REPTILES & AMPHIBIANS

Morphological Variation in the European Pond Turtle, *Emys orbicularis* (Linnaeus 1758), on the Island of Menorca

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Abstract.—The European Pond Turtle (*Emys orbicularis*) is a widely distributed species in Menorca (Balearic Islands, western Mediterranean) and it likely was introduced in historical times. In this study, we evaluated sexual dimorphism and morphological variation of *E. orbicularis* on the island from a sample of 455 individuals captured at ten survey sites. In these individuals, we measured seven linear traits related to body size. In addition, we measured two shape descriptors (carapace and plastron shape) and iris and plastral coloration. The results showed very pronounced sexual dimorphism, with females being larger than males and also having longer and broader plastra with higher carapaces. The site of capture had significant effects on carapace, plastral shape, and coloration. Both males and females had dark- or light-colored plastra, but some males had almost completely dark plastra (to 99% dark), whereas in females, the maximum extent of black plastra was lower (79%). The sexes also differed in iridal chromatic attributes, with females more frequently having yellow and males having red or orange irises. However, this was not diagnostic of sexual identity, as 13% of females had red irises and 15% of males had yellow irises.

The Mediterranean Islands have a rich reptilian fauna, much of which is endemic; however, some species have been translocated historically or even more recently (the second half of the twentieth century) (Corti et al. 1999; Grano and Cattaneo 2019; Escoriza 2021). Some of these introduced species have an important effect on island reptiles and amphibians, which frequently show tameness and lack deceptive strategies to reduce predation (Mencía et al. 2017; Edwards et al. 2021). Therefore, the management and conservation of island herpetofauna must prioritize the removal of introduced species, unless they demonstrate no impact on native species and cultural or scientific value that justifies their conservation (Carpaneto 2006; Vamberger et al. 2011).

The European Pond Turtle, *Emys orbicularis* (Linnaeus 1758) is one of four species of freshwater turtles native to Europe and, until recently, was the only species present on the islands of the western Mediterranean (Speybroeck et al. 2016). This turtle has been recorded on the islands of Corsica, Sardinia, Mallorca, and Menorca (Keller and Andreu 2002). In Menorca, the first records of its presence date from the 9th Century, although no fossils have been recorded from prehuman times. Hence, *E. orbicularis* almost certainly has been introduced to Menorca (Alcover and Mayol 1981; Keller and Andreu 2002). Moreover, Menorcan populations of *E. orbicularis* are clustered with those of the subspecies *E. o. galloitalica*, which is native to western Italy, southern France, and the northeastern Iberian Peninsula (Velo-Antón et al. 2008).

Populations of *E. orbicularis* from Mallorca (the closest island with populations of *E. orbicularis*), also allochthonous, originated from the Tyrrhenian Islands (subspecies *E. o. galloitalica*) and central-eastern Europe (subspecies *E. o. orbicularis*) (Fritz et al. 1998). This mixed origin of the Mallorcan populations could explain why individuals from this island show phenotypic traits of both parental subspecies, for example, yellow or dark plastral coloration and yellow or reddish irises in males (Fritz et al. 1998). In the case of Menorca, this variability had not been assessed.

In this study, we examined morphological variability and sexual dimorphism of *E. orbicularis* on Menorca. Semiaquatic turtles display several sexually dimorphic traits involving coloration of the carapace, limbs, and head, body size, and shapes of the carapace and plastron (Ernst and Lovich 2009; Gradela et al. 2017). This variability is related to mating behaviour (e.g., plastral concavity and long tails in males enhance the holding of females during copulation), honest sexual signals, and fecundity (larger body size in females favors the

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production of larger egg clutches) (Litzgus and Smith 2010; Thomson et al. 2021). Marked sexual dimorphism involving body size, relative weight, and iris and plastral coloration has been reported in mainland populations of E. orbicularis (Fritz et al. 1998; Ayres Fernández and Cordero Rivera 2001). We hypothesized that populations on Menorca would also show significant morphological variability, depending on sexual identity and reflecting the mixed origin of these populations.

Methods

Surveys were conducted at ten locations across the island (Fig. 1) during spring and summer 2022, coinciding with peak activity of these turtles (González 2015). Survey sites included brackish bodies of water, permanent freshwater ponds, artificial reservoirs, and streams (Table 1).We used funnel traps (110 × 50 cm) baited with fresh or frozen fish, partially submerged to prevent accidental suffocation. The number of traps at each site was proportional to the surface of the water (Table 1). Traps were monitored and turtles removed every 24 hours.

Captured individuals were marked using different combinations of holes drilled in the marginal scutes of the carapace (Plummer 1979) to prevent processing individuals twice. We measured linear variables that described the shape of the carapace and plastron (Mosimann and Bider 1960). These measurements included straight carapace length (SCL), shell height, bridge width, plastral length, and carapace curvature. We also measured precloacal tail length, a highly dimorphic character in sexes of turtles. Precloacal tail length is preferable to the full tail length, because tail tips of turtles are frequently shortened by predators or other injuries (Walde et al. 2003). Linear distances were measured to the nearest millimeter with

Table 1. Habitats surveyed for European Pond Turtles (*Emys orbicularis*) in Menorca with number of traps and trap density in each.

Wetland type	Surface area (m^2)	Number of traps	Trap density (n/m^2)
Freshwater pond	25.50	1	0.039
Artificial reservoir	2404.68	3	0.001
Stream	1742.59	2	0.001
Stream	3044.73	5	0.002
Drainage channel	385.49	5	0.013
Stream	1022.40	3	0.003
Brackish water pond	3101.87	7	0.002
Brackish water pond	7543.56	5	0.001
Freshwater pond	1380.99	6	0.004
Freshwater pond	3491.07	8	0.002

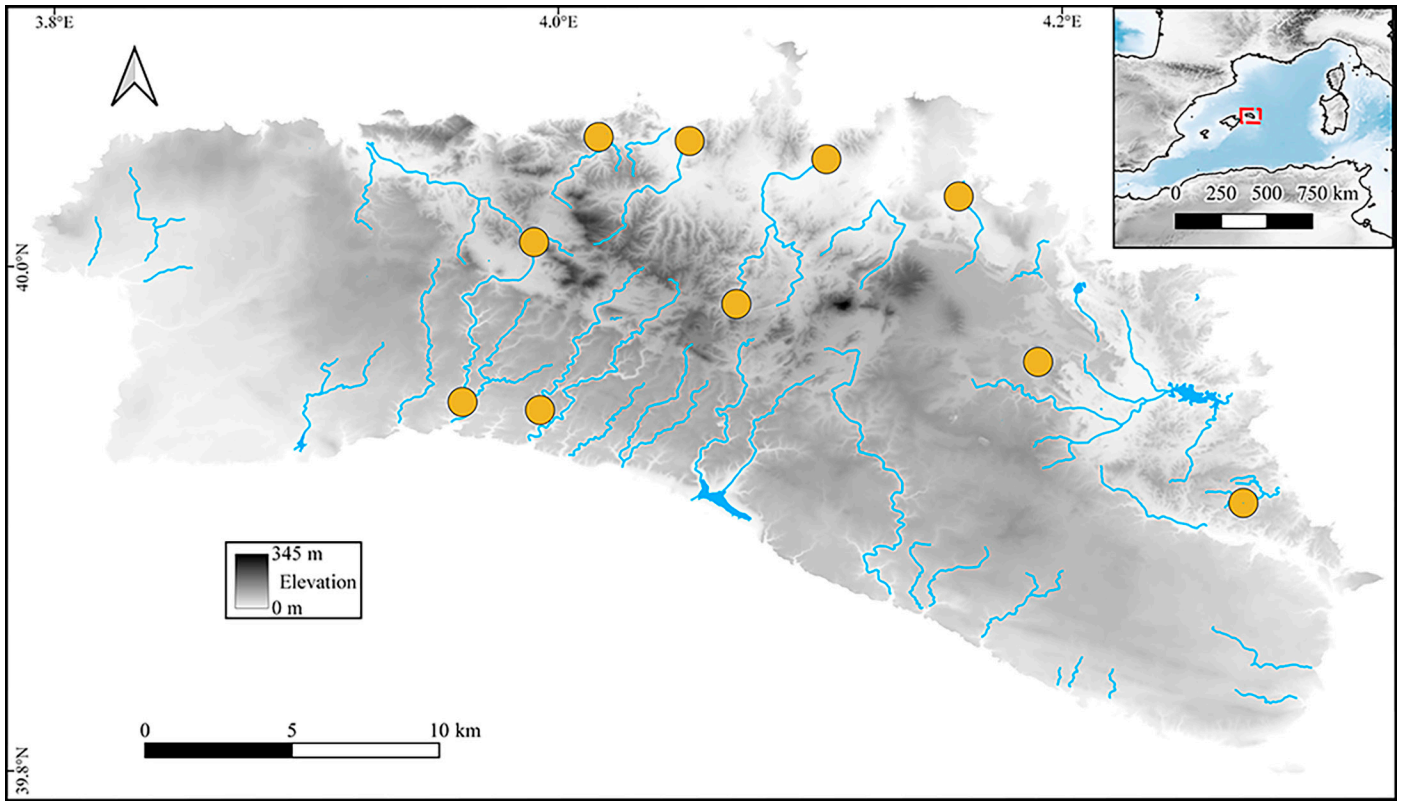

Figure 1. Map of the island of Menorca, indicating the sampling sites (yellow circles) for European Pond Turtles (*Emys orbicularis*) and the main hydrographic network (blue). Sampled locations are slightly displaced for conservation reasons.

a digital calliper (± 0.01 mm). In addition, all individuals were weighed with a digital scale (± 0.1 g).

We categorized individuals with a SCL smaller than 100 mm as juveniles (Escoriza et al. 2020). Adults were assigned to sexes based on external traits, such as the length of the tail, the position of the cloaca relative to the anal plates, and head coloration (Alarcos et al. 2019). Dorsal and ventral photographs were taken to measure carapace shape, plastral shape, and coloration. In a subsample of 122 turtles (n_{males} = 45, $n_{females}$ = 46, $n_{juveniles}$ = 31) we also took photographs of heads to assess iridal chromatic variations.

Shape variation of the carapace and plastron was evaluated using a roundness index ($4 \times \text{area}/\pi \times [\text{major axis}]^2$), the value of which ranges from 0 to 1, with 1 being a mathematically perfect circle (Bolden et al. 2020). For traits highly collinear with body size (carapace width and height, bridge width, plastral length, precloacal tail length, carapace curvature, and body mass), we used ANCOVA to determine intersexual differences. Variables were first log-transformed, and we then used carapace length as a covariate with two factors: sex (fixed effect with two levels) and site (random effect with 10 levels) (Schutz et al. 2009). For each sexual group, we estimated the adjusted mean for the covariate effect.

Plastral coloration was quantified as the ratio of black to lighter background color after converting photographs to binary images (Chong et al. 2019). Individuals with plastra infested by epizoophytic algae were excluded from analyses. Image analysis was conducted using ImageJ (Schneider et al. 2012). Significant differences in total length, carapace roundness, plastral roundness, and plastral black ratio of adults were evaluated using two-way ANOVA tests. This analysis included two factors: sex (fixed effect with two levels) and site (random effect with 10 levels) to account for the possible effects of site conditions on shell morphology (Lovich et al. 1990; Zuffi et al. 2017).

Variability in iris coloration was examined by building a contingency table and comparing relative proportions with chi-squared tests. We determined iris coloration that best explained the intersexual differences by estimating the cell-standardized residuals and adjusting their statistical significance with the Bonferroni method (Sharpe 2015). These analyses were performed with the package lmerTest (Kuznetsova et al. 2017) in R (R Core Team 2023).

Results

We captured a total of 455 individuals: 39.5% males (180 individuals), 47.9% females (218 individuals), and 12.5% juveniles (57 individuals). ANOVA tests showed that females were significantly more elongated, whereas males tended to have darker plastra (Table 2). However, plastral coloration showed great variability, ranging from prevailing vanilla-yellow to almost entirely gray-black, including some individuals with predominantly orange-brown plastra (Fig. 2). In addition, carapace length, carapace roundness, plastral roundness, and plastral coloration were also statistically influenced by the site of capture (Table 2).

ANCOVA indicated that females tended to be heavier and have broader and higher carapaces and longer plastra but shorter precloacal tail lengths than males (Table 3). Iris coloration differed between males and females (Chi-squared test = 106.48, $P < 0.001$) (Table 4). Yellow iris coloration was more frequent in females whereas orange-red was more frequent in males, but this was not diagnostic of sexual identity (Table 4) (Figs. 3 and 4). All juveniles and hatchlings had yellow-dotted irises.

Discussion

The results of this study demonstrate substantial morphological variability in populations of *E. orbicularis* on Menorca. Females are larger and proportionally heavier than males, as in other populations of the species throughout its extensive range (Zuffi and Gariboldi 1995; Kaviani and Rahimibashar 2015; Escoriza et al. 2021). Both sexes also differ in relative proportions of carapace and plastron. Females have wider and taller carapaces and longer plastra. These differences arise from the greater intra-abdominal space necessary to accommodate a large number of eggs (to 16 eggs per clutch) (Bonin et al. 2006).

As had been previously documented for this species, males have longer tails, making it easier to hold females during mating (Fattizzo 2004; Alarcos et al. 2019). Additionally, males,

Table 2. Two-way ANOVA comparing non-collinear variables with body size in European Pond Turtles (*Emys orbicularis*) in Menorca. Females (n = 218), males (n =180).

	Females (mean ± SD)	Males (mean ± SD)	Factor: Sex P-value	Factor: Site P-value
Carapace length (mm)	130.700 ± 0.800	117.500 ± 0.700	0.0002	0.0001
Carapace roundness	0.784 ± 0.004	0.785 ± 0.004	0.3150	0.0350
Plastral roundness	0.625 ± 0.002	0.611 ± 0.002	0.0820	0.0003
Plastral black ratio (%)	21.506 ± 1.283	29.064 ± 1.650	0.0150	0.0001

Figure 2. Variability in the plastral coloration of female (upper four photos) and male European Pond Turtles (*Emys orbicularis*) in Menorca. In the left side, the plot showed the variability in plastral black ratio (%) in both sexes. Photographs by Santiago Poch and Daniel Escoriza.

Table 3. Results of the comparison of collinear variables with body size in European Pond Turtles (*Emys orbicularis*) in Menorca using ANCOVA (df = degree of freedom). Adjusted means (means of each sex category adjusted for carapace length) are shown in the female and male columns. The effect of site was incorporated in the model as a random effect. Measurements are in mm and mass in g.

	Females	Males	df	F	P-value
Carapace width	100.20	98.52	386	4.221	0.0020
Carapace height	48.51	42.42	386	19.930	0.0001
Bridge width	34.40	29.96	386	15.432	0.0001
Plastral length	119.99	109.34	386	26.317	0.0001
Precloacal tail length	12.44	23.24	346	13.299	0.0005
Shell curvature	14.19	14.10	378	1.113	0.2400
Body mass	356.10	316.77	385	6.020	0.0100

Table 4. Contingency table showing the chromatic variability of iris coloration in a subsample of 122 European Pond Turtles (*Emys orbicularis*) in Menorca (including 31 juveniles and neonates with uniform iris coloration). The standardized residuals provided an estimate of the color category that contributes most to the differences between sexes, and the statistical significance of these intersexual differences are shown with P values adjusted for multiple testing using the Bonferroni procedure.

Iris coloration	Females n = 45	Males n = 46	Standardized residuals	Adjusted P
Yellow	0.87	0.15	5.04	< 0.0001
Orange	0.00	0.28	3.74	< 0.0001
Red	0.13	0.57	3.72	< 0.0001

on average, have darker plastra. Some males have almost uniformly black plastra (to 99% dark), whereas dark color on plastra in females never exceeded 79% of total plastral cover. Some of these results are similar to those reported in Mallorca and southern Italy (Fritz et al. 1998; Fattizzo 2008), although those authors used discrete categories to quantify variations in plastral coloration. In southeastern Italian populations, males also tend to be darker than females (but rarely completely black) (Fattizzo 2008). On Mallorca, both sexes had dark- and light-colored plastra but, as on Menorca, females do not have completely dark plastra (> 2/3 dark) (Fritz et al. 1998). In our sample, 23% of individuals (encompassing both sexes) had to be excluded from the analyses due to an epizoophytic algal infestation on the plates, which is common in this species and other freshwater turtles (Hulse 1976; Fayolle et al. 2016).

The general assumption is that variation in plastral coloration in *E. orbicularis* is determined by the phenotypic

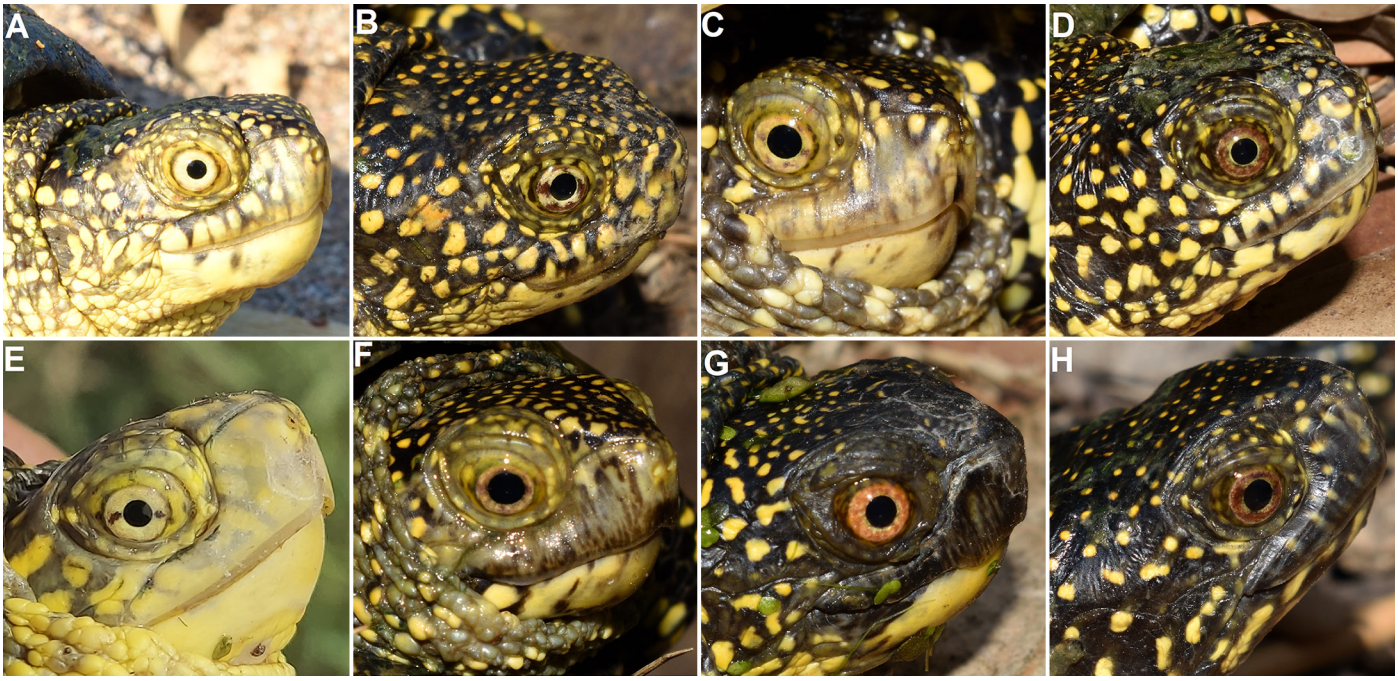

Figure 3. Variability in iris coloration of female (A–D) and male (E–H) European Pond Turtles (*Emys orbicularis*) in Menorca. Photographs by Daniel Escoriza (A, B, C, D, F, G, H) and Santiago Poch (E).

variation among subspecies (Fritz 1998; Fritz et al. 1998). If this is the case, the variability observed in Menorca should be attributable to the existence of hybrids that express traits of the mixed parental clades. However, this characteristic also might show some level of environmental variability, for example, depending on the amounts of minerals and tannins in the water, as has been observed in other emydids (Lovich et al. 1990). Our analysis indicates that variation in plastral coloration is influenced by site of capture, but we have not determined whether this depends on exogenous (e.g., water quality or habitat characteristics) or endogenous factors (e.g., genetic isolation or interclade hybridization).

Another distinctive characteristic of Menorcan populations is the considerable range of iridal chromatic variation. In *E. orbicularis*, iris coloration is usually a sexually dimorphic character (Fritz et al. 1998), although it is not diagnostic for sexual identity in all populations. For example, in Puglia (southeastern Italy), both females and males have yellow irises (Fattizzo 2008). In Menorca, males have red-orange irises, lighter transitions to yellow ("pink" or yellow reddish-dotted), and more rarely yellow (yellow-black dotted), whereas females have predominantly yellow irises (clean or more frequently dotted), darker transitions ("pink" or yellow reddish-dotted), and more rarely red, which is a pattern previously unreported in female *E. orbicularis*. These findings indicate that the iris chromatic pattern is therefore suggestive, albeit not diagnostic of the sexual identity of *E. orbicularis* in Menorca as in other regions of the species' distribution (Fattizzo 2008).

Iris coloration in male *E. orbicularis* is assumed to follow a geographic pattern defined by subspecies (Fritz 1998; Fritz et al. 1998). The reddish coloration in the iris of males would be a typical character of eastern clades (southwestern Asia and the Balkans), appearing much more rarely in individuals from Sicily, mainland Spain, and Mallorca (Fritz et al. 2006). In the Iberian Peninsula, southeastern France, and northwestern Africa (the geographically closest natural populations to Menorca), males usually have yellowish-white irises (Fritz et al. 1998). However, in our sample, the reddish-orange coloration was present in 85% of our subsample of 46 males.

Given the threatened situation in which numerous endemic island species occur, the management and conservation of island herpetofaunas must prioritize the removal of introduced species. Our results highlight the morphological uniqueness of these island populations of *E. orbicularis*. Despite the fact that this species is almost certainly introduced, Menorcan populations show several characteristics that make them particularly suitable as models to study phenotypic variability in semiaquatic turtles. Therefore, the conservation of these introduced populations should be prioritized, not only because of their unquestionable scientific value but also as part of the cultural heritage of the Mediterranean islands.

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Figure 4. Details of iris coloration of atypical European Pond Turtles (*Emys orbicularis*) of both sexes in Menorca. A male with yellow-dotted irises; the length of the tail, among other characters, clearly identifies this individual as a male (top). A female with red irises; the distance from the cloaca to the anal plates identifies this individual as a female (bottom). Photographs by Santiago Poch.

Insular de Menorca and the Reserva de Biosfera de Menorca, as part of the biodiversity monitoring program (Exp. C4300-2022-000134). Authorizations for fieldwork, captures, and individual marking were provided by the Servei de Protecció d'Espècies CMAT-CAIB (CEP 12/2022) and fieldwork was supervised by the nature protection service of the Balearic Government. Part of this research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 869296 – The PONDERFUL Project.

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