

Sexual Dimorphism and Morphological Differentiation in European Pond Turtle (*Emys orbicularis*) Populations from Northwestern Spain

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ABSTRACT. – The populations of the European pond turtle in northwestern Spain are isolated from the most important nuclei of this species in the Iberian peninsula, and are suffering a marked retreat. To determine whether these turtles are morphologically different from core Iberian populations, we did a biometric analysis of 99 individuals measured during 1996–2000 in the last known large population in this area. Our results suggest that animals from northwestern Spain resemble Central European specimens in some morphological features, and clearly depart from the phenotype typical of eastern and southern Spain. Analysis of sexual dimorphism indicates that males differ from females mainly in tail length and plastron shape, there being no clear dimorphism in body length. Males are usually darker than females. We suggest that northwestern Spain (Galicia) might have an undescribed subspecies of *E. orbicularis*, but the taxonomic status of these populations cannot be definitively established without genetic studies.

KEY WORDS. – Reptilia; Testudines; Emydidae; *Emys orbicularis*; turtle; systematics; sexual dimorphism; morphological variability; subspecific differentiation; Galicia; Spain

As is usual in many non-migratory species with wide geographic ranges, local populations of the European pond turtle (*Emys orbicularis*) show extensive regional differentiation. This observation has recently led to the description of 13 subspecies (see review in Fritz, 1998), the validity of some of which have been confirmed by genetic studies (Lenk et al., 1999). According to Fritz (1993a) and Fritz et al. (1996) two subspecies are found in the Iberian peninsula, one in the eastern area (*E. o. fritzjuergenobsti*) and the other in the southwest (*E. o. hispanica*) (Fig. 1). The taxonomic status of populations from northwestern Spain remains unresolved since no morphometric or genetic studies have ever been published. Fritz et al. (1996) indicated the possibility that a third subspecies of *E. orbicularis* occurs in the northwestern part of the Iberian peninsula (Galicia), this area having served as a glacial refuge. Northwestern Spain harbors several endemic taxa of birds, butterflies, mammals, fishes, reptiles, and amphibians, including *Chioglossa lusitanica*, *Rana iberica*, *Lacerta lepida iberica*, *L. schreiberi*, and *Podarcis bocagei* (Fritz et al., 1996). Possible differentiation of the turtles of the northwestern Iberian peninsula is supported by the extensive isolation between the populations of *E. orbicularis* from southern and eastern Spain and those of Galicia (Fig. 1; Andreu, 1997). *Emys orbicularis* is very rare in northern Portugal, with only 2–3 records north of Porto (Rito de Araújo et al., 1997).

Emys orbicularis is probably the most threatened reptile in northwestern Spain (Galán, 1999), and recently the local government has initiated protection measures for the last populations. Our first objective was to test the hypothesis that the geographical isolation of these populations resulted

in Galician animals having developed different phenotypes from those described for the populations of the southern and eastern Iberian peninsula. To this end we carried out a biometric study to describe the phenotype of Galician adults and hatchlings and analyze their sexual dimorphism.

METHODS

This study was carried out in three localities in 1996–2000 in southwestern Galicia, northwestern Spain, near the wetlands of Gándaras de Budiño (O Porriño, Pontevedra), a protected natural area where *E. orbicularis* maintains the last large populations in the region (Fig. 1). Turtles were captured using meat bait and hand nets. Captured individuals were weighed with a dynamometer (± 1 –5 g) and measured with calipers (± 1 mm). When possible, individuals were photographed to obtain a visual reference of their phenotype. Animals were individually marked by means of numbered plastic cards attached to the carapace (C. Utzeri, *pers. comm.*). To determine sex we used the shape of the plastron (concave in males) and tail length (longer in males). Behavior of marked animals was always in agreement with their determined sex. We identified as juveniles all animals whose carapace length (CL) was less than 80 mm (Mazzotti, 1995), but a single individual (CL = 82 mm) that was intermediate in sexual characters was not included in the adult sample.

Measurement points and morphometric variables followed Fritz (1995a) and Zuffi and Gariboldi (1995). Statistical tests utilized the most recent measurements for each animal. Data are presented as mean \pm SE (n). The statistical analyses were carried out with SPSS 10.0 for Windows.

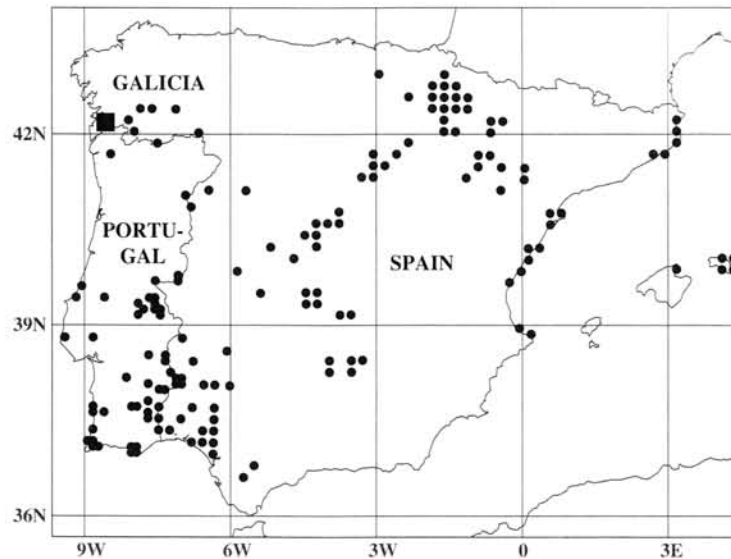


Figure 1. Distribution map of *Emys orbicularis* in the Iberian peninsula. The square indicates the studied populations in southwestern Galicia. Note the relative absence of the species in northern Portugal and the isolation of the Galician distribution from the rest of the species' range. Populations in southwestern Spain and southern Portugal have been described as *E. o. hispanica* (Fritz et al. 1996) and northeastern Spain populations as *E. o. fritzjuergenobsti* (Fritz, 1993a). This map is based on Sociedade Galega de Historia Natural (1995) for Galicia, Andreu (1997) and Fritz (2001) for Spain, and Rito de Araújo et al. (1997) for Portugal.

RESULTS

Phenotypic Description. — We obtained biometric data on 99 specimens of *E. orbicularis* from southwestern Galicia. Table 1 shows the results of biometric analyses, and typical animals are illustrated in Fig. 2. The maximum CL found was 181 mm for males and 173 mm for females. Females above 120 mm CL are heavier than males of equal length (Fig. 3). The only clearly dimorphic trait is tail length (conspicuously greater in males). If the effect of body size is removed, including CL as covariate, the sexes also differ significantly in all variables that describe plastron shape, as well as weight, shell depth, tail length, and length of gular, abdominal, and

anal scutes (Table 1). For the same CL, females show narrower (Fig. 4a) and deeper shells (Fig. 4b). We found significant differences between the sexes in the length of the gular, abdominal, and anal scutes (Fig. 5). Most animals showed some asymmetrical scutes in their carapace (Fig. 2).

Table 2 summarizes the variability observed in the coloration of the animals. The coloration of the shell of mature animals is black with yellow marks. Forty-nine percent show a phenotype in which dark colors prevail (the "orbicularis" type, according to Fritz, 1995a), and 41% have clear and dark colors in similar proportion ("transition" type). Individuals with clear shells with dark stains ("maculosa" type) represent only 10% of the sample. The plastron is highly variable, with

Table 1. Results of the biometric analysis (in mm and g, mean \pm SE, *n*). Variable names and landmarks after Fritz (1995a) or Zuffi and Gariboldi (1995). P1 = probability after a *t*-test comparing means of both sexes. P2 = probability from an ANOVA comparing sexes but including carapace length (CL) as covariate. After Bonferroni correction of type I errors, significant results are those with $p < 0.0014$.

Variable	Males	Females	P1	P2
Carapace length (CL)	136.4 \pm 3.54 (45)	122.6 \pm 5.75 (25)	0.035	—
Carapace width (CW)*	104.0 \pm 2.33 (45)	95.2 \pm 3.72 (25)	0.039	0.918
Plastron length (PL)	115.6 \pm 2.68 (45)	113.8 \pm 5.14 (25)	0.763	< 0.001
Plastron width (D-D)	77.2 \pm 1.59 (45)	74.8 \pm 2.85 (25)	0.463	< 0.001
Shell depth (PH)	52.3 \pm 1.29 (45)	53.0 \pm 2.81 (24)	0.835	< 0.001
Total length of the tail (F-F)	81.0 \pm 2.56 (42)	63.7 \pm 2.53 (23)	< 0.001	< 0.001
Length cloaca-tail apex (G-G, H-H)	60.5 \pm 1.65 (43)	56.3 \pm 2.29 (23)	0.147	0.231
Length of gular scute (GuL)	24.5 \pm 0.65 (44)	24.0 \pm 1.06 (25)	0.663	0.002
Length of humeral scute (HumL)	7.8 \pm 0.39 (44)	7.6 \pm 0.47 (25)	0.784	0.294
Length of pectoral scute (PecL)	20.4 \pm 0.52 (44)	18.9 \pm 0.79 (25)	0.110	0.628
Length of abdominal scute (AbdL)	16.4 \pm 0.47 (44)	18.4 \pm 0.92 (25)	0.054	< 0.001
Length of femoral scute (FemL)	13.4 \pm 0.41 (44)	12.0 \pm 0.45 (25)	0.027	0.401
Length of anal scute (Anal)	33.4 \pm 0.77 (44)	32.5 \pm 1.64 (25)	0.949	< 0.001
Anterior width of plastron (PBI)**	67.1 \pm 1.42 (45)	66.0 \pm 2.78 (25)	0.714	< 0.001
Posterior width of plastron (PBII)**	71.6 \pm 1.65 (45)	68.8 \pm 3.08 (25)	0.438	< 0.001
Length of nuchal scute (NuL)	10.1 \pm 0.32 (43)	9.6 \pm 0.40 (25)	0.284	0.174
Width of nuchal scute (NuB)	6.2 \pm 0.15 (43)	6.2 \pm 0.21 (25)	0.947	0.166
Weight	408.0 \pm 24.59 (45)	352.6 \pm 45.05 (24)	0.288	0.001

* measured between the 7th and 8th marginal scutes.

** PBI measured at pectoral scutes; PBII measured at femoral scutes.

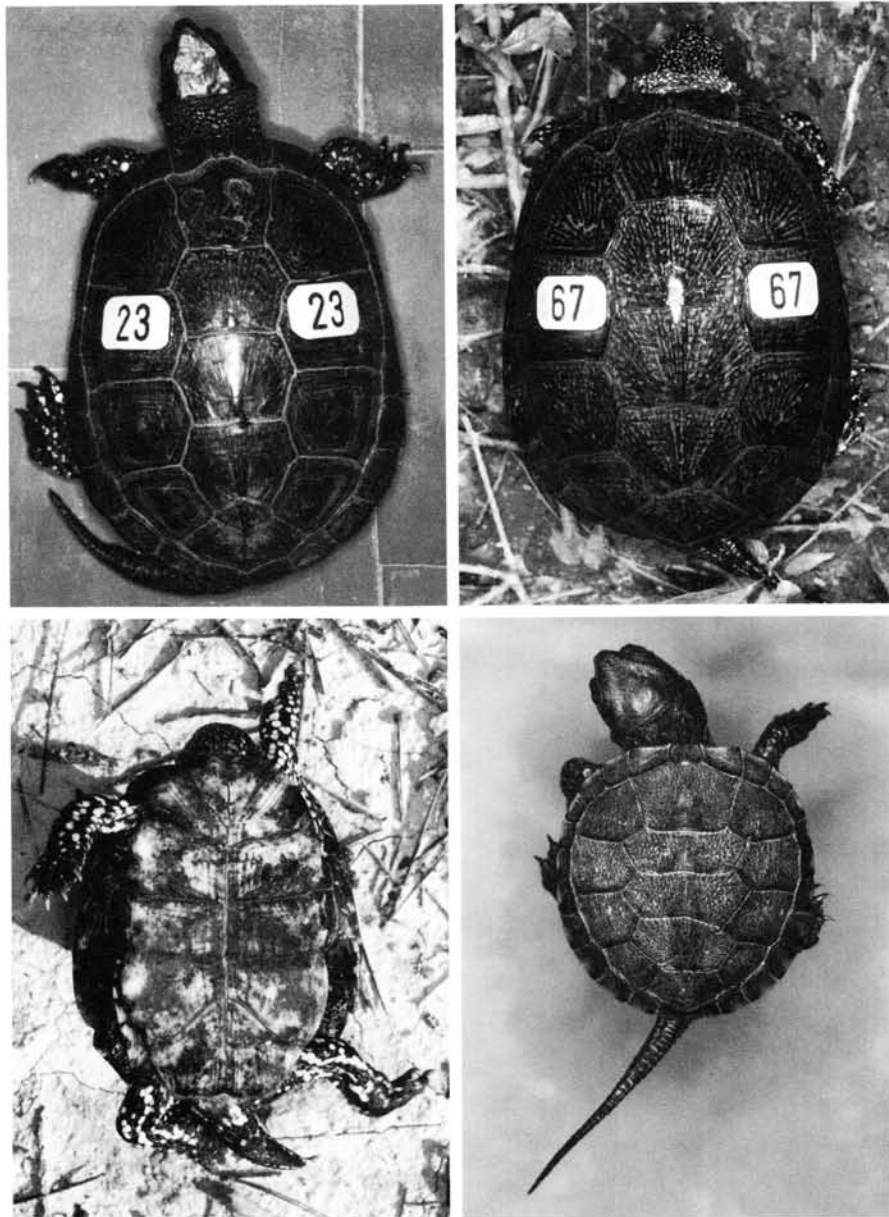


Figure 2. Representative individuals from the studied Galician populations. **Upper left:** male 23 (note the azygous scute between vertebrals 4 and 5). **Upper right:** female 67. **Lower left:** ventral view of male 17. **Lower right:** one of the 1999 hatchlings (note the division of vertebral 4).

clear and dark colored animals equally abundant (Table 2). In general males are darker than females. This difference is very evident in the dorsal ($\chi^2 = 12.47$, d.f. = 3, $p = 0.006$) and ventral coloration of the head ($\chi^2 = 11.19$, d.f. = 1, $p < 0.001$), in such a way that it is possible to distinguish males and females at a distance even if only the head is visible. The coloration of the iris is brown in most males and yellow or yellow with a brown horizontal band in females and juveniles.

Hatchlings (Fig. 2) are mainly blackish with white dots on the front legs, neck, and head, that are larger ventrally than dorsally. Carapace length is 28–31 mm. The plastron shows a narrow black central figure, typical of the *E. o. occidentalis* subspecies group (Fritz, 1993a). After a few weeks shell coloration changes to mainly brown with black dots, the marginals showing a yellow half-moon in the middle of

each scute, and the white dots on the head, neck, and front legs begin to change to yellow. The upper jaw shows a line of white dots.

Subspecific Differentiation. — Table 3 shows seven morphological ratios used by Fritz et al. (1996) to distinguish between *Emys orbicularis occidentalis*, *E. o. hispanica*, *E. o. fritzjuergenobsti*, and *E. o. orbicularis*. We have estimated the means and confidence intervals for these subspecies from graphs presented by Fritz et al. (1996) and compared these with our animals in Fig. 6. The relationship length/width (CL/CW; see Table 1 for explanation of variables) of the shell in Galician animals is more similar to the subspecies *orbicularis* than to any of the Iberian subspecies. Galician animals are thus more rounded than those of eastern and southern Spain and northern Africa, but there is ample

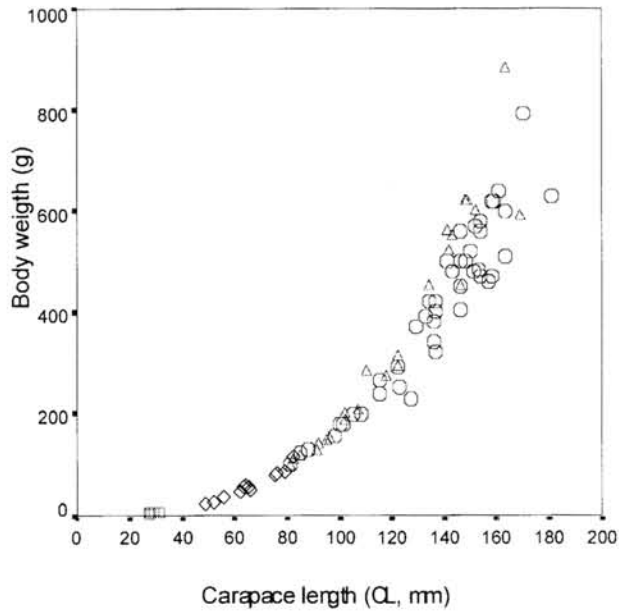


Figure 3. The relationship between carapace length and weight for *E. orbicularis*. Note that the relationship is curvilinear, and that the weight of the females is greater above 120 mm of length, when it is probable that they are adult. Δ = females, \circ = males, \diamond = juveniles, \square = hatchlings.

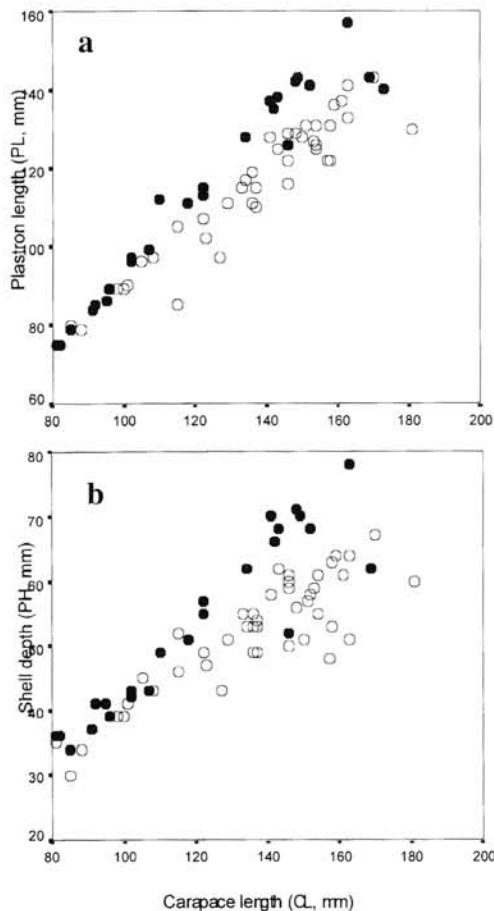


Figure 4. The relationship between carapace length and (a) plastron length and (b) shell depth. \bullet = females, \circ = males.

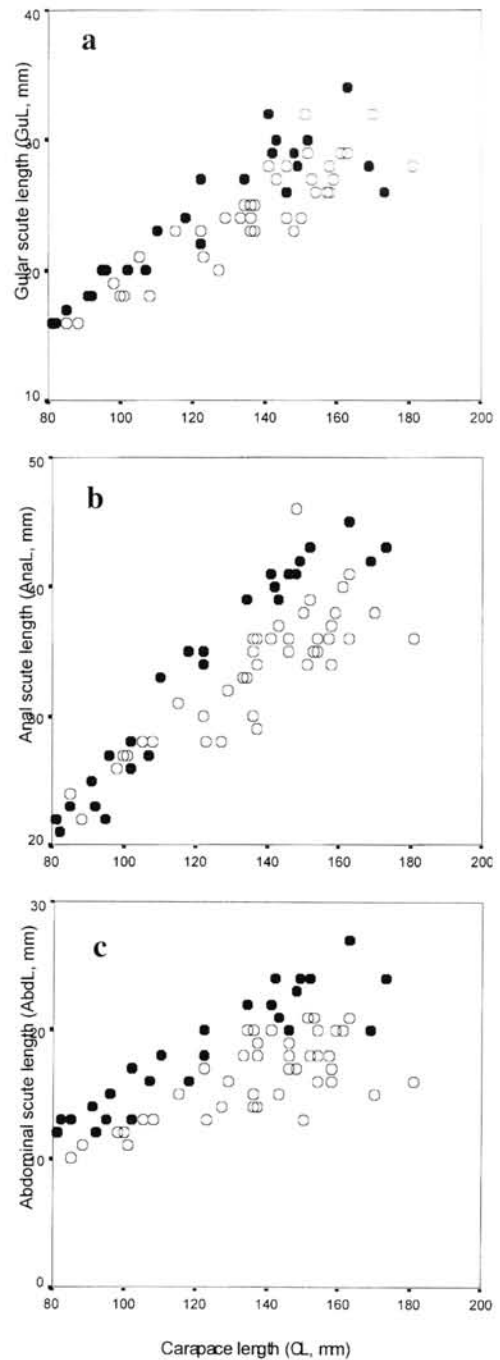


Figure 5. The relationship between carapace length and the length of (a) the gular scute, (b) the anal scute, and (c) the abdominal scute. \bullet = females, \circ = males.

overlap in confidence intervals with *occidentalis* and *hispanica*. Galician animals are significantly different from all subspecies in the nuchal (CL/NuB and CL/NuL ratios), anal (PL/AnL), and gular (PL/GuL) scutes. Significant differences are also observed in comparison with some subspecies in the femoral and humeral scutes. On the other hand, the percentage of animals with a convergent nuchal scute (45%) is halfway between the values of the subspecies *orbicularis* (80%) and *hispanica* (< 10%) and *fritzjuergenobsti* (10%), being identical to the percentage observed in African animals (45%) (Fritz et al., 1996).

Table 2. Patterns of coloration of adults of *E. orbicularis* from the northwestern Iberian peninsula (Galicia). Phenotypic categories according to Fritz (1995a).

Character	Males	Females	Juveniles
Carapace			
“orbicularis” type	12	10	3
transition type	8	9	4
“maculosa” type	5	0	3
Plastron			
less than 1/3 dark	2	8	5
1/3–2/3 dark	12	7	4
more than 2/3 dark	9	2	1
Head dorsum			
dotted	3	9	7
intermediate	4	5	0
reticulate	14	2	0
monochrome	1	0	0
Throat			
predominantly yellow	8	10	3
predominantly black	18	1	3

Table 3. Morphological ratios for Galician animals (juveniles and hatchlings not included), calculated as in Fritz et al. (1996), to allow direct comparison.

Ratio	n	Mean	SE	Minimum	Maximum
CL/CW	84	1.27	0.009	1.11	1.53
CL/NuB	82	20.32	0.594	11.20	38.25
CL/NuL	82	13.29	0.164	9.80	17.57
PL/AnL*10	83	34.93	0.208	28.04	39.09
PL/FemL*10	83	88.30	1.447	61.11	143.75
PL/GuL*10	83	47.32	0.297	40.94	56.09
PL/HumL*10	83	165.91	6.312	68.75	433.33

DISCUSSION

Sexual Dimorphism. — The results of the work of Fritz and co-workers on different populations of *E. orbicularis* (Fritz, 1989, 1992, 1993a,b, 1994a,b, 1995a,b, 1996; Fritz and Obst, 1995; Fritz et al., 1995, 1996), indicate that there is sexual dimorphism in body size and weight, females being larger. These results agree with those obtained by C. Utzeri (*pers. comm.*), Zuffi and Gariboldi (1995), Mazzotti (1995), Zuffi and Ballasina (1998), and Rovero et al. (1999) for Italian populations of *E. orbicularis*, as well as by Servan (1998) in France, Roessler (1999) in Austria, and Snieshkus (1998) in

Lithuania. This is not always the case in the *occidentalis* subspecies group of *E. orbicularis* (Keller, 1997; Keller et al., 1998; Fritz, 1998). In Galician populations we found that for animals longer than 120 mm CL, females are heavier than males, but there is no dimorphism in body length. This negative result could be explained by the paucity of females in our sample, because only three females were larger than 160 mm. Sexual dimorphism in body size is quite frequent in turtles and has many ecological interpretations, but seems most related to sexual selection (Berry and Shine, 1980). One such cause is a relationship between temperature during embryonic development and size, with females coming from hotter nests. Nevertheless, if age of animals is not known, many confounding variables can account for sexual dimorphism in body size. For example, size-dependent mortality in only one sex may produce sexual size dimorphism, even if neither sex directly benefits from larger size (St.Clair, 1998). Surprisingly, we found clear sexual dimorphism in head coloration, males being darker. This dichromatism seems

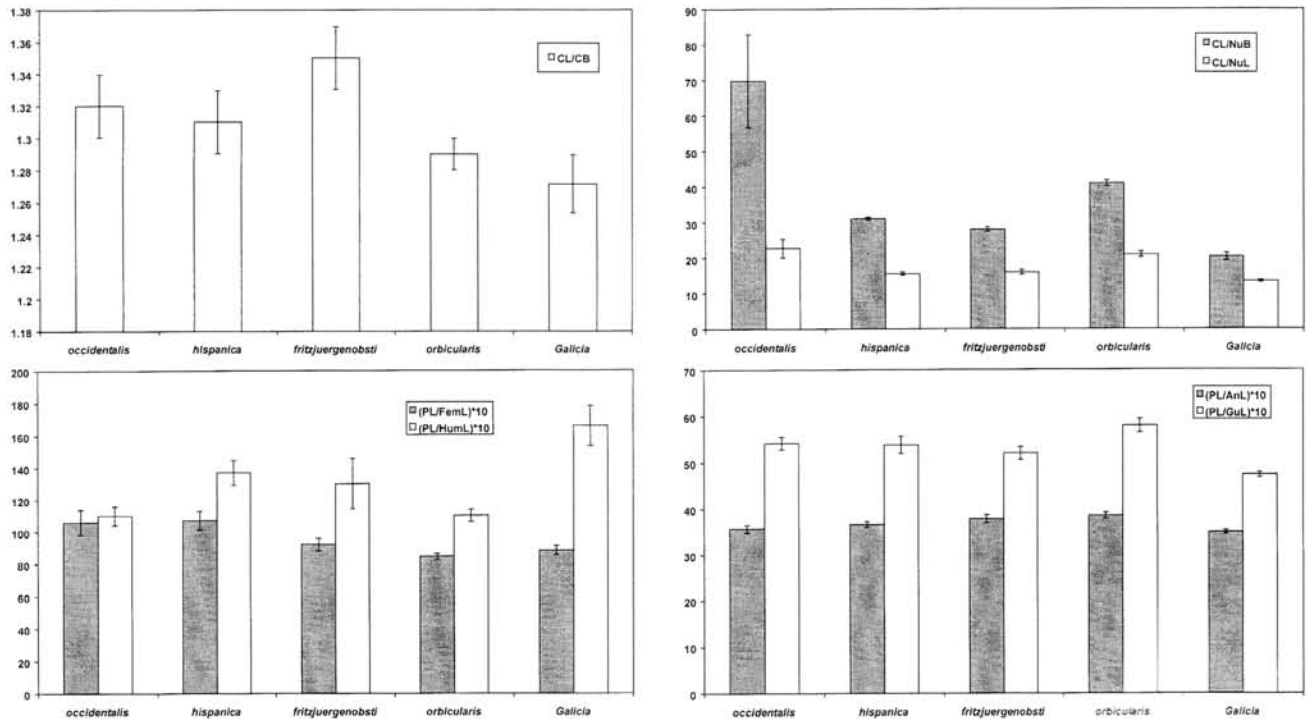


Figure 6. Morphometric proportions for different subspecies of *E. orbicularis* (data extrapolated from the graphs of Fritz et al., 1996) and the Galician populations analyzed in this study (mean \pm 1.96 SE). CL = carapace length, CB = carapace width, NuB = width of nuchal scute, NuL = length of nuchal scute, PL = plastron length, FemL = length of femoral scute, HumL = length of humeral scute. AnL = length of anal scute, GuL = length of gular scute.

a peculiarity of Galician populations of *Emys orbicularis*, but male age-related melanism is known in other species of turtles (Zangerl, 1969; Lovich et al., 1990). Sexual dimorphism in eye coloration has been described in other populations of *E. orbicularis* (Pritchard, 1966; Fritz, 1995).

Taxonomic Status. — The main differences between the *Emys orbicularis* subspecies described for the Iberian Peninsula (*E. o. fritzjuergenobsti* and *E. o. hispanica*) and North Africa (*E. o. occidentalis*) are the width of the shell, the length of the femoral scute, the length of the humeral scute, and the form of the nuchal scute (Fritz et al., 1996). Our preliminary results, using the same methodology as Fritz and co-workers, suggest that the animals from the northwestern Iberian peninsula do not resemble any of the subspecies described for Iberia. These results are to be interpreted with caution, because our study animals came from a reduced geographical area in southwestern Galicia, and might not be representative of all northwestern Spain *E. orbicularis*. Unfortunately the rarity of this species in the area impedes comparison with other populations. The differentiation we have recorded could result from the geographic isolation of Galician populations. However, we cannot discard the possibility that their similarity with the subspecies *E. o. orbicularis* in some morphological ratios is due to a genetic influence of Central Europe animals, either introduced by humans or not. In that case, Galician specimens would be an intergrade of the subspecies *orbicularis* and *hispanica*. In northeastern Spain, recent studies have indicated that four subspecies of *Emys orbicularis* intergrade there, and four haplotypes (characteristic of *hispanica*, *fritzjuergenobsti*, *galloitalica*, and *orbicularis*) were found in animals from the Ebro delta (Mascort, 1999). It is even possible that all turtles have been introduced in Galicia, as apparently has happened in the Balearic islands (Braitmayer et al., 1998; Pieh and Sättele, 1998; Fritz et al., 1998). Thus, we conclude that only a genetic analysis could justify the description of a new subspecies for northwestern Spain pond turtles.

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RESUMEN

El galápago europeo es muy raro en el noroeste de España, donde presenta poblaciones aisladas de los núcleos principales de la península Ibérica. En este trabajo presentamos un análisis biométrico de 99 individuos medidos durante 1996–2000 en la última gran población conocida del área. El objetivo es probar la hipótesis de que los animales gallegos son morfológicamente diferentes de las otras

subespecies de la península. Los resultados sugieren que nuestros especímenes se parecen en algunas características morfológicas a los animales de Europa Central, y son claramente diferentes del fenotipo dominante en el este y sur de España. El análisis del dimorfismo sexual indica que los machos se diferencian de las hembras principalmente en la longitud de la cola y en la forma del plastrón, pero no hay un claro dimorfismo de tamaño. Los machos son usualmente más oscuros que las hembras. Sugerimos que noroeste de España (Galicia) puede albergar una subespecie no descrita de *E. orbicularis*, pero el estatus taxonómico de estas poblaciones no puede ser establecido con seguridad sin la realización de estudios genéticos.

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