	<i>n</i>	77	47			55	33		

(figs 5-6). On this background colour, a radiating pattern of black dots and streaks may occur. This so-called ‘*maculosa* morph’ (Fritz, 1992) frequently occurs also in several southern *E. orbicularis* subspecies, among others in *E. o. galloitalica* (Fritz, 1995a, 2003; Fritz et al., 2005a). In the Torre Salsa population also plastral coloration is lighter than in Lago Preola turtles (fig. 5, bottom) in that dark plastral blotches, if present, are in most cases very small. In a few Lago Preola turtles, the plastron is even predominantly black coloured, and a few turtles have a streaked or mottled, diffuse dark pattern instead of massive black distal blotches on single plastral scutes. We registered in both populations males with whitish or yellowish iris coloration, as mentioned in the original description for *E. trinacris*, but found also males with an intensely reddish iris. This is the first record of reddish iris coloration in *Emys* not associated with the *E. orbicularis* subspecies *E. o. orbicularis* sensu lato, *E. o. colchica*, *E. o. iberica*, *E. o. persica*, or *E. o. luteofusca* (Fritz, 2003), and a rare record

in the western Mediterranean Basin (fig. 7). All other regions in the western Mediterranean where a reddish iris coloration is known to occur (Spanish Mediterranean coast, introduced populations on Balearic Islands; Fritz, 2003) are natural or artificial hybrid zones of *E. o. orbicularis* sensu lato and Mediterranean *E. orbicularis* subspecies. Parenthetically it may be noted that the pupil in some *E. trinacris* lies in the centre of a dark five-point star, a very rare iris pattern in pond turtles.

Coloration and pattern of Sicilian pond turtles from other populations, mainly in southwestern and western Sicily, but also of 11 individuals from Agrigento, Catania, Enna, and Siracusa Provinces in central and eastern Sicily, resemble variation observed at Lago Preola and Torre Salsa.

The captive turtle with haplotype IVa, an adult male, was displaying all typical coloration and pattern characteristics of *Emys orbicularis hellenica*, a taxon morphologically clearly different from *E. o. galloitalica* and *E. trinacris*

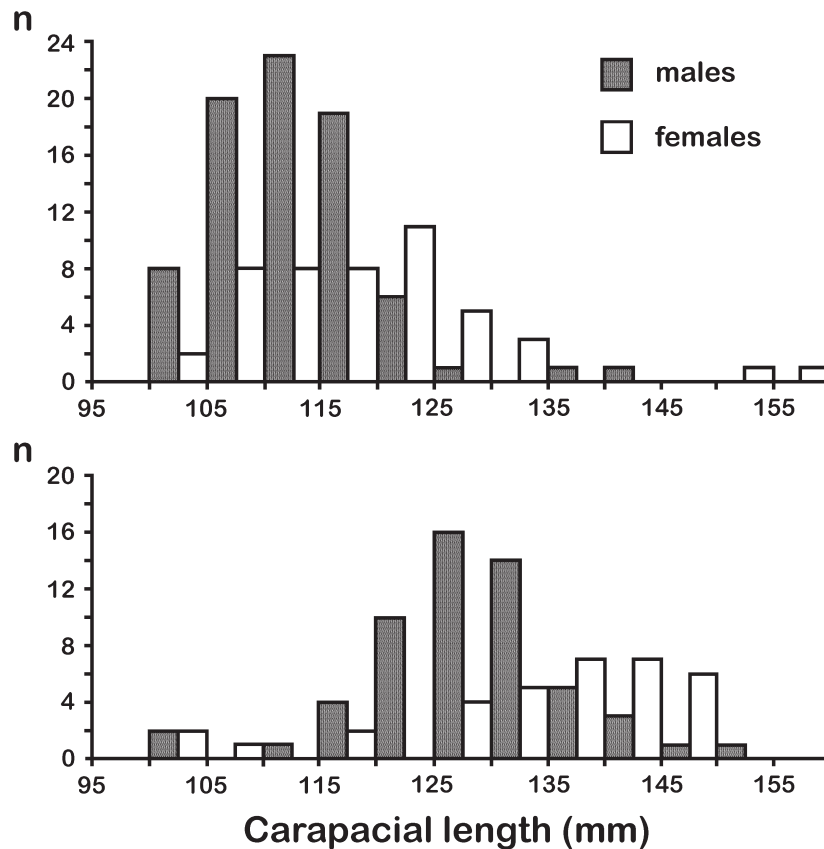


Figure 2. Carapacial size variation in two *Emys trinacris* populations (straight line measurements). Top: Lago Preola (males $n = 79$; females $n = 47$). Bottom: Torre Salsa (males: $n = 57$; females $n = 34$).

(see descriptions and figures in Fritz, 2003; Fritz et al., 2005a). This record underlines that pond turtles are caught as pets and transported over considerable distances in southern Italy.

Hatchlings

Morphometry. *Emys trinacris* hatchlings are on average smaller and lighter than hatchlings of *E. orbicularis* subspecies (fig. 8). *Emys trinacris* hatchlings reach a mean carapace length of 23.1 mm and an average body mass of 3.4 g, while mean carapacial lengths and body masses range in hatchlings of different *E. orbicularis* subspecies between 23.4 and 30.8 mm or 3.7 and 6.7 g, respectively (table 5).

Coloration and pattern. Hatchlings of *Emys trinacris* have a uniform loamy brown cara-

Table 3. Unstandardized and standardized canonical discriminant coefficients and percentages of Eigenvalues for two *Emys trinacris* populations and *Emys orbicularis galloitalica*.

	Unstandardized			Standardized		
	DA1	DA2	DA3	DA1	DA2	DA3
CL	-0.16	-0.08	0.13	-1.08	-0.52	0.86
CW	-0.04	-0.08	0.09	-0.25	-0.47	0.51
SH	0.20	0.04	-0.09	0.67	0.15	-0.28
PL	0.00	0.03	0.01	0.01	0.42	0.11
GuL	0.16	0.21	0.22	0.32	0.42	0.43
HumL	0.26	-0.27	0.11	0.46	-0.48	0.20
PecL	0.28	-0.14	-0.23	0.56	-0.28	-0.46
AbdL	0.20	0.30	-0.10	0.30	0.46	-0.16
FemL	0.07	0.33	-0.10	0.14	0.62	-0.18
AnL	0.24	0.17	-0.03	0.57	0.41	-0.08
NuL	0.04	-0.49	-0.30	0.04	-0.54	-0.33
NuW	0.24	-0.65	-0.20	0.22	-0.60	-0.19
Constant	-8.50	3.52	-15.92			
% Eigenvalue	7.21	3.40	1.77	7.21	3.40	1.77

Table 4. Classification of pond turtles from two *Emys trinacris* populations and from the type locality of *Emys orbicularis galloitalica* by discriminant analysis, based on 12 morphometric characters.

Group	n	Predicted grouping		
		<i>Emys trinacris</i> Lago Preola	<i>Emys trinacris</i> Torre Salsa	<i>Emys orbicularis</i> <i>galloitalica</i>
Males				
<i>Emys trinacris</i> Lago Preola	17	17 (100%)	0 (0%)	0 (0%)
<i>Emys trinacris</i> Torre Salsa	12	0 (0%)	10 (83.3%)	2 (16.7%)
<i>Emys orbicularis galloitalica</i>	11	0 (0%)	1 (9.0%)	10 (91%)
Females				
<i>Emys trinacris</i> Lago Preola	3	3 (100%)	0 (0%)	0 (0%)
<i>Emys trinacris</i> Torre Salsa	6	0 (0%)	6 (100%)	0 (0%)
<i>Emys orbicularis galloitalica</i>	10	0 (0%)	0 (0%)	10 (100%)

Table 5. Carapacial straight line lengths (CL) and body masses of *Emys orbicularis* and *Emys trinacris* hatchlings. If available, means are followed by the standard error of the mean and the range (bracketed). Only literature data for true hatchlings, i.e. turtles that did not start growing yet, were used. *Emys orbicularis orbicularis* I and *E. o. orbicularis* II refer to distinct subspecies which are presently lumped due to nomenclatural problems (Fritz, 2003). Values rounded to the nearest 0.1 mm or 0.1 g. The small *E. o. hellenica* from Istria are captive bred individuals that died shortly after hatching (vouchers in the Museum of Zoology Dresden, MTD 32045-32048, 35561, 41630). It could be that these turtles are therefore undersized. ZSM = Zoologische Staatssammlung München.

Taxon	Region	n	CL (mm)	n	Body Mass (g)	Reference / Voucher
<i>Emys orbicularis orbicularis</i> I	Central Poland	81	26.5 ± 0.1 (23.3-29.2)	81	5.3 ± 0.1 (4.0-6.0)	Mitrus and Zemanek (2000)
<i>Emys orbicularis orbicularis</i> I?	Danube Delta, Ukraine	114	25.6 (22.5-28.0)	105	4.7 (3.6-5.6)	Kotenko (2000)
<i>Emys orbicularis orbicularis</i> II?	Charente-Maritime, France	8	24.2	8	4.0	Duguay and Baron (1998)
<i>Emys orbicularis orbicularis</i> II	Brenne, France	80	26.2 (23.2-29.6)	80	4.9	Servan and Pieau (1984)
<i>Emys orbicularis orbicularis</i> II	Brandenburg, Germany	6	25.0 (22.2-26.0)	–	–	Fritz (2003)
<i>Emys orbicularis orbicularis</i> II	Southeast Slovakia	20	27.5 (25.4-28.6)	–	–	Fritz (2003)
<i>Emys orbicularis orbicularis</i> II	Fruška Gora, Serbia	7	26.7 (23.0-29.0)	–	–	Fritz (2003)
<i>Emys orbicularis orbicularis</i> II	Balaton, Hungary	5	23.4 (21.3-25.3)	–	–	Konok (1961)
<i>Emys orbicularis galloitalica</i>	Latium, Italy	18	24.0	18	3.7	Zuffi (2000)
<i>Emys orbicularis hellenica</i>	Istria, Croatia	6	24.2 (23.0-25.8)	–	–	Fritz (2003)
<i>Emys orbicularis hellenica</i>	Dalmatia, Bosnia Herzegovina	6	30.8 ± 0.5 (28.5-32.2)	–	–	ZSM-SLM 162
<i>Emys orbicularis hispanica</i>	Doñana, Spain	7	29.4 ± 0.6 (27.2-31.1)	6	6.7 (6.1-7.3)	Fritz et al. (1996), Keller (1997)
<i>Emys orbicularis iberica</i>	Azerbaijan	?	? (23.3-25.6)	?	? (4.4-4.8)	Alekperov (1978)
<i>Emys trinacris</i>	Lago Preola, Sicily	6	22.7 ± 0.5 (20.4-24.3)	1	3.0	This study
<i>Emys trinacris</i>	Torre Salsa, Sicily	8	23.5 ± 0.6 (20.4-25.0)	8	3.5 ± 0.3 (2.0-4.0)	This study
<i>Emys trinacris</i>	both localities combined	14	23.1 ± 0.4 (20.4-25.0)	9	3.4 ± 0.2 (2.0-4.0)	This study

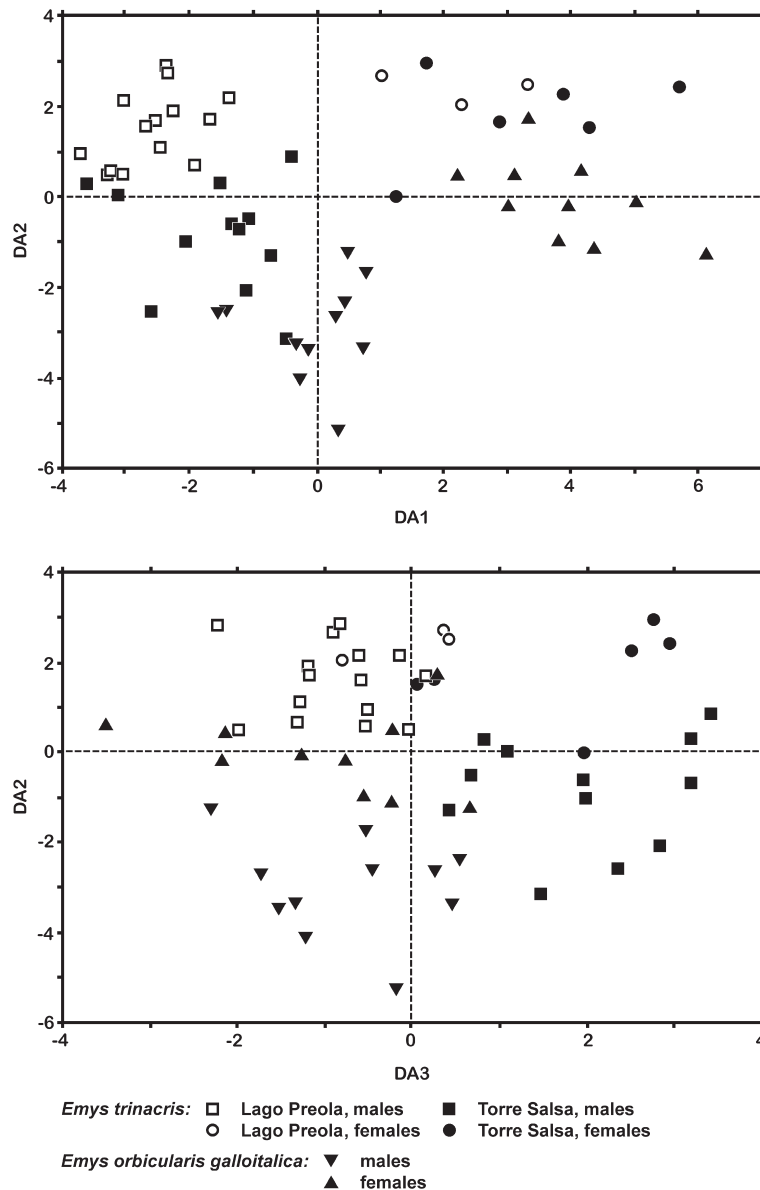


Figure 3. Plot of scores of *Emys trinacris* and *Emys orbicularis galloitalica* on three discriminant axes (DA1-3).

pace coloration, sometimes with a few very small black speckles. The edge of the marginal scutes is slightly lighter coloured, but without a well-defined yellow pattern as in hatchlings of some Mediterranean *E. orbicularis* subspecies. Plastral coloration of *E. trinacris* hatchlings is markedly different from *E. orbicularis*, allowing immediate species determination. In some hatchlings the plastron is immaculate yellow.

When a dark plastral blotch is present, it is small and confined to the central part of the plastron or its hind-lobe. In hatchlings of *E. orbicularis* the plastron is distinctly darker (fig. 9). At least half of the plastron is covered by an extensive black central blotch; only the lateral rim is yellow. This dark blotch covers approximately 50% of the plastron in *E. o. fritzjuergenobsti* (see figure in Fritz, 2001, 2003) and in all other sub-

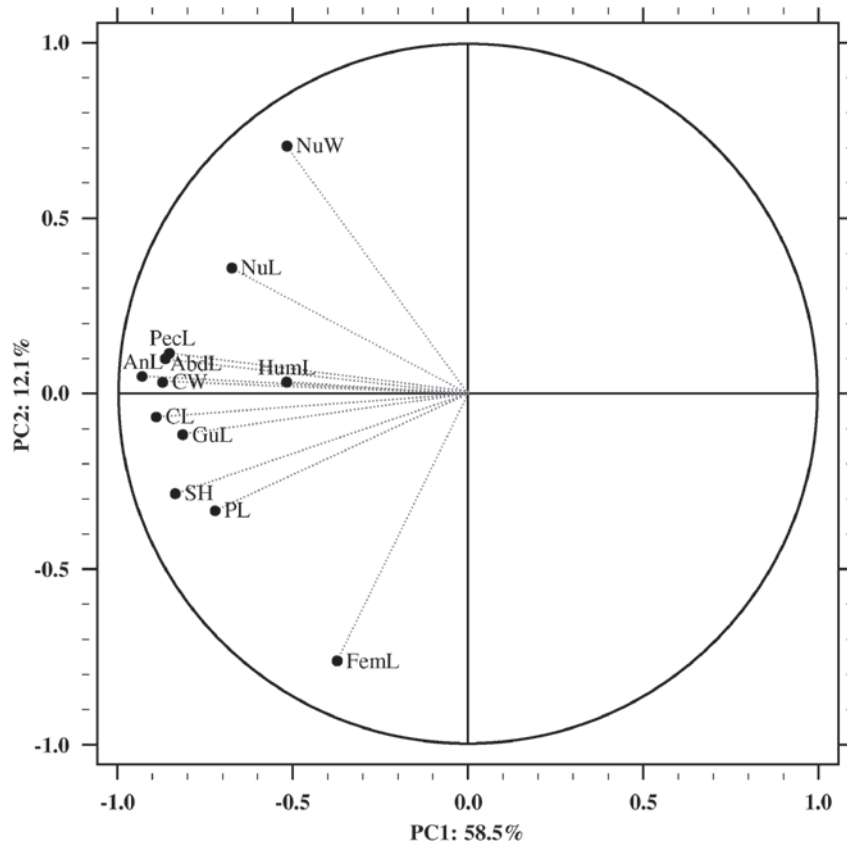


Figure 4. Plot of scores of variables on first two principal component axes for two *Emys trinacris* populations and *Emys orbicularis galloitalica*.

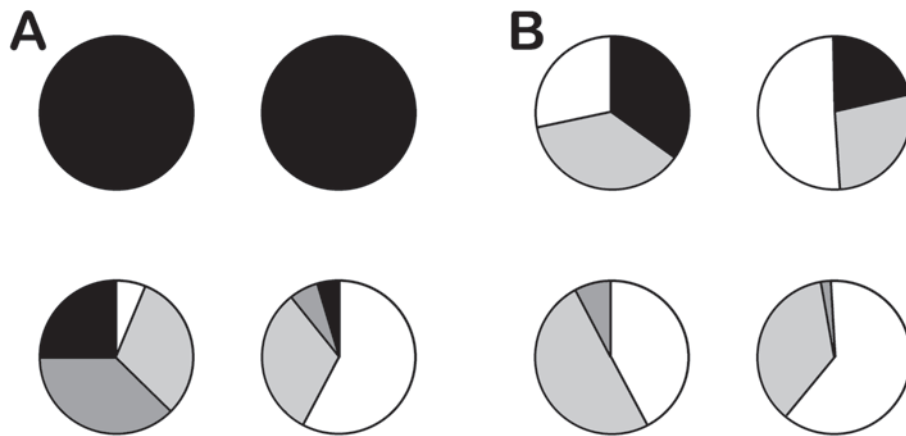


Figure 5. Shell coloration and pattern in two *Emys trinacris* populations (percentages). A: Lago Preola (left – males $n = 64$; right – females $n = 47$). B: Torre Salsa (left – males $n = 59$; right – females $n = 43$). Top: carapace (black: black primary colour; white: light brownish to chestnut primary colour = *maculosa* morph; grey: intermediate). Bottom: plastron (black: mainly black; dark grey: huge black, in part confluent distal blotches or dark elements, covering more than 1/3 of plastron; light grey: small, isolated black distal blotches or dark elements, covering less than 1/3 of plastron; white: entirely yellow).

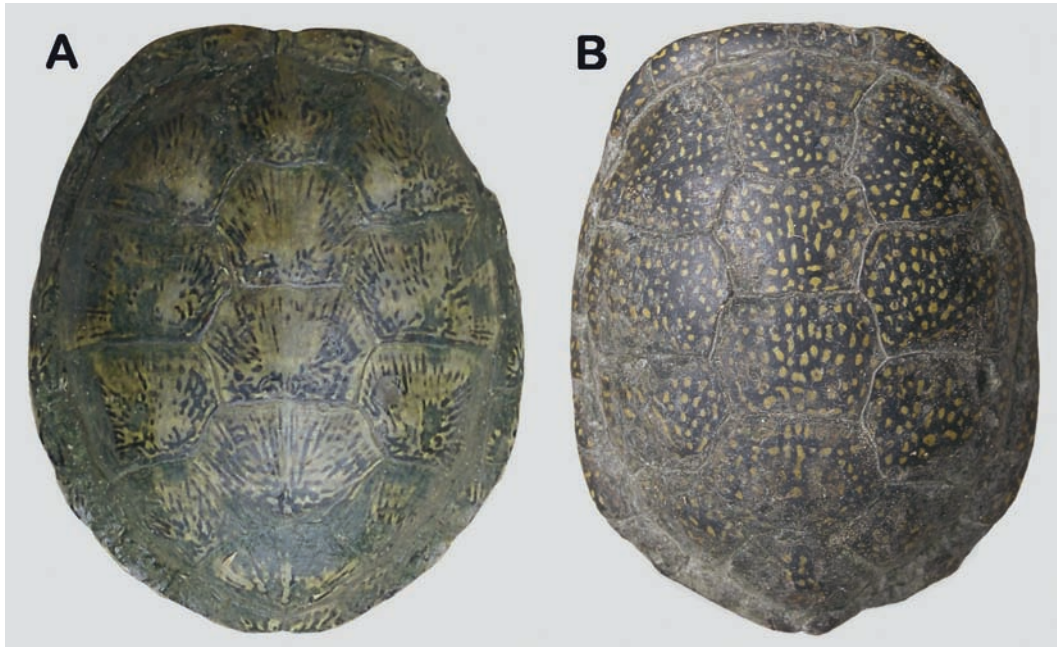


Figure 6. Carapacial coloration morphs in *Emys trinacris*. A: Light coloured turtle (*maculosa* morph; black pattern may be entirely absent). B: Dark coloured turtle.

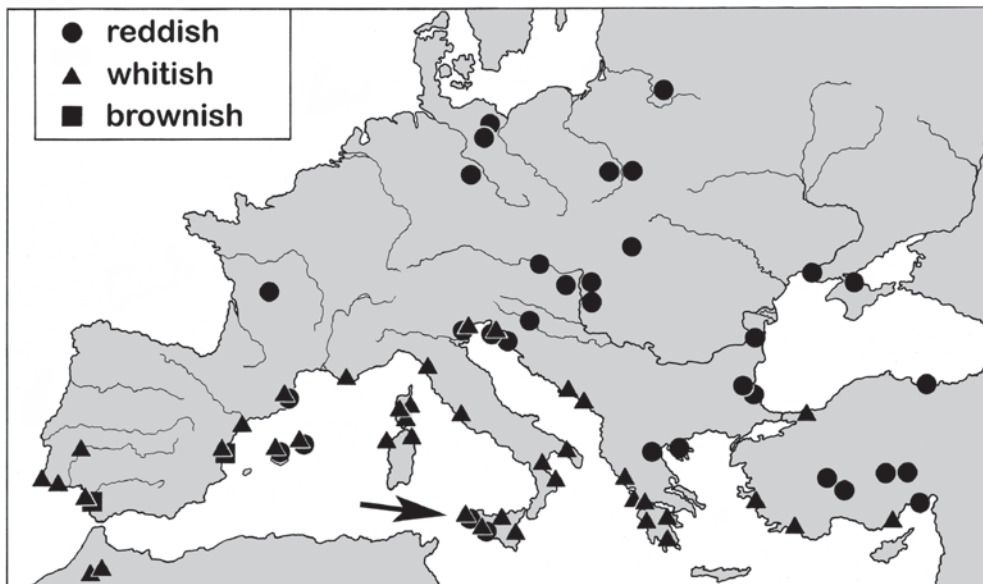


Figure 7. Distribution of different iris colorations in males of *Emys orbicularis* and *E. trinacris* (modified from Fritz, 2003). With exception of *E. trinacris* on Sicily (arrow), red iris colour results in the western Mediterranean Basin from hybridization of Mediterranean *E. orbicularis* subspecies with *E. o. orbicularis* sensu lato.

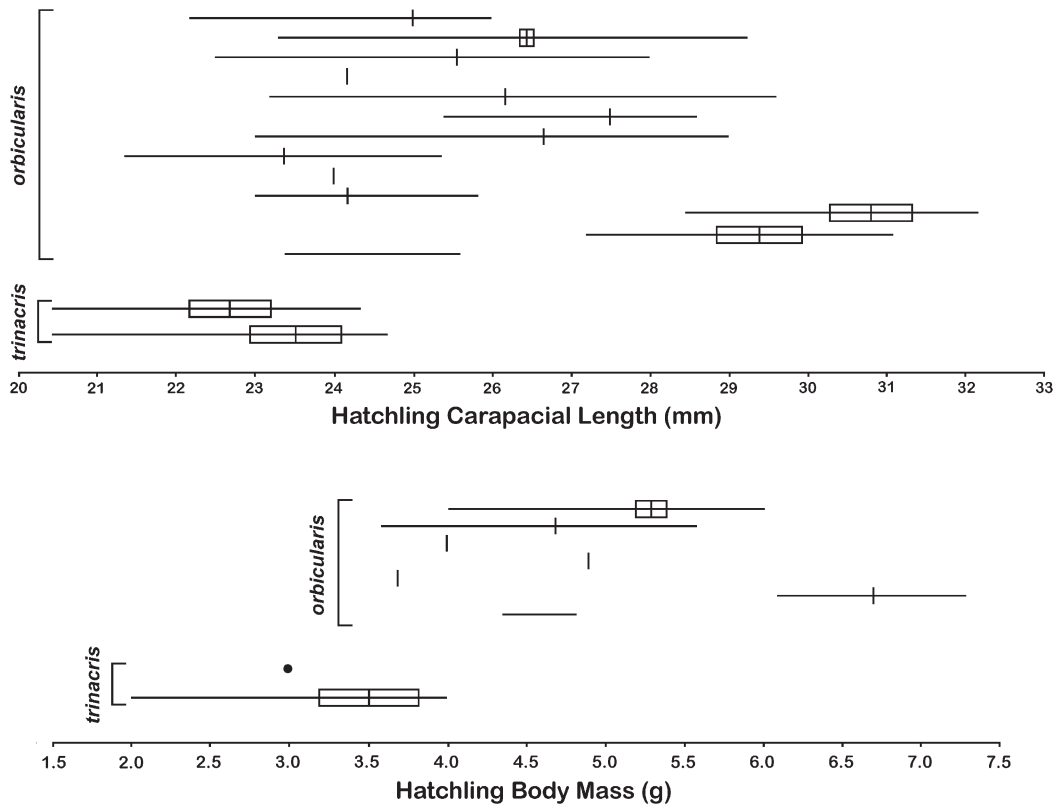


Figure 8. Hatchling carapacial lengths (straight line) and body masses in *Emys orbicularis* and *E. trinacris*. Samples correspond from top to bottom to the ones given in table 5 (*E. trinacris* populations not combined). Horizontal lines denote minimum and maximum values and vertical lines means. Boxes represent one standard error above and below mean. Dot represents body mass of an *E. trinacris* hatchling from Lago Preola.

species at least two-thirds of the plastron (Fritz, 2003 and U. Fritz, unpubl. data).

Discussion

Morphological variation and what makes the species cryptic?

We observed significant differences between adult *Emys trinacris* from the Torre Salsa and Lago Preola populations. Torre Salsa turtles are larger and distinctly lighter coloured than Lago Preola turtles. Moreover, coloration of Lago Preola turtles is rather uniform, whereas turtles from Torre Salsa exhibit considerable coloration polymorphism.

Despite this unexpected polymorphism we are confident dealing with native Sicilian tur-

tles in both localities. In natural or artificial hybrid populations of different *E. orbicularis* subspecies haplotypes of distinct mtDNA lineages occur (Lenk et al., 1999; Fritz et al., 2004, 2005a, b; U. Fritz, unpubl. data), reflecting different origins, while 11 individuals from Torre Salsa harboured mtDNA haplotypes characteristic for *E. trinacris*; among the sequenced turtles were representatives of all coloration morphs.

The morphological distinctness of turtles from Lago Preola and Torre Salsa is remarkable because both localities are only 80 km away, and there are no obvious environmental differences. This suggests pronounced population-specific differences and that local kinship relation could play an important role in phenetic variation.

The coloration polymorphism of adult *E. trinacris* comprises a fair part of variation known from *E. orbicularis*. Although certain southern *E. orbicularis* subspecies are also polymorphic, especially in regard to shell coloration, variation is, as a rule, lower than in *E. trinacris*. For example, in different *E. orbicularis* subspecies adult males have either a reddish or a whitish iris coloration. In *E. trinacris* males both coloration types occur. We speculate that in *E. trinacris* the complete array of morphological variation of the last common ancestor of *E. trinacris* and *E. orbicularis* may have survived until today and that selection or genetic bottlenecks reduced the original range of coloration and pattern variation in subspecies of *E. orbicularis*, resulting in different subspecies in character displacement.

The small size of *E. trinacris* adults matches Mediterranean *E. orbicularis* subspecies. Northern *E. orbicularis* subspecies and European pond turtles from central Turkey reach distinctly larger shell lengths of up to 230 mm (Fritz, 2003). We doubt, however, that the small adult size of *E. trinacris* and many *E. orbicularis* subspecies is indicative of a small size of the last common ancestor. Fossil *Emys*, also from Italy, often reach considerable shell lengths, in part exceeding size records of extant turtles (Fritz, 1995b). We assume that in northern regions or under continental climates, like in central Turkey, large turtles are favoured by selection. This hypothesis is supported by the observation that, according to Bergmann's rule, size increases with geographic latitude also in other turtle species (Ashton and Feldman, 2003). Dark coloration combined with large size, as found in the northern parts of the distribution range of *E. orbicularis*, supports body-warming during basking and allows longer storage of up-taken energy. While dark coloration seems thus to be beneficial in the north, light coloration and small size might be simply not disadvantageous in the south, allowing coloration polymorphisms in *E. trinacris* and southern *E. orbicularis* subspecies. This situation is contribut-

ing to morphological similarity of *E. trinacris* and *E. o. galloitalica*.

In contrast to adults that resemble *E. o. galloitalica* morphologically, hatchlings of *E. trinacris* are conspicuously different from hatchlings of all *E. orbicularis* subspecies. A similar phenomenon is observed in hatchlings of New World cooter, slider and map turtles (genera *Pseudemys*, *Trachemys*, *Graptemys*; Emydidae). Also in these species adults, that have lost their distinctive hatchling colour patterns, are much more difficult to determine compared to newly hatched turtles (see photos in Pritchard, 1979 and Ernst et al., 2000). *Emys trinacris* hatchlings seem, moreover, to be generally smaller than hatchlings of *E. orbicularis*. Although size and body mass data are not available for hatchlings of all *E. orbicularis* subspecies, it is obvious that *E. trinacris* is in the lowermost part of the range of *E. orbicularis* (fig. 8, table 5). Within *E. orbicularis* regional or subspecific variation in hatchling size and body mass could exist. This is suggested by data for *E. o. hispanica* and in part for *E. o. hellenica*, exceeding considerably other subspecies and being far removed of the small and light *E. trinacris* hatchlings.

Until now, virtually nothing is known about reproduction in *E. trinacris*. Hatchlings and very small juveniles (carapace length below 3-4 cm) were found during field-work either in September or in spring (March to June). Probably some hatchlings overwinter in their nests and surface not before next spring, like in *E. orbicularis* (Fritz, 2003). It is well-known that different *E. orbicularis* subspecies have distinct reproductive strategies. Northern, large-bodied subspecies produce normally only one large clutch per year, while small-bodied southern subspecies lay two or more clutches, each with distinctly fewer eggs. Obviously, clutch size is limited by body size, as egg size seems to be fairly constant in different subspecies (Fritz, 2003). The small hatchling size of *E. trinacris* suggests that also eggs could be smaller than in *E. orbicularis*. If so, this raises the question

whether *E. trinacris* produces a larger number of eggs per clutch compared to similar-sized Mediterranean *E. orbicularis* subspecies.

Cryptic species, species concepts and species delineation

Adults of *Emys trinacris* resemble *Emys orbicularis galloitalica* so much that determination by morphological means is virtually impossible, while nuclear genomic fingerprinting indicates reproductive isolation. Therefore, *E. trinacris* was considered to represent a 'cryptic species' (Fritz et al., 2005a). Morphological similarity of *E. trinacris* and *E. o. galloitalica* was generally confirmed in the present study, although red-eyed males, like in *E. trinacris*, never occur in *E. o. galloitalica* and such individuals are easy to distinguish.

Multivariate statistics using more characters than in the original description were not helpful for differentiation of *E. trinacris* and *E. o. galloitalica*. Discriminant analysis classified to approximately the same high rate individuals of both *E. trinacris* populations and of *E. o. galloitalica* properly. Although such multivariate methods are often applied in taxonomy (e.g. in chelonians: Iverson and McCord, 1992, 1997; Perälä, 2002; Schulze and Fritz, 2003; Fritz et al., 2005a), our results argue for caution. A high rate of correct classification could reflect differences between populations rather than taxonomic differences, may the studied populations be natural populations or statistical populations created by sampling in a wider geographic area. We suggest researchers should focus more on detecting first-class qualitative morphological differences between taxa than relying on statistical differences produced by an – in the worst case – biased sampling or even wrong application of powerful statistical methods (cf. James and McCulloch, 1990). A refined morphological approach using qualitative characters may even lead in cryptic species to discovery of previously overlooked diagnostic characters, as is the case with the distinctive coloration of *E. trinacris* hatchlings or the earlier recognized col-

oration differences of hatchlings of *Lacerta bilineata* and *L. viridis* (Rykena, 1991; Amann et al., 1997). These examples underline also that distinct life stages of the same species may differ much regarding their diagnosability, making certain stages 'cryptic' and others not.

With the recent advent of so-called DNA taxonomy and DNA bar-coding, a renaissance in interest for cryptic species became evident (e.g. Hebert et al., 2003a, b, 2004a, b; Sáez et al., 2003; Zhang et al., 2004; Sáez and Lozano, 2005). Among the first authors highlighting the occurrence of morphologically indistinguishable or very similar species was Ernst Mayr (1942), coining for them the term 'sibling species', derived from the German 'Geschwisterarten'. 'Sibling species' was later more and more replaced by 'cryptic species', perhaps to avoid confusion with 'sister taxon' from phylogenetic terminology. Mayr (1942, 1963 and later works) believed 'sibling species' confirming existence of species as natural entities, corresponding to reproductively isolated groups (or better genetically isolated groups; Bock, 2004) of populations of bisexual animals. Mayr contrasted his species concept, later known as Biological Species Concept (e.g. Mayr, 1963, 2000), explicitly to higher taxonomic units, which were considered by him classification systems created by man (see also Mayr and Bock, 2002) – a clear difference to the perception of Phylogenetic Systematics treating monophyla of all hierarchical levels as natural kinds (e.g. Ax, 1995). Mayr took the existence of sibling species, possessing intrinsic mechanisms enabling them to maintain their genetic integrity, as support for reality of the species entity in nature – regardless whether species might represent paraphyletic or polyphyletic assemblages of populations.

Today usage of the replacement term 'cryptic species' erodes, and this is related with competing species concepts. Mayr's original idea was that genetically isolated entities of bisexual animals exist that are hardly or not distinguishable by cognitive abilities of man. To-



Figure 9. Ventral aspects of hatchlings of *Emys trinacris* (top; live turtles), Torre Salsa near Siculiana, Sicily, and *Emys orbicularis hellenica* (bottom; preserved specimens, Zoologische Staatssammlung München, ZSM-SLM 162), Metković, Bosnia Herzegovina. Not to scale. Note different plastral pattern.

day, monophyletic mtDNA lineages are often used for species delineation, in accordance with Phylogenetic Species Concepts defining species as diagnosable, ideally monophyletic units (e.g. Cracraft, 1983, 1987; Crowe, 1999; Mishler and Theriot, 2000; Wheeler and Platnick, 2000; review in Coyne and Orr, 2004), but without appreciating whether investigated populations are reproductively isolated or not. A fair part of recently unravelled 'cryptic species' certainly correspond to taxa harbouring monophyletic mtDNA clades. However, animal mtDNA is not always an ideal tool for delineating species boundaries. For example, Funk and Omland (2003) demonstrated that 23% of 2319 sur-

veyed animal species are characterized by paraphyletic or polyphyletic mitochondrial gene trees. Already Avise (1989) was aware of this 'gene tree and species tree' problem, caused by many factors, including lacking recombination in mtDNA as well as maternal vs. biparental inheritance and different effective population sizes of mitochondrial and nuclear genomes that result in distinct patterns for incomplete lineage sorting and introgression (Funk and Omland, 2003; Ballard and Whitlock, 2004). If congruent results of bar-coding and species borders are desired, adoption of a Phylogenetic Species Concept is required and species delineation often is

reduced to measuring distances between monophyletic mitochondrial lineages.

However, there are cases known where distinct Biological Species are hidden behind the same monophyletic mtDNA lineage. A recently discovered herpetological example is the Carpathian newt (*Triturus montandoni*), whose original mitochondrial genome was replaced by the mitochondrial genome of the closely related *T. vulgaris* through hybridization (Babik et al., 2005), and several turtle species share the same or extremely similar mitochondrial haplotypes, suggestive of slow evolution rates, recently split species, incomplete lineage sorting or introgression. Lamb et al. (1994) found no or only low divergence between the twelve species of *Graptemys* using cytochrome b (0-1.5%) and restriction site data (0-3.2%), with several species indistinguishable by these markers. Stuart and Parham (2004) reported similarly weak mtDNA differentiation between three *Cuora* species using COI and ND4 sequence data (identical sequences in *C. aurocapitata* and *C. pani*; maximum uncorrected p distance between *C. aurocapitata*, *C. pani* and *C. trifasciata* 0.45%).

On the other hand, taxa with distinct monophyletic mitochondrial gene trees are not necessarily genetically isolated and, therefore, such taxa are not necessarily 'Biological Species'. Within *Emys orbicularis* eight monophyletic mtDNA lineages are known with sequence divergences (cytochrome b gene, uncorrected p distances) from 0.48 to 1.84%, while sequence divergences between the eight mtDNA lineages of *E. orbicularis* and *E. trinacris* fall within this range (0.98-1.66; Fritz et al., 2005a). However, in nuclear genomic fingerprinting with ISSR PCR a clear gap between *E. trinacris* and *E. orbicularis* was detected, while matrilineages and subspecies of *E. orbicularis* were indistinguishable. This suggests extensive gene flow between *E. orbicularis* harbouring different mtDNA lineages and suggests under the Biological Species Concept subspecies status, while the nuclear genomic gap between *E. or-*

bicularis and *E. trinacris* is indicative of reproductive isolation and species status.

It is beyond the scope to discuss this in detail; however, these examples underline that species delineation should not be reduced to differences or even thresholds of distinctness of the mitochondrial genome.

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