Rev. Esp. Herp. (1999) 13:7-16

Morphology, geographic variation and taxonomy of *Emys orbicularis* L. 1758, in the northeast of the Iberian Peninsula.

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Abstract: The study of the biometry of 131 specimens captured, measured and re-released, compared with genetic data, shows us that NE Iberia is a crossroads of different subspecies of *Emys orbicularis*, which intergrade here. Two groups can be separated in our analysis: a northern one with two populations in Girona (province), and a southern one with the Ebro delta and Castellón (province) coastal populations.

In addition to these two groups, there are populations in the Ribagorzana basin (in the province of Huesca) and on the coast of Tarragona (province) which cannot be included in either group. According to our morphometrical analysis, the Ribagorzana basin population appears to be more closely related to the southern group, while the coastal Tarragona population appears to be more closely related to the northern one, although genetic data examined suggests the opposite.

The "northern group" specimens are referable to *E. o.* cf. *orbicularis*, a presumably recent eastern European invader of central Europe which reaches its south-western limit here, intergrading with *E. o. galloitalica* (in the province of Girona) and *E. o. hispanica* (in the province of Huesca). The "southern group" includes pure *E. o. galloitalica* (Tarragona coastal population), the presumably autochthonous pleistocenic substrate, and intergrades of this subspecies with described *E. o. fritzjuergenobsti* (in Castellón) or with this latter and cf. *orbicularis* and *hispanica* (which appear in the Ebro river basin) in the Ebro delta.

Key words: *Emys orbicularis*, Biometry, Morphology, Canonical Variate Analysis, Subspecies, Intergradation, Biogeography.

Resumen: Morfología, variación geográfica y taxonomía de *Emys orbicularis* L. 1758, en el nordeste de la Península Ibérica.- El estudio de la biometría de 131 ejemplares de galápago europeo, *Emys orbicularis*, capturados, medidos y posteriormente liberados nos muestra que el Noreste ibérico es una encrucijada donde entran en contacto y se intergradan diversas subespecies. Tras nuestro análisis, en primer lugar cabe separar dos grupos: uno septentrional, con poblaciones en la provincia de Girona; y otro meridional, con las poblaciones del delta del Ebro y la provincia de Castellón. Además de estos dos grupos, las poblaciones de la cuenca de la Ribagorzana (en la provincia de Huesca) y de la costa de Tarragona no pueden ser asignados a ningún grupo. De acuerdo con nuestro análisis morfométrico, la población de la cuenca de la Ribagorzana aparece más cercana al grupo meridional, mientras que la población costera de Tarragona aparece más cercana al grupo septentrional. Sin embargo, los datos genéticos sugieren una relación inversa a la que ofrecen los datos morfométricos en el caso de estas dos poblaciones.

Los ejemplares del grupo septentrional son referibles como *E. o.* cf. *orbicularis*, presumiblemente subespecie invasora reciente desde el centro y este de Europa, alcanzando en nuestra área de estudio el límite suroeste conocido, e intergradándose aquí con *E. o. galloitalica* (en Girona) y *E. o. hispanica* (en Huesca).

El grupo meridional incluye ejemplares correspondientes a la subespecie *E. o. galloitalica* en la costa de Tarragona, subespecie que constituye probablemente el sustrato primitivo pleistocénico en la zona. Intermedios de esta subespecie *con E. o. fritzjuergenobsti* se dan en Castellón, y junto con estos últimos, aparecen en el Delta del Ebro *E. o*. cf. *orbicularis* y *E. o. hispanica* que habrian llegado a través de la extensa cuenca del mencionado rio.

Palabras clave: *Emys orbicularis*, biometria, morfología, análisis canónico de poblaciones, subespecies, intergradación, biogeografía.

INTRODUCTION

The European pond turtle is widespread, although very scarce, and is present in scattered populations in most of the Iberian Peninsula. It is only locally common or abundant in some points of the south of the territory, being represented in the remaining areas only by isolated individuals or scattered and usually endangered populations. It is often difficult to ascertain if the rare finds of isolated specimens represent introductions or are the relics of previous larger populations, still present in the area.

In Catalonia, and by extension in the northeastern part of the Iberian Peninsula, the species is very rare and rapidly disappearing. Its presence has been known for almost a century since the references of BOSCÁ (1916) and MALUQUER (1919) for one location near the Ebro river mouth (Sant Carles de la Rapita, province of Tarragona). In addition to these two classic references there are general references in VIVES-BALMAÑA's Atlas (1990), LLORENTE *et al.* (1995), and SAN MIGUEL (1975) which usually mix true populations with isolated specimens. Two papers which deal specifically with this species in the area are those by ARRIBAS (1991) and MASCORT (1998).

MATERIAL & METHODS

A total of 131 specimens with lengths exceeding 90 mm straight caparace length (SCL) were measured and studied. Specimens were captured by hand, with dip net or funnel traps. They were measured, blood samples were taken, and they were then released immediately. All captures were made with permit nº 066/97, issued by the *Departament d'Agricultura, Ramaderia i Pesca - Generalitat de Catalunya* to one of the authors.

Localities of sampling:

- Ter basin population (henceforth "TER").
- Girona inland population (henceforth "GIR").
- Ribagorzana basin population (henceforth "RIBA").
- Tarragona coastal population (henceforth "TARR").
- Ebro delta population (henceforth "EBRE").
- Castellón coastal population (henceforth "CAST").

Precise localities are not given to avoid illegal collection of specimens.

Features studied:

W (weight); SCL (straight caparace length); CB (caparace width); CH (caparace height); PL (plastron length); GUL (gular suture length); HUML (humeral suture length); PECL (pectoral suture length); ABDL (abdominal suture length); FEML (femoral suture length); ANL (anal suture length); KL (head length); KB (head width); KH (head height); NuL (nuchal length); NuB (nuchal width).

All linear measurements were made by the senior author (R.M.) with a calliper to the nearest mm, to avoid interobserver variability.

The following ratios were calculated: CL/CB; CL/PH; PL/GUL; PL/HUM; PL/PECL; PL/ABDL; PL/ANL; CL/NuL; CL/NuB; CL/KL, CL/KB.

All ratios are usually given multiplied by 100 to avoid excessive decimal points; and measurements were taken as in FRITZ, 1993, and FRITZ *et al.*, 1996.

Statistical Procedures:

Linear measurements were transformed to their Log (x+1), and ratios by means of Arcsin square-root transformation (SOKAL & ROHLF, 1969). However, we have observed that transformed and raw data give almost identical results. Also results with linear measurements and indexes yield largely similar output (see also BERRY & BERRY, 1984).

Statistical analyses performed in the present study are phenetic, based on the relative similarity or dissimilarity of populations with many characters considered simultaneously and without *a priori* weighting (JARDINE & SIBSON, 1971; SNEATH & SOKAL, 1973).

Univariate statistics: Descriptive statistics (mean, standard error of mean and range) are given. Comparisons between populations were done by means of ANOVA (Analysis of variance) (SOKAL & ROHLF, 1969). If significant differences were present, Scheffé's test was run at $p < 0.05$ (significant differences) and $p < 0.01$ (highly significant differences) for multiple comparisons between the means.

Multivariate analyses include three steps (CUADRAS, 1981): 1) a comparison of variance-covariance matrices by means of the Bartlett test. If this test is not significant, as is the case, then 2) comparison of populations by MANOVA (Multivariate Analysis of the Variance), that is, considering all measurements at the same time; and 3) a Canonical Variate Analysis (CVA), a method for population representation along orthogonal axes (Canonical variates) with maximum discriminative power using Mahalanobis' distance. Each population is represented by a centroid (a hypothetical median individual). We have run analyses for males and females separately to test congruence in the results.

Despite its widespread use to discriminate well-differentiated samples (e.g. different species), our results with Principal Components Analysis have been very unsatisfactory due to the fact that this detects outlying specimens or measurements but does not discriminate well between very similar populations.

The relative distances between population centroids can be represented numerically by the Mahalanobis' distance D2. The D2 values between each population mean centroids are used as the basis for the dendrograms. The closer two populations appear in the branching sequence, the closer their population centroids are in the hyperspace (smaller D2). This is an intuitive unidimensional representation of the different degrees of affinity between populations. For cluster aggregation of these distances, we use the UPGMA method that is widely used and has been found to be superior to other clustering algorithms (ROHLF, 1962; SNEATH & SOKAL,1973). Although D2 values are greatly exaggerated for small samples and even more so for isolated individuals, MST (minimum spanning tree)(SNEATH & SOKAL, 1973) is a good method of ascertaining population affinities, independently of the scale of the distances.

Finally we discuss our results in comparison with mitochondrial DNA genetic results obtained from these same specimens (LENK *et al*., 1998, 1999).

RESULTS

Anova

Descriptive statistics from SCL and indexes are in table 1. It can be seen that there are three indexes that clearly separate the "northern populations" (TER, GIR and RIBA) from the "southern populations" (TARR, EBRE and CAST). These characters are PL/PECL, PL/ABDL and PL/ANL (see table 1). Other characters only discriminate isolated populations from others, without such a congruent pattern.

Canonical Variate Analysis:

Males:

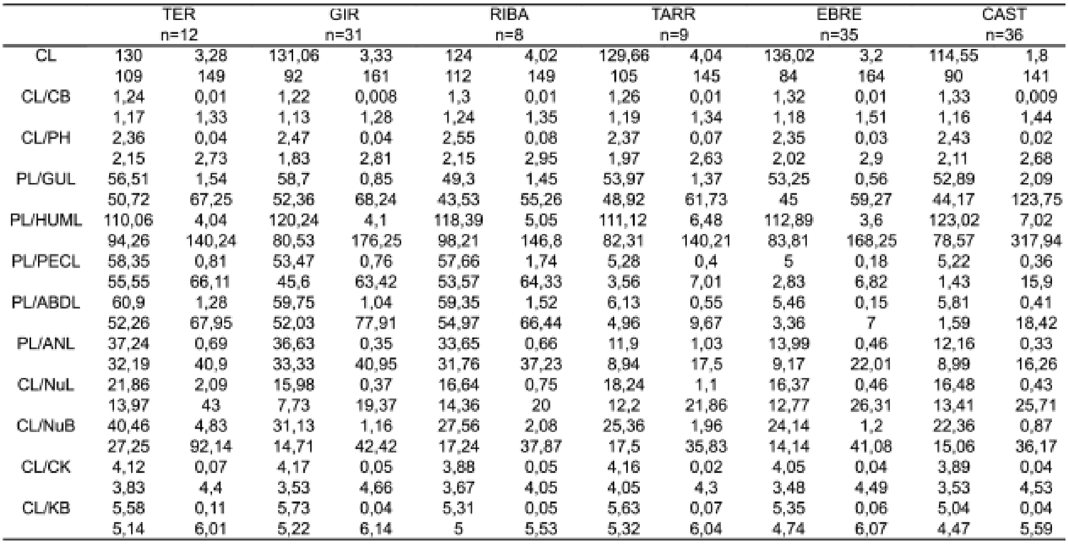
The analysis of males was made with four samples and two isolated specimens (n = 59 specimens) and 15 characters.

MANOVA has a value of F = 3.1854 (with 75 and 191 d.f., $p < 0.0001$), thus we reject the null hypothesis and assume that there are significant differences between samples. Wilk's Lambda is 0.0205.

The three first axes have eigenvalues greater than one (29.9, 12 and 5.16, respectively) and account for 88.4% of the total variance between samples.

The first three axes are represented in figure 1 and account for 88.4% of all variability (56.1, 22.6 and 9.7% respectively) thus giving a good representation of the sample discrimination.

Table 1: Statistical parameters from the populations studied (Mean and Standard deviation in the first line; Maximum and Minimum in the second line). Abbreviations are shown as in text.
Tabla 1: Parámetros estadísticos de las poblaciones estudiadas (Media y desviación estándar en la primera línea; máximo y mínimo en la segunda línea). Las abreviaturas se muestran como en el texto.



	TER		GIR		RIBA		TARR		EBRE		CAST	
	n=12		n=31		n=8		n=9		n=35		n=36	
CL	130	3,28	131,06	3,33	124	4,02	129,66	4,04	136,02	3,2	114,55	1,8
	109	149	92	161	112	149	105	145	84	164	90	141
CL/CB	1,24	0,01	1,22	0,008	1,3	0,01	1,26	0,01	1,32	0,01	1,33	0,009
	1,17	1,33	1,13	1,28	1,24	1,35	1,19	1,34	1,18	1,51	1,16	1,44
CL/PH	2,36	0,04	2,47	0,04	2,55	0,08	2,37	0,07	2,35	0,03	2,43	0,02
	2,15	2,73	1,83	2,81	2,15	2,95	1,97	2,63	2,02	2,9	2,11	2,68
PL/GUL	56,51	1,54	58,7	0,85	49,3	1,45	53,97	1,37	53,25	0,56	52,89	2,09
	50,72	67,25	52,36	68,24	43,53	55,26	48,92	61,73	45	59,27	44,17	123,75
PL/HUML	110,06	4,04	120,24	4,1	118,39	5,05	111,12	6,48	112,89	3,6	123,02	7,02
	94,26	140,24	80,53	176,25	98,21	146,8	82,31	140,21	83,81	168,25	78,57	317,94
PL/PECL	58,35	0,81	53,47	0,76	57,66	1,74	5,28	0,4	5	0,18	5,22	0,36
	55,55	66,11	45,6	63,42	53,57	64,33	3,56	7,01	2,83	6,82	1,43	15,9
PL/ABDL	60,9	1,28	59,75	1,04	59,35	1,52	6,13	0,55	5,46	0,15	5,81	0,41
	52,26	67,95	52,03	77,91	54,97	66,44	4,96	9,67	3,36	7	1,59	18,42
PL/ANL	37,24	0,69	36,63	0,35	33,65	0,66	11,9	1,03	13,99	0,46	12,16	0,33
	32,19	40,9	33,33	40,95	31,76	37,23	8,94	17,5	9,17	22,01	8,99	16,26
CL/NuL	21,86	2,09	15,98	0,37	16,64	0,75	18,24	1,1	16,37	0,46	16,48	0,43
	13,97	43	7,73	19,37	14,36	20	12,2	21,86	12,77	26,31	13,41	25,71
CL/NuB	40,46	4,83	31,13	1,16	27,56	2,08	25,36	1,96	24,14	1,2	22,36	0,87
	27,25	92,14	14,71	42,42	17,24	37,87	17,5	35,83	14,14	41,08	15,06	36,17
CL/CK	4,12	0,07	4,17	0,05	3,88	0,05	4,16	0,02	4,05	0,04	3,89	0,04
	3,83	4,4	3,53	4,66	3,67	4,05	4,05	4,3	3,48	4,49	3,53	4,53
CL/KB	5,58	0,11	5,73	0,04	5,31	0,05	5,63	0,07	5,35	0,06	5,04	0,04
	5,14	6,01	5,22	6,14	5	5,53	5,32	6,04	4,74	6,07	4,47	5,59

The first axis discriminates populations in a roughly north-south direction. The negative part of the axis, characterised by greater values for CB (–.456), PL (–.300), CH (–.272), ANL (–.238) and CL (–.236) is occupied by the "northern populations" (Girona inland and Ter basin) plus the Tarragona coastal sample. The positive part of the axis, with the lower scores for the specified variables, is occupied by the Castellón coastal population, the Ebro delta sample being in an intermediate position closer to the Castellón sample. The Ribagorzana basin sample appears near these "southern populations" in this first axis. As almost all coefficients of this first axis are negative, this is probably due to general size, with the "northern populations" being larger and specimens becoming progressively smaller towards the south. The "northern group" also shows a greater length of anal suture.

The second axis has higher loadings of FEM (.458) CH (.345), and lower loadings of NuB (.234) and ABDL (–.152), other variables being less important, and only discriminates the Ribagorzana population towards its negative part, characterised by lower values of CH (flatter caparace) and FEML (shorter femoral sutures).

The third axis has higher loadings of PL (.381) and ANL (.364), and in its lower values discriminates the "northern populations" and Castellón from the remaining populations.

The tridimensional representation of the three axes (Figure 1) shows the "northern populations" very close, and differentiated from the "southern populations". The Tarragona coastal sample is well differentiated from the "northern populations", and the Ribagorzana population also appears differentiated. The Ebro delta sample has an intermediate position between the Castellón and Tarragona populations.

Co-ordinates of centroids (Figure 1) and confidence radia (not shown) are:

Table 2: ANOVA results (F and p) with intersample comparison (1 to 6). Abbreviations are shown as in text.
Tabla 2: Resultados del ANOVA (F y p) y comparación entre las muestras (1 a 6). Las abreviaturas se muestran como en el texto.

	ANOVA																
	F	P	1.2	1.3	1.4	1.5	1.6	2.3	2.4	2.5	2.6	3.4	3.5	3.6	4.5	4.6	5.6
CL	7,59	0									**						**
CL/CB	16,82	0				**	**	*		**	**						
CL/PH	1,99	0,085															
PL/GUL	3,34	0,0072															
PL/HUML	0,78	0,567															
PL/PECL	1936,44	0			**	**	**	**	**	**	**	**	**	**			
PL/ABDL	1409,45	0			**	**	**		**	**	**	**	**	**			
PL/ANL	627,11	0			**	**	**		**	**	**	**	**	**			
CL/NuL	6,71	0	**	*		**	**										
CL/NuB	12,64	0	*	*	**	**	**			*	**						
CL/CK	5,74	0,0001									**						
CL/KB	21,07	0					**	*		**	**					**	**

	Axis 1	Axis 2	Axis 3	radius (95%)
TER	–2.90	–.483	–.272	- - -
GIR	–2.33	.127	–1.16	2.87
RIB	1.82	–2.87	.308	- - -
TARR	–1.11	.874	1.76	4.96
EBRO	1.45	1.31	.0943	3.51
CAST	3.07	1.04	–.729	2.87

The Mahalanobis' distances between centroids are:

	Ter	Gir	Riba	Tarr	Ebre	Cast
TER	0					
GIR	5.52	0				
RIBA	30	29	0			
TARR	11.1	11.4	25.3	0		
EBRE	26.4	20.2	19.6	14	0	
CAST	39.6	33.2	19.9	24.4	10.6	0

The MST (minimum spanning tree) clusters the Ter basin sample with the Girona inland population (5.52) and also, at a greater distance, with the Tarragona coastal population (11.1). The Tarragona coastal population clusters with the Ebro delta sample (14.0). The Ebro delta population clusters with the Castellón population (10.6), and finally this latter with the Ribagorzana basin sample (19.6).

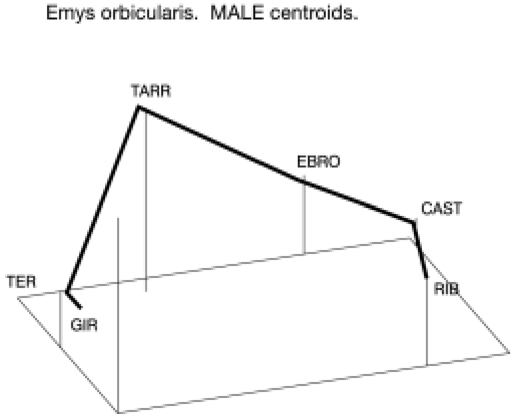


Figure 1.- Tridimensional representation of male centroids from the populations studied. Minimum Spanning Tree (MST) is superimposed.
Figura 1.- Representación tridimensional de los centroides de los machos de las poblaciones estudiadas. El árbol de mínima extensión (MST: Minimum Spanning Tree) se representa sobreimpresionado.

An UPGMA cluster of the studied populations is shown in figure 2, and shows the two above-mentioned groups: a northern group, with the Ter basin and Girona inland populations very closely related, and a more differentiated population from the Tarragona coast. The second group includes the Castellón and

Ebro delta samples and again, a more differentiated population from the Ribagorzana basin.

Females:

The analysis of females was also made with six samples (a total of 72 specimens) and 15 characters.

MANOVA gives us an F = 4.5797 (75 and 253 d.f., $p < 0.0001$), which indicates that we must reject the null hypothesis and accept that there are significant differences between samples. Wilk's Lambda is 0.0165.

The three first axes have eigenvalues greater than one (37.3, 9.87 and 6.93, respectively). The first two axes account for 78.3% of all the variability between populations, which reaches 89.8% with the third axis (61.9%, 16.4% and 11.5% respectively). The tridimensional representation of these centroids is in figure 3.

The first axis has higher scores for CB (–.181), FEML (–.265), NuB (.144) and PECL (–.120) and discriminates the "northern populations" (Girona inland and Ter basin) plus the Tarragona coast sample, with higher scores of these values, from the "southern populations" (Ebro delta and Castellón), plus the Ribagorzana basin sample. This axis is also probably related to size as almost all coefficients are of the same sign.

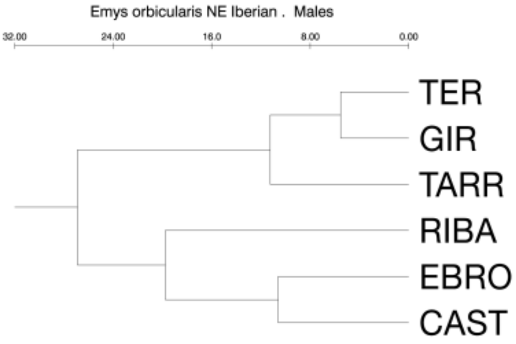


Figure 2.- Dendrogram (by UPGMA method) of the relationships between males from different populations based on their Mahalanobis' distances.

Figura 2.- Dendrograma (método UPGMA) de las relaciones entre los machos de las diferentes poblaciones estudiadas basándose en sus distancias de Mahalanobis.

The second axis discriminates very few individuals among these two groups, with the extreme northern and southern populations (also the best represented) having the highest scores of FEML (.286), NuB (.576), and KH (–.205). The Tarragona and Castellón samples are represented towards the positive part of this axis, characterised by relatively higher values of NuB and FEML, and lower values of KH.

The third axis has higher loadings for FEML (.387), KH (.217) and CH (.189) and discriminates mainly the Ribagorzana basin specimens with lower values of these characters.

The two first axes of the analysis of female specimens clearly discriminate two groups without overlap: one with the "northern populations" (Girona inland and Ter basin) plus the Tarragona coastal population, and a second, with the "southern populations" (Castellón, Ebro delta) plus the Ribagorzana basin population. The third axis separates the Ribagorzana population from the others. In the tridimensional representation of these axes (Figure 3), there are four groups: first, the northern populations; second, the Tarragona sample, somewhat differentiated from the former; third, and well differentiated, the Castellón population; and fourth the Ribagorzana population; the Ebro delta sample is somewhat intermediate between the Castellón and Ribagorzana samples, but clearly more closely related to the first.

Co-ordinates of centroids (Figure 3) and confidence radia (not shown) are:

	Axis 1	Axis 2	Axis 3	radius
TER	–1.86	–1.43	.451	1.88
GIR	–2.96	–1.01	.01	1.65
RIB	2.43	–.572	–2.10	2.43
TARR	–2.56	2.42	–.415	3.44
EBRO	1.99	–.187	1.18	1.27
CAST	2.95	.783	.867	1.40

The Mahalanobis' distances between populations are:

	Ter	Gir	Riba	Tarr	Ebro	Cast
TER	0					
GIR	9.73	0				
RIBA	27.8	35.8	0			
TARR	17.7	14.9	36.8	0		
EBRO	22	28.7	12.1	31.2	0	
CAST	30.6	41.3	11.8	35.8	5.55	0

The MST between samples clusters the river Ter sample with the neighbouring Girona inland population (9.73), the latter with the Tarragona coastal population (14.9). The link between the two groups is between the Ter and Ebro delta populations (22.0). The Ebro delta population clusters with the Castellón population (5.55) and finally this latter with the Ribagorzana basin sample (11.8).

The UPGMA cluster relationships are shown in the dendrogram in figure 4.

DISCUSSION

The results from male and female analyses are very coincident. Both show the presence of two differentiated morphological groups, both of them containing two fairly closely related populations and a third which is more differentiated.

In our study, we were able to distinguish between a "northern" and a "southern" group. The first comprises the closely related Ter basin and Girona inland populations and the more differentiated Tarragona coastal population. The second includes the closely related Castellón and Ebro delta populations and the relatively differentiated Ribagorzana basin population.

In view of these results, one could expect the presence of two subspecies of *Emys orbicularis* in the area. In fact, FRITZ (1993) describes from the region of Valencia (which includes Castellón) *Emys orbicularis fritzjuergenobsti*, and FRITZ *et al.* (1996) postulate for the remaining Iberian Peninsula the probable presence of *Emys orbicularis hispanica* (described from the province of Huelva, in the extreme south-west of Spain).

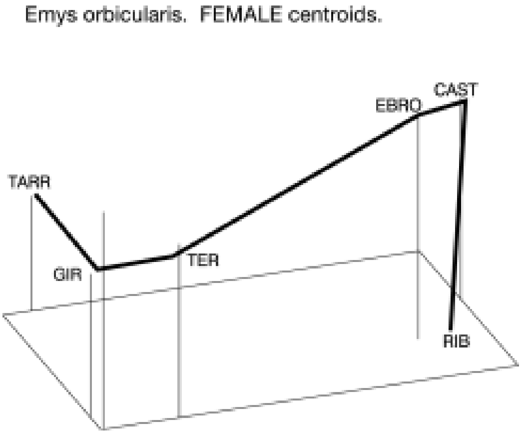


Figure 3.- Tridimensional representation of female centroids from the populations studied. Minimum Spanning Tree (MST) is superimposed.
Figura 3.- Representación tridimensional de los centroides de las hembras de las poblaciones estudiadas. El árbol de mínima extensión (MST: Minimum Spanning Tree) se representa sobreimpresionado.

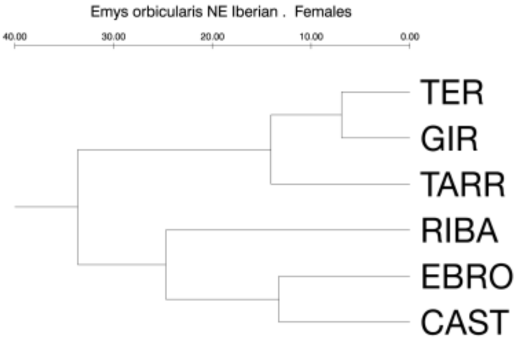


Figure 4.- Dendrogram (by UPGMA method) of the relationships between females from different populations based on their Mahalanobis' distances.
Figura 4.- Dendrograma (método UPGMA) de las relaciones entre las hembras de las diferentes poblaciones estudiadas basándose en sus distancias de Mahalanobis.

In our area however, close to the Pyrenees and very far from the *E. o. hispanica* type locality, there is some doubt as to which subspecies really occurs. In France two subspecies are reported: *E. orbicularis galloitalica* from the eastern Mediterranean coast and *E. orbicularis orbicularis* from the west-central regions (see FRITZ, 1996); thus the presence of these taxa in north-eastern Spain is very probable.

Parallel to the morphological study, samples of blood were taken and sent to Heidelberg University in Germany for genetic analyses of MtDNA. These analyses have revealed the presence of several different haplotypes in north-eastern Iberia (LENK *et al*., 1998, 1999). In fact the "northern populations" show, almost without exception, haplotype IIa, typical of the *orbicularis s. str.* populations. This haplotype appears as dominant in the Ribagorzana basin population, but VId haplotype also appears there. The Tarragona coastal population shows haplotype V, and the Castellón population shows the V and the VIa haplotypes. Finally, in the Ebro delta population up to four haplotypes were found (IIa, VId, VIa and V).

In our analysis it appears clear that the "northern populations" (Ter basin and Girona inland) are very close to *Emys orbicularis orbicularis*, characterised by the IIa haplotype. The morphological characteristics corroborate this classification in the central European subspecies. French and north-eastern Spanish specimens referable to *orbicularis* are clearly smaller than the true central and eastern European *E. o. orbicularis* (Fritz, pers. comm.), so we consider these populations as *E. o.* cf. *orbicularis.*

The Tarragona coast and Ebro delta populations which cluster at some distance with these cf. *orbicularis* populations, are *E. orbicularis galloitalica,* described from southern France (FRITZ, 1995) and widespread in a large part of Italy, the eastern Mediterranean coast of France and parts of the north-eastern Iberian coast. This subspecies is represented by the MtDNA V haplotype.

The Ribagorzana basin population seems to be intermediate between *E. o.* cf. *orbicularis* and *E. o. hispanica*. In fact, the majority of the haplotypes of this population are IIa (orbicularis-type) but the shell shape and general appearance seem very different and although habitat peculiarities could be related to some differences, they appear in fact quite different from the Girona populations close to *orbicularis*. We regard this as a secondary intergradation zone between *hispanica* and cf. *orbicularis*.

In the Castellón coastal population two haplotypes appear: V (for *galloitalica*) and VIa (characteristic of the *fritzjuergenobsti* area). This population could be regarded as intermediate between these subspecies, and constitutes the southern limit of *galloitalica* and probably also the natural northern limit of *fritzjuergenobsti*.

The Ebro delta population merits mention apart; although it clusters with the second group (the southern localities), morphologically it shows an intermediacy between the southern and northern samples. There is an astonishing diversity of haplotypes in this area (in fact, the greatest known up to now in Europe). This is due to the fact that, apart from its position as the destination of the enormous Ebro watershed, escapes and releases of specimens have been made in this area. In this population one can find the coastal *galloitalica*, *hispanica* and cf. *orbicularis* –present certainly in the Ribagorzana which belongs to the same basin– and introduced and escaped specimens of *fritzjuergenobsti* coming from the nearby Castellón province.

In fact, the relationships expressed by the Minimum Spanning Tree (see above) are very congruent with this interpretation, the two "northern populations" *(E. o.* cf. *orbicularis*) are very close, and related at a moderate distance to the Tarragona coastal population (*E. o. galloitalica*).

The "southern populations" cluster is composed in fact of populations intermediate between different subspecies, but all with a different degree of contribution from "Iberian groups" haplotypes. Among these, in the male analysis, the most closely related to the northern ones are the Ebro delta specimens (with individuals of all haplotypes) and the coastal Tarragona (*galloitalica*) specimens; and the Ebro delta (all haplotypes) and river Ter (cf. *orbicularis*) populations in the female analysis.

In the case of Castellón (*galloitalica* × *fritzjuergenobsti*) this population is more closely related to the Ebro delta (all haplotypes) population in both sexes, probably explaining a stronger presence of these haplotypes in this latter sample. Finally, the Ribagorzana population (cf. *orbicularis* × *hispanica*) appears moderately distant but more closely related to the Ebro delta (all haplotypes) in the males, thus confirming the closer relation of cf. *orbicularis* to *galloitalica* which also appears among the "northern populations", thus pointing out once again the probable greater influence of *galloitalica* in the Ebro delta male sample (see above Castellón conclusions). In the case of the females, the Ribagorzana population (cf. *orbicularis* × *hispanica*) appears more closely related to –but clearly different from– the Castellón samples (*galloitalica* × *fritzjergenobsti*) the only possible explanation of this remote similitude being the common presence of related "Iberian subspecies" haplotypes, *fritzjuergenobsti* in the case of the Castellón sample and *hispanica* in the case of the Ribagorzana samples.

Although our area appears as one of the most diverse from a genetic and morphologic point of view, the scenario for the present distribution of subspecies seems to be clear.

First we can imagine the area populated by a primitive autochthonous *galloitalica* substrate, which presents today a seemingly relictual chorologic pattern in areas probably suitable for the species during the Pleistocene (forested with evergreen and broad leafed forests). This substrate, which appears in a pure form today only in the Tarragona population in our area, probably came into contact with *fritzjuergenobsti* in the province of Castellón, although this contact may have occurred a very long time ago.

The "Iberian subspecies" probably differentiated in two distinct areas of the Iberian Peninsula: *E. o. fritzjuergenobsti* 'in situ' in the Spanish Levantine area in which it occupies today an area of coastal marshes, more developed during Pleistocene by the low sea level, and from which it was probably unable to spread; and *E. o. hispanica* in southern Spain from which it spread to the greater part of the Iberian Peninsula, reaching the Ebro basin probably from its southern tributaries where the species is poorly known.

In contrast, *E. o.* cf. *orbicularis*, which is an eastern European immigrant, could have reached western Europe via the Danube-Rhône corridor, well known in zoological and historic-anthropological literature, reaching French territory and later our area via the Eastern Pyrenees, cutting off and contacting with the primitive *galloitalica* area, and probably intergrading with it in southern France and perhaps in the study area (see FRITZ, 1996). It appears almost pure in the province of Girona. At the same time, in the Ebro basin it contacts with the spreading *hispanica* haplotypes, producing intermediate populations. This intermediate *hispanica* × cf. *orbicularis* from the river Ebro basin plus the primitive substrate of coastal *galloitalica* populations makes up the mixed population of the Ebro delta, completed by the escape and release, if not yet present in the area, of Castellón specimens (with *fritzjuergenobsti* haplotypes).

In this respect morphometric and genetic results seem to complement each other very well, but it should be borne in mind that haplotypes (MtDNA) are always maternally inherited, whereas morphology expresses inheritance from both parents (nuclear DNA). The presence of two haplotypes in the same locality indicates a past contact (if they are morphologically –thus DNA nuclearly– uniform) or recent introductions (if discordant haplotypes also present different morphologies and thus different nuclear DNA).

Acknowledgements

To Enric Adroguer, Xavier Capalleras, J. F. Lluís Solà, Ramon Ribas and Javier Valverde for their helpand support in the field work. To Dr. Uwe Fritz for his suggestions and correction of the manuscript; to Peter Lenk for sharing with us the results of the genetic study from the blood samples we sent him; and finally, to Sheila Hardie and Jeff Humm for their corrections of the English text.

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Recibido: 09/10/98
Aceptado: 23/12/99