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Morphological and genetic evidence of the full species status of *Iberolacerta cyreni martinezricai* (Arribas, 1996)

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Abstract

Iberolacerta cyreni martinezricai is elevated to the species level (*I. martinezricai*) based on both morphological and molecular data. The phylogenetic analysis using two mitochondrial and one nuclear gene shows *I. martinezricai* is more closely related to *I. monticola* than to *I. cyreni*. A multivariate analysis of the morphological data also supports the affinities between *I. martinezricai* and *I. monticola* but, at the same time, clearly indicates that *I. martinezricai* is morphologically distinct from both *I. monticola* and *I. cyreni*. The molecular data suggests *I. cyreni* and the clade formed by *I. monticola* + *I. martinezricai* split approximately 6.1 Mya, during the Mesinian Salinity Crisis, when climatic conditions around the Mediterranean area changed dramatically as a result of the desiccation of the Mediterranean Sea. Separation between *I. martinezricai* and *I. monticola* occurred approximately 2 Mya but, with at least two equally plausible alternative hypotheses, their biogeography is still unclear. New data on the habitat and distribution of *I. martinezricai* indicates its distribution area is very small (12–15 km^2), and that it lives in a climatically extreme habitat for this kind of mountain species. As a result of that and the low numbers of individuals, *I. martinezricai* is considered here as Critically Endangered.

Key words: *Iberolacerta*, *Lacerta*, taxonomy, phylogeny, evolution, biogeography, conservation, morphology, mountain restriction, cytochrome *b*, 12S rRNA, c-*mos*, mitochondrial DNA, nuclear DNA

Introduction

Klemmer (1957) was the first herpetologist to report the presence of Rock lizards on the Peña de Francia. He based his study on a single male specimen, captured by A. Epple in September 1954, which he classified as *Lacerta monticola cantabrica* Mertens, 1929. Latter on, Martinez-Rica (1979) reclassified Peña de Francia's Rock lizards as *L. monticola*

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cyreni Müller & Hellmich, 1937. This situation has remained unchanged until recently, when Arribas (1996) elevated the Rock lizards from the Sistema Central to the species level (*L. (=Iberolacerta) cyreni*), and described two new subspecies within its distribution range: *Iberolacerta cyreni cyreni* from the Sierra de Guadarrama, *I. cyreni castiliana* from the Sierra de Gredos and *I. cyreni martinezricai* from the Peña de Francia, province of Salamanca, Spain.

Subspecific status for the Rock lizards from the Peña de Francia was suggested by Arribas (1996) on the basis of the existence of clear differences in scalation (lack of contact between the rostral and internasal scales) and presence of blue ocelli on the shoulders, characteristics radically different to the other *I. cyreni* populations. In spite of these differences and its geographical isolation, the validity of *I. cyreni martinezricai* was questioned on the basis of the small sample size (one male, one female and a juvenile) used for its original description (Pérez-Mellado, 1997; Barbadillo *et al*. 1998). In a follow-up study, Arribas (1999b) reanalysed the subspecific status of Peña de Francia's population including new data from 23 live specimens, which were not preserved for conservation reasons. In this study, using mainly scalation and coloration, he arrived to exactly the same conclusions as in his previous analysis (Arribas, 1996), reinforcing the validity of the taxon *I. cyreni martinezricai*. Recent molecular studies based on mitochondrial DNA sequences including representatives of all species and subspecies of *Iberolacerta* (Mayer and Arribas, 2003; Crochet *et al*. 2004; Carranza *et al*., *in press*) have shown that all *I. cyreni martinezricai* specimens from the Peña de Francia analysed were genetically distinct from the rest of Iberian Rock lizard taxa. However, and contrary to what was thought, in both analyses *I. cyreni martinezricai* was part of the *I. monticola* clade, rendering *I. cyreni* paraphyletic.

The known distribution range of *I. cyreni martinezricai* has been extended very recently (Arribas, *in press*; Arribas and Odierna, *in press*). It now includes several other localities close to the Peña de Francia, all within a very small polygon formed by the Peña de Francia-Hastiala, Rongiero and Orconera Mountains. It probably also extends westwards towards the Sierra de Gata. In the Peña de Francia itself, *I. c. martinezricai* is present from the bottom of the mountain up to the summit at 1723 m a.s.l. In one of the new localities, the Batuecas Valley, it is common between 1000 and 1400 m a.s.l., with some records even at lower altitudes (840 m a.s.l). It is also present on the northern slopes of the Sierra de las Mestas (Rongiero – 1627 m a.s.l. at the summit-), at altitudes ranging between 1200 and 1400 m a.s.l., and most probably also on the southern slopes of this mountain, which lie in Extremadura. Despite *I. cyreni martinezricai* consisting of more individuals and occupying a much larger area than it was originally thought, it is still probably one of the most threatened vertebrates in Europe. This is as a result of the habitat where it lives (block boulders inside forests) and the ever-increasing touristic pressure in the area. The distribution range of this species lies in the Meso- and Supramediterranean oak and evergreen-oak forest belts (mainly occupied by *Quercus ilex* and *Quercus pyrena-*

ica), at least from 840 to 1730 m a.s.l., in fully Mediterranean climate type (Meso- and Supramediterranean belts) with very high summer temperatures and only marginally entering into Oromediterranean areas. This contrasts with the Atlantically-influenced and/or high mountain habitats inhabited by the other two species (Colline to Alpine in *I. monticola* and Oromediterranean in *I. cyreni*, although *I. m. monticola* from Serra da Estrela inhabits Supramediterranean areas and *I. m. cantabrica* from Orense, Zamora and S. León provinces penetrate a bit into Oromediterranean areas).

In this paper, we use morphological (biometry and scalation) and molecular data (mitochondrial and nuclear DNA) together with karyology and osteology (Arribas and Odierna, *in press*) to assess the taxonomic status of *I. cyreni martinezricai*.

Material & Methods

Morphology

A total of 535 measured specimens from Oscar Arribas' (OA) database with snout-vent length greater than 45 mm were included in the analyses. These were studied from Pedro Galan's collection (University of La Coruña, Spain), Manuel Meijide's collection (Soria, Spain), the author scientific collection from Barcelona, and the collections of the Estación Biológica de Doñana, C.S.I.C. (Sevilla, Spain). The majority of specimens are the same as in Arribas (1996 and 1999b).

Acronyms used for the different OTU's and their current taxonomic allocation included in the morphological analysis are as follows:

GUA: Sierra de Guadarrama (Madrid and Segovia provinces, Spain). 32 males and 39 females [*I. cyreni cyreni*]

GRE: Sierra de Gredos (Avila province, Spain). 21 males and 37 females [*I. cyreni castiliana*]

FRA: Sierra de la Peña de Francia (Salamanca province, Spain). 22 males and 28 females [*I. cyreni martinezricai*]

EST: Serra da Estrela (Beira Alta district, Portugal). 17 males and 21 females [*I. monticola monticola*]

GAL: Galicia lowland areas (La Coruña and Lugo provinces; Spain). 33 males and 24 females [*I. monticola cantabrica*]

WCA: West Cantabrian Mountains (Lugo province, Spain). 35 males and 19 females [*I. monticola cantabrica*]

CCA: Central Cantabrian mountains (Leon and Oviedo provinces, Spain). 67 males and 69 females. [*I. monticola cantabrica*]

PIC: Picos de Europa and more eastern areas (Oviedo, Santander and Palencia provinces, Spain). 38 males and 33 females [*I. monticola cantabrica*]

A complete list of specimen localities studied is available from O.A. upon request.

Characters studied

Biometric characters

Snout-vent length (SVL); Forelimb length (FLL); Hindlimb length (HLL); Pileus length (PL); Pileus width (PW); Parietal length (PaL); Masseteric scale diameter (DM); Tympanic scale diameter (DT); Anal width (AW) and Anal length (AL). All linear measurements were made with a digital calliper to the nearest 0.01 mm by O.A. to avoid inter observer variability. These measurements were transformed to the following more informative and not dimensional-depending ratios: FLL/SVL (relative forelimb length; "FLL index"); HLL/SVL (relative hindlinb length, "HLL index"); PL/PW (pileus shape, "Pileus index"); DM/PaL (relative masseteric plate size, "Masseteric index"); DT/PaL (relative tympanic size, "Tympanic index"); AL/AW (anal plate surface, "Anal form index") and AS/SVL (v(AL*AW)*100/SVL, relative anal plate size in respect to total legth, "Anal size index") (see Arribas, 1996, 2001). Results of linear measurements and indexes yielded largely similar results. All ratios were given multiplied by 100 to avoid excessive decimal scores.

Scalation characters

Supraciliar Granulae (GrS) for the right and left sides; Gularia (GUL); Collaria (COLL); Dorsalia (DORS); Ventralia (VENT); Femoralia rigth (FEMr) and left (FEMl); 4th. digit Lamellae (LAM); and Circumanalia (CIRCA). The full presence (2), contact at one point (1) or absence (0) of contact between Rostral-Internasal (R-I) and Postocular-Parietal (Po-Pa) plates was also studied.

Pattern and coloration

The pairs of ventral plates ranges (symmetric) with black dots were recorded (PV), as well as the presence (1) or absence (0) of blue ocelli on the shoulders (BO). In general, only specimens for which a full set of characters was available were included in the numerical analyses, but in cases where only one value was missing, it was estimated using lineal regression. Given that these populations present sexual dimorphism (Arribas, 1996, 1999b), analyses were carried out for males and females independently.

Statistical Procedures

Statistical analyses used in the morphological study are the same as in Arribas (1996, 1999b) and included both Univariate (ANOVA for SVL, scalation characters and indexes, and ANCOVA with SVL as a covariate for the linear measurements, both of them with *post-hoc* Tukey-Kramer tests at $P < 0.05$ and $P < 0.01$ to detect differences among samples) and Multivariate techniques (Canonical Discriminant Analysis, CDA). In this later analysis, each population is represented by a centroid (a hypothetical middle individual) and a confidence interval (spheroid) of 95 %. The Minimum-length spanning tree (MST) computed from the Mahalanobis' distance matrix is represented superimposed to the CDA and helps to detect the nearest neighbors based in their position in the multidimensional space. Trees were constructed with the UPGMA method, based also on Mahalanobis distances (D^2) derived from CDA.

Multivariate analyses (CDA) were performed with CANP and DISC programs from MULTICUA Package (Arenas, Cuadras & Fortiana, 1991). UPGMA trees were calculated with NTSYS 2.1© (Rohlf, 2000). Univariate statistics were processed with NCSS 2001© package (Hintze, 2001).

Genetic study

Samples, DNA extraction and amplification

Two mitochondrial (cytochrome *b* and 12S rRNA) and one nuclear (c-*mos*) gene from 6 individuals of *I. martinezricai* from two different localities (Peña de Francia and Puerto El Portillo, Salamanca, Spain) plus one representative of all species and subspecies of Iberian *Iberolacerta* described to date (Arribas, 1996; Arribas, 1999c; Arribas, 2000, 2001) were used in the phylogenetic analyses. *'Lacerta' oxycephala* was used to root the tree. Specimen data are given in Table 1 and some localities are shown in Fig. 1. DNA extraction, PCR amplification and sequencing of the PCR products followed procedures described elsewhere (Carranza *et al.*, 1999; 2000). Primers used in both amplification and sequencing were 12Sa and 12Sb (Kocher *et al.*, 1989) for the 12S rRNA gene, cytochrome *b*1 and cytochrome *b*2 (Kocher *et al.*, 1989) for the cytochrome *b* (cytb) gene, and G73 and G74 (Saint *et al.*, 1998) for the nuclear c-*mos* gene.

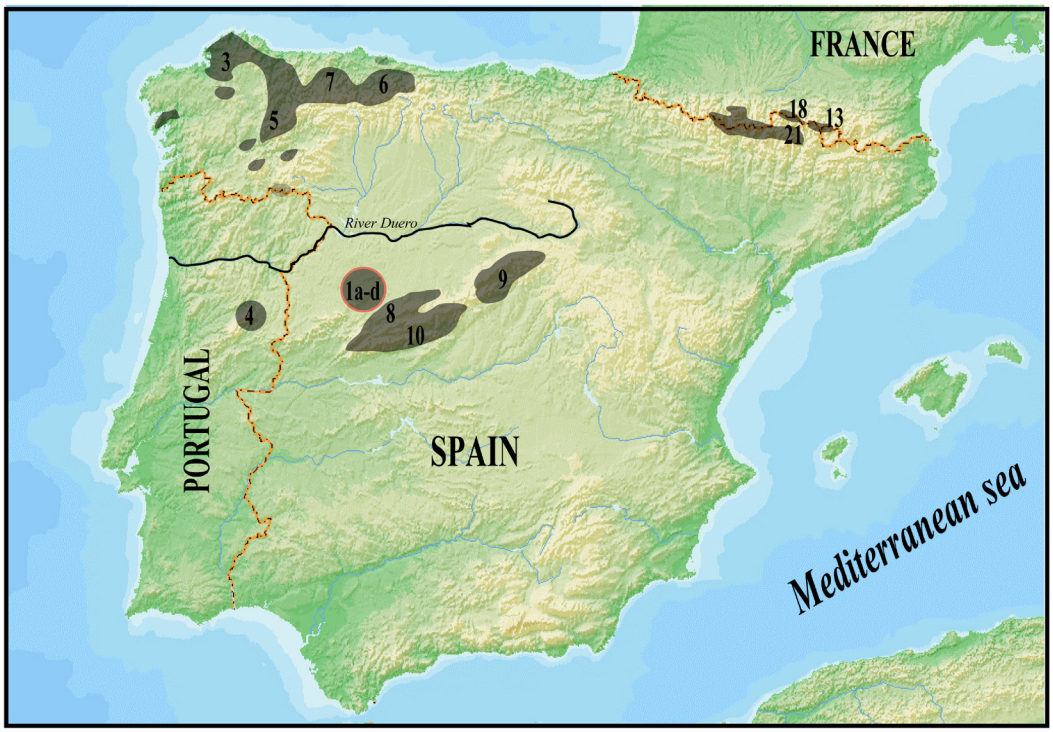


FIGURE 1.— Map of the Iberian Peninsula showing localities of *Iberolacerta* included in the DNA study. See Table 1 and Fig. 2 for further details.

TABLE 1.— Details of material and sequences used in the present study. Numbers after species names identify particular individuals sequenced from the same species Numbers between brackets after locality names refer to localities shown in Figure 1. Code refers to the code used for Genbank submission.

TAXA	LOCALITY	ACCESSION NUMBERS Cyt *b*/12SrRNA / C-*mos*	Codes
"Lacerta" oxycephala	Bosnia-Herzegovina	AY256652 / AY256657 / AY256660	E230923
Iberolacerta cyreni martinezricai-1	Maillo (Peña de Francia) (Spain) [1a]	AY151895 / AY151975 / AY152009	E2106123
Iberolacerta cyreni martinezricai-2	200m del Monasterio P. de Francia (Spain) [1b]	AY683631 / AY683635 / AY683639	E410.26
Iberolacerta cyreni martinezricai-3	Santuario Peña de Francia (Spain) [1c]	AY151897 / AY151977 / AY683640	E410.28
Iberolacerta cyreni martinezricai-4	Santuario Peña de Francia (Spain) [1c]	AY683632 / AY683636 / AY683640	E3107.1
Iberolacerta cyreni martinezricai-5	Pto. Portillo (Spain) [1d]	AY683633 / AY683637 / AY683641	E3107.2
Iberolacerta cyreni martinezricai-6	Pto. Portillo (Spain) [1d]	AY683634 / AY683638 / AY683642	E3107.3
Iberolacerta monticola cantabrica-1	Rio Eume (Spain) [3]	AY151865 / AY151945 / AY152011	E50614
Iberolacerta monticola cantabrica-2	Sierra de Caurel (Spain) [5]	AY151857 / AY151937 / AY152013	E4109
Iberolacerta monticola cantabrica-3	Sierra de Caurel (Spain) [5]	AY151858 / AY151938 / AY152014	E41010
Iberolacerta monticola cantabrica-4	Sierra de Caurel (Spain) [5]	AY151860 / AY151940 / AY152015	E41012
Iberolacerta monticola cantabrica-5	Somiedo (Spain) [7]	AY151864 / AY151944 / AY152016	E140611
Iberolacerta monticola cantabrica-6	Somiedo (Spain) [7]	AY151856 / AY151936 / AY152017	E50612
Iberolacerta monticola cantabrica-7	Puerto de Vegarada (Spain) [6]	AY151861 / AY151941 / AY152018	E41015
Iberolacerta monticola monticola	Serra da Estrela (Portugal) [4]	AY151870 / AY151950 / AY152012	E140618
Iberolacerta cyreni cyreni	Navacerrada (Spain) [9]	AY151845 / AY151925 / AY152021	E140612
Iberolacerta cyreni castiliana-1	Sierra de Bejar (Spain) [8]	AY151849 / AY151929 / AY152019	E41022
Iberolacerta cyreni castiliana-2	Sierra deGredos (Spain) [10]	AY151852 / AY151932 / AY152022	E140615
Iberolacerta bonnali	Port de Rus (Spain) [21]	AY151889 / AY151969 / AY152035	E4108
Iberolacerta aranica	Muntanyes de Barlongere (France) [18]	AY151873 / AY151953 / AY152030	E4101
Iberolacerta aurelioi	Circ de Comapedrosa (Andorra) [13]	AY151880 / AY151960 / AY152025	E40617

Phylogenetic analyses

A total of 1013 bp of mitochondrial (303 bp of cytb and 375 bp of 12S rRNA) and nuclear (335 bp of c-*mos*) DNA for all specimens listed in Table 1 were included in the phylogenetic analyses.

DNA sequences were aligned by hand using the alignment editor BIOEDIT v. 5.0.9 (Hall, 1999) and taking into account the published secondary structure (Hickson *et al.*, 1996). Alignment gaps were inserted to resolve length differences between sequences, and positions that could not be unambiguously aligned were excluded. Cytb sequences were translated into amino acids prior to analysis and did not show any stop codons, suggesting that all were functional. Two different methods of phylogenetic analysis were employed: maximum-likelihood (ML) and maximum parsimony (MP). MODELTEST (Posada & Crandall, 1998) was used to select the most appropriate model of sequence evolution for the ML analysis, under the Akaike Information Criterion. For the data set including all three genes this was the General Time Reversible (GTR) model, taking into account the shape of the Gamma distribution (G) and the number of invariable sites (I), while for the data set including the c-*mos* nuclear gene only it was the K80.

Both ML and MP analyses were performed in PAUP* 4.0b10 (Swofford, 1998) and included heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 10 and 100 random stepwise additions of taxa respectively. In all MP analyses, gaps were included as a fifth state. In order to correct for the estimated (using ML) transitions (ts) : transversions (tv) ratio, transversions were given the same weight as transitions and four times that weight in different analyses. Nodal support for all MP and ML trees was assessed using bootstrap analysis (Felsenstein, 1985) involving 1000 and 100 pseudoreplications respectively.

Where appropriate, topological constraints were generated with MacClade v. 4.0 (Maddison & Maddison, 1992) and compared to our optimal topologies using the Shimodaira-Hasegawa (SH) (Shimodaira & Hasegawa, 1999) test implemented in PAUP * 4.0b10 (Swofford, 1998) and employing RELL bootstrap with 1000 replicates.

Species recognition criteria

The criteria for recognizing species used in this work is primarily based on that outlined by Good and Wake (1993), following Frost and Hillis (1990) and applied to other similar papers (Garcia-París and Wake, 2000). In short, we consider as new species genetically cohesive units that are evolutionarily independent entities. Our main goal has been to be able to recognize and diagnose the taxa using morphological criteria, but we also present biochemical evidence that is diagnostic. When molecules suggest there is substantially more variation between samples than within accepted species, morphology is investigated and, if it also supports divergence, we recognize the unit as a new species.

Results

Molecular analysis

The results of the molecular analyses using mitochondrial and nuclear genes are presented in Fig. 2A and B. Contrary to what had been suggested before (Martinez-Rica, 1979; Pérez-Mellado, 1983; 1997; Barbadillo *et al.* 1998; Arribas, 1996; 1999b), both trees suggest that *I. c. martinezricai* is more closely related to the *I. monticola* clade than to the *I. cyreni* clade. To further test the phylogenetic position of *I. c. martinezricai*, a constraint analysis was carried out in which it was constrained to branch with *I. cyreni*. The results of the Shimodaira-Hasegawa (1999) test show that the likelihood value of the tree presented in Fig. 2A (-2570.17092) is significantly different from the likelihood value of the alternative topology (-2594.64252), indicating it is not supported by our data set (P = 0.013). The results of the phylogenetic analysis using the *c-mos* nuclear gene alone (Fig. 2B) also support that *I. monticola* and *I. c. martinezricai* are sister taxa. Moreover, in this analysis, all *I. monticola* samples sequenced consistently present a bp change in the same position (position 15 in our alignment) that differentiate them from *I. c. martinezricai*.

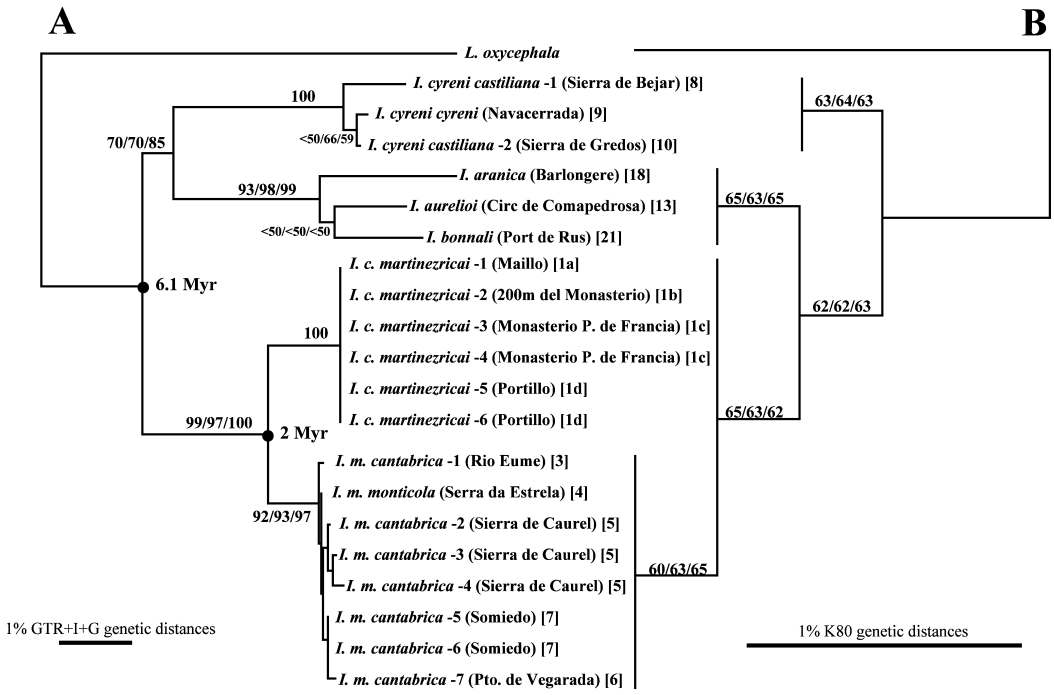


FIGURE 2.— ML trees of *Iberolacerta*. A) ML (GTR+I+G) tree (-log likelihood = 2570.17092) using two mitochondrial (cytb and 12S rRNA) and one nuclear gene (c-*mos*). Values above branches are bootstrap supports and, from left to right, they refer to the results using the following methods and models: ML (GTR+I+G) / MP (Ts=Tv) / MP (Ts=1, Tv=4). The black dot indicates the inferred age of the split between *I. martinezricai* and *I. monticola*. B) ML (K80) tree (-log likelihood = 541.28927) using the c-*mos* nuclear gene only. Values above branches are bootstrap supports and, from left to right, they refer to the results using the following methods and models: ML (K80) / MP (Ts=Tv) / MP (Ts=1, Tv=4).

In order to incorporate rough dates to our phylogeny, the ML tree from Fig. 2A (log likelihood = -2570.17092) was compared with a ML with clock-like branch lengths (log likelihood = -2580.7388). The results of the likelihood ratio test indicate the difference between both trees is not statistically significant ($-2\log\Delta = 21.1358$, which approximates to a X^2_{19} distribution under the null hypothesis; $P > 0.05$) and therefore the tree from Fig. 2A could be used to infer dates. This was done using the evolutionary rate of another lacertid lizard (the genus *Gallotia* from the Canary Islands), for which a reliable calibration for exactly the same gene regions used in the present work was available (Carranza *et al. in press*). The rates used to calibrate the molecular clock obtained with the three genes together was 0.9% per million years (My). Independent rates for each one of the data partitions were: 2.3% per My for the cytb alone, 0.5% for the 12S rRNA gene alone and 1.35% for the combination of the two mitochondrial genes (cytb and 12S rRNA).

The results of the clock analysis suggest *I. c. martinezricai* split from its sister clade (*I. monticola*) approximately 2 million years ago (Mya) (approximately 1.8% of genetic

divergence for the dataset containing the three genes together), during the Upper Pliocene. Despite evolving as independent lineages for all this time, variability within both clades is very low: 0% for *I. c. martinezricai* and 0.02% for *I. monticola*. These results suggests the patchy distribution of *I. monticola* with several isolated populations across more than 600 Km (see Fig. 1) might have been propitiated by a recent extinction of intermediate populations as a consequence of climatic changes occurred during the Pleistocene.

The phylogenetic tree presented in Fig. 2 A, suggests that the clade containing the Pyrenean Rock lizards is sister to *I. cyreni*. This result however, is neither supported by our c-*mos* analysis (Fig. 2 B) nor by previous, more complete, molecular analyses (Mayer and Arribas, 2003; Carranza *et al*., *in press*). So, it is most probably an artefact related to the lack of the only member of *Iberolacerta* from outside the Iberian Peninsula (*I. horvathi*) in our analysis.

Mophological analysis

Canonical Discriminant Analysis (males)

The analysis distributes the samples on the plane defined by the first two axes, which account for 75.9 % of the total variance. This is a good representation of all the inter-sample variability, especially in their first axis. These two axes together (Fig. 3A) indicate the presence of four groups: a) *I. cyreni* (GUA+GRE); b) *I. c. martinezricai* (FRA); c) *I. monticola* (EST); and d) the remaining *I. monticola* populations (GAL+WCA+CCA+PIC). MANOVA test shows the existence of significant differences between samples ($F_{147,1592}$=8.83, P<0.0001; Wilk's Lambda=0.0187).

The first axis (eigenvalue of 46.7, accounts for 64.9 % of the total inter-sample variability) separates in its positive part between *I. monticola* (WCA +GAL +PIC +CCA+ EST) and *I. cyreni* (GUA+GRE) populations. The *I. monticola* populations are characterized by (variable contributions to this axis -loadings- among parenthesis) having higher values of BO (0.800), PV (0.351) and VENT (0.243) than the *I. cyreni* populations (GUA+GRE) situated on the opposite extreme of the axis and characterized by the lower values for these characters. In this axis, *I. c. martinezricai* (FRA) is well differentiated from both and situated in an intermediate position between *I. monticola* and *I. cyreni* (also with intermediate values in the discriminant characters). The first axis can be interpreted as an "species discriminating axis". There are three clearly differentiated groups along it (*I. cyreni; I. c. martinezricai* and *I. monticola*).

The second axis (eigenvalue of 7.90, 11 % of intersample variability) has a much lower discriminative power than the first one and separates populations within the same species. FRA (*I. c. martinezricai*) samples appear on the positive extreme of the axis, close to EST (*I. monticola*). Both populations are characterized by their lower values of R-I (0.532), LAM (0.427) and MD (0.270). Increasing values in these characters are found from CCA, through PIC and GAL to WCA (all *I. monticola* populations from their main area). GUA and GRE (*I. cyreni*) appear largely undifferentiated in the central part of the

axis. The second axis can be interpreted as a "geographical axis", separating populations from west (on the negative part of the axis) to east (on the positive one). Only EST appears in a slightly more eastern position than expected from a strict geographical sense.

The third axis (graphically not represented, eigenvalue of 6.24, 8.7 % of total variability) has an even lower discriminative power but also outstands FRA in its most negative part, characterized by the lower values of VENT (0.547) and MD (0.494).

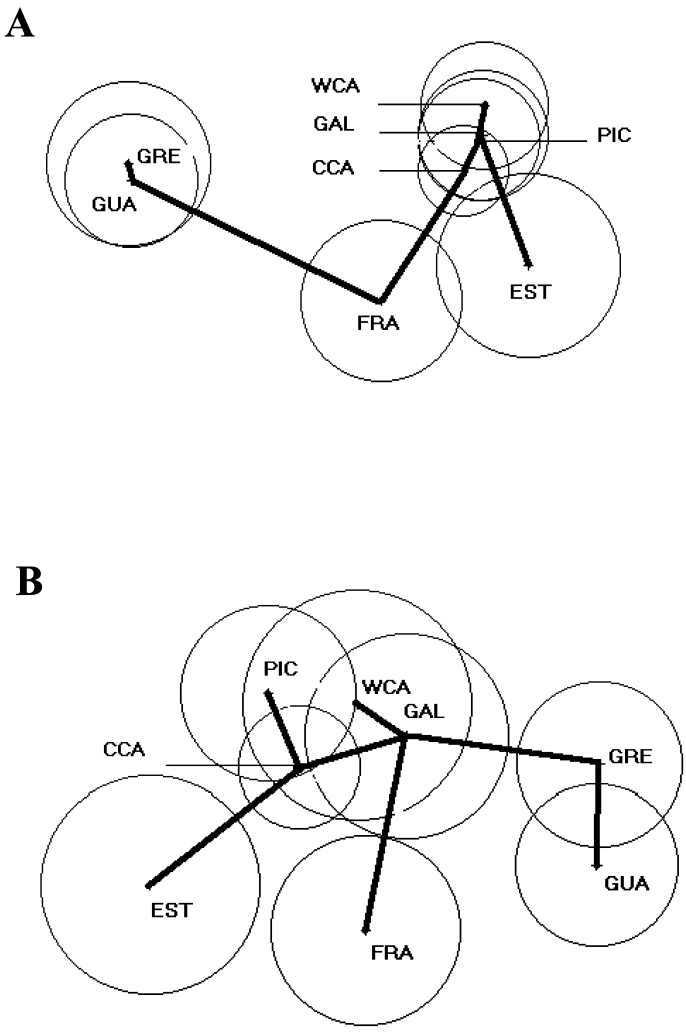


FIGURE 3.— Canonical Variate Analysis (CVA). Bidimensional representation of the first two canonical axes. OTUs (same abb. as in text) are represented by the centroid and a 95% confidence area. The Minimum-length Spanning Tree (MST) is superimposed. A) Male analysis (75.9% of all variability explained). B) Female analysis (67.3% of all variability explained).

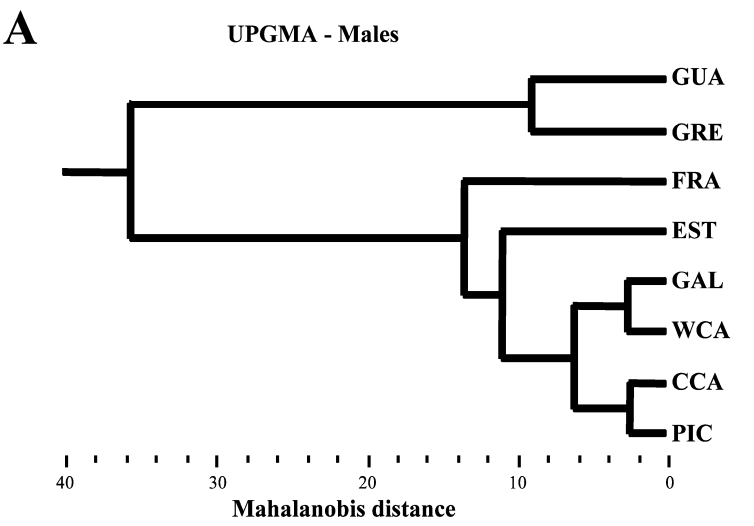
The discriminant function derived from these three canonical axes classify specimens as follows: of a total of 53 *I. cyreni* males studied, 51 were correctly classified, one was incorrectly classified as *I. c. martinezricai* and another one as *I. monticola*. The correct classification value therefore was 96.2 %. Out of 22 male specimens of *I. martinezricai* included in the study, 14 were correctly classified and seven incorrectly (four as *I. cyreni* and three as *I. monticola* - correct classification value of 63.6 %). Finally, from 260 *I. monticola* males analysed, 252 were correctly classified and only eight were incorrectly placed in other taxa (four as *I. cyreni* and four as *I. c. martinezricai* – correct classification value of 96.9 %). The total number of specimens that were incorrectly classified was 71 (26.7 % of wrong classification).

The minimum-length spanning tree (MST) (superimposed over Fig. 3 A) clusters together the two populations of *I. cyreni* (GUA and GRE, at D^2=9), as well as all populations of *I. monticola* (CCA-PIC at D^2=2.45, GAL-WCA at D^2=2.64, PIC-WCA at D^2=3.99 and PIC-EST more distant at D^2=9.41). The most similar population to *I. c. martinezricai* is the Central Cantabrian *I. monticola* (FRA-CCA, at D^2=10.5), while the geographically closer *I. cyreni* population more than doubles this distance (FRA-GUA, at D^2=24.7). See Table 2 for all OTUs reciprocal distances .

TABLE 2: Mahalanobis' distances among populations derived from CDA. MALES above-rigth diagonal and FEMALES below-left diagonal.

F\M	GUA	GRE	FRA	EST	GAL	WCA	CCA	PIC
GUA	**.000**	9.01	24.7	46.8	36.9	35.9	32.6	33.7
GRE	8.40	**.000**	26.9	46.5	35.8	36.7	33.8	35.9
FRA	14.6	15.3	**.000**	16.6	12.4	14.2	10.5	13.4
EST	32.3	31.7	11.7	**.000**	10.9	12.0	11.0	9.41
GAL	13.4	8.37	7.29	15.5	**.000**	2.64	7.22	6.41
WCA	18.6	13.6	10.1	14.7	4.60	**.000**	6.67	3.96
CCA	16.1	18.5	8.97	10.3	6.99	8.50	**.000**	2.45
PIC	21.1	21.0	13.6	10.9	7.63	9.12	3.26	**.000**

The cluster analysis (UPGMA) based on Mahalanobis' distances (Fig. 4 A) situates the two *I. cyreni* OUT's on one side of the tree, and *I. c. martinezricai* plus all *I. monticola* populations on the other side. *I. c. martinezricai* is sister to all *I. monticola* populations analysed. The cophenetic Correlation Coefficient (r = 0.95) shows that the dendrogram has a very good fit (r > 0.9; Rohlf, 2000) with the original distance matrix.



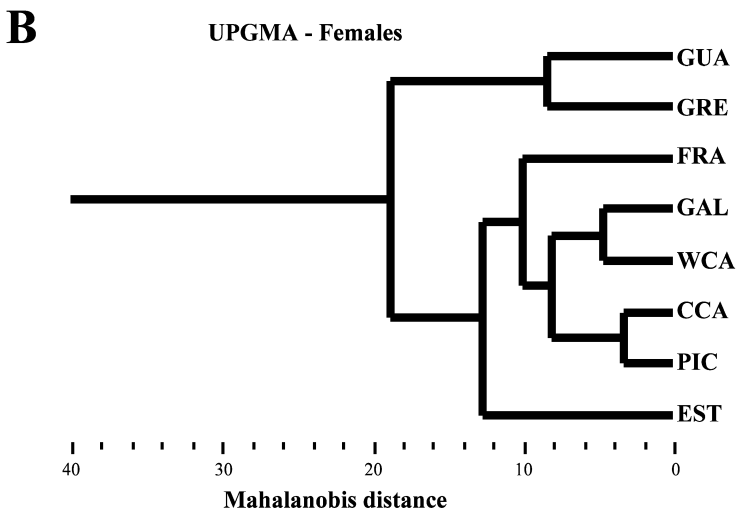


FIGURE 4.- UPGMA trees derived from distances among OTUs centroids. Abbreviations as in Fig. 3 .

MALES. Dendrogram showing three different clusters, one with *I. cyreni* (GUA and GRE), another with the different *I. monticola* OTUs clustered progressively and, basal to this, *I. martinezricai*. This tree reflects very well the original distance matrix (r=0.95).

FEMALES. *I. cyreni* (GRE, GUA) cluster together in a different group to all the rest of *I. monticola* + *I. martinezricai* samples analysed. *I. monticola* from EST appears basal to the other two clusters recovered in the analysis ((FRA + (PIC, CCA, GAL and WCA)). This second tree has a poor fit to the original distance matrix (r=0.73).

Canonical Discriminant Analysis (females)

The analysis distributes the female samples on a plane defined by the two first axes which cover 67.3 % of all interspecies variability (Fig. 3 B). The MANOVA test indicates the existence of significant differences among samples ($F_{147,1626}$=7.60, P<0.0001; Wilk's Lambda=0.0305). These two axes separate four groups: a) the first group includes all the Cantabrian *I. monticola* samples from populations PIC, CCA, WCA and GAL (clinally ordered in the graph in a geographical sense); b) a second group that includes the two relatively well differentiated *I. cyreni* populations (GRE and GUA); c) the EST population (an *I. monticola* locality); and finally, d) the FRA population of *I. c. martinezricai*.

First axis (eigenvalue of 23.9, accounts for 50.8 % of the total intersample variability). This axis separates two groups: one that includes *I. cyreni* and another one with *I. monticola* plus *I. c. martinezricai*. The *I. cyreni* populations appear on the positive side and are characterized by high values of "ANAL size index" (0.361) and R-I contact (0.279), and very low values of PV (-0.477) and BO (-0.366) and DT (-0.246). The remaining populations of *I. monticola* and *I. c. martinezricai* appear widespread along the central (FRA, GAL and WCA) and the negative parts (CCA, PIC and EST) of the axis, increasingly characterized by the contrary values to the *I. cyreni* samples. *Iberolacerta martinezricai* females appear among the samples of the two other species with a sligth overlap in this axis with *I. monticola*. As in the male analysis, this axis has an interpretation as a "species discriminating axis".

Second axis (eigenvalue of 7.78, 16.5 % of the total variability) has a lower discriminative power, but separates *I. c. martinezricai* (FRA) in their negative extreme, characterized by the lower values of R-I contact (0.511), LAM (0.504), BO (0.279) and MD (0.247). The remaining populations (both from *I. cyreni* and *I. monticola*) also appear widespread along the axis, especially the *I. monticola* ones. *Iberolacerta cyreni* samples are less differentiated in the central parts. As in the male analysis, this axis has an imperfect interpretation as a "geographic position axis".

The third axis (graphically not represented) also has a low discriminative power (eigenvalue of 7.03, 15 % of the total variability) and separates towards its positive part the easternmost populations (PIC, CCA and GUA) from the remaining ones. These samples are characterized by greater values of "Anal size index" (0.405) and lower of DORS (-0.699).

The discriminant function derived from these canonical axes reclassificates female specimens as follows: from 76 *I. cyreni*, it classifies 72 correctly and 4 incorrectly (three as *I. monticola* and one as *I. c. martinezricai*, 94.7 % of correct classification). From 28 *I. c. martinezricai*, it classifies 22 specimens correctly and 6 incorrectly (one as *I. cyreni* and five as *I. monticola*, 78.5 % of correct classification). Finally, from 166 *I. monticola*, it classifies 153 correctly and 13 incorrectly (seven as *I. c. martinezricai* and 6 as *I. cyreni*; 92.1 % of correct classification). The total number of confused (misidentified) specimens in the female analysis is 64 (23.7 % of wrong classification).

Minimum-length spanning tree (MST) (superimposed over Fig. 3 B) clusters all *I. monticola* populations together (CCA-PIC at D^2=3.26, GAL-WCA at D^2=4.6, GAL-CCA at D^2=6.99, and CCA-EST at D^2=10.3) on one side, and the two *I. cyreni* ones (GUA-GRE at D^2=8.4) on the other. *Iberolacerta martinezricai* (FRA) clusters with GAL (*I. monticola*) (FRA-GAL at D^2=7.29), and this later appears also as the most similar one to GRE (*I. cyreni*) (GRE-GAL at D^2=8.37). See Table 2 for all reciprocal distances among OTUs.

Cluster Analysis (UPGMA) (Fig. 4 B) based on Mahalanobis' distances gives a poor fit to the original distance matrix (r= 0.73) ($0.8<P<0.7$; Rohlf, 2000), and thus a less reliable representation of relationship among OTUs. The UPGMA tree derived from distances among OTUs centroids clusters all the Cantabrian *I. monticola* samples (PIC, CCA, GAL and WCA). *Iberolacerta martinezricai* (FRA) appears basal to this group of "Cantabrian monticola" but EST (belonging to *I. monticola*) appears even more basal to all them.

ANOVA-ANCOVA

The study of scalation characters by univariate methods (ANOVA-ANCOVA, with Tukey-Kramer MCT post-hoc tests at $P<0.05$ and $P<0.01$) gave the following results (see Tables 3 & 4).

TABLE 3.— ANOVA/ANCOVA results of the morphometric, scalation and biometric indexes from MALES of ***I. monticola, I. martinezricai*** and ***I. cyreni***.

	I. cyreni (1) (n=49)	*I. martinezricai* (2) (n=17)	*I. monticola* (3) (n=181)	*F*	*P*	1-2	1-3	2-3
SVL	63.48±0.95 45.1–74.1	59.71±1.61 50.69–68.15	61.12±0.49 45.81–72.69	3.07	0.048			
FLL	22.14±0.16 17.53–26.79	21.20±0.27 18.5–22.5	21.69±0.08 15.87–26.9	5	0.007	*	*	
HLL	31.50±0.28 23.1–38.23	30.78±0.47 24.5–33.6	31.51±0.14 22.41–53.17	1.08	0.23			
PL	15.04±0.08 10.7–18.74	15.73±0.13 13.01–17.77	15.32±0.04 10.7–18.2	9.69	0.00009	**	**	*
PW	7.07±0.06 5.2–11.9	7.04±0.11 5.91–7.75	7.25±0.03 5.43–8.7	4.06	0.018		*	
PaL	5.03±0.05 3.1–6.65	5.33±0.08 4.32–6.23	5.43±0.026 3.39–7.27	23.41	0.00000	**	**	
DM	2.02±0.04 1.2–2.95	1.59±0.08 0.76–2.52	2.16±0.02 0.68–3.2	23.62	0.00000	**	*	**
DT	1.79±0.04 0.93–3	1.93±0.07 1.4–2.51	1.92±0.02 0.82–3.36	3.35	0.62		*	
AW	4.49±0.06 2.7–6.87	4.23±0.10 3.06–5.07	4.12±0.03 2.6–5.5	15.05	0.00000		**	
AL	2.53±0.04 1.4–3.8	2.22±0.07 1.63–3.11	2.28±0.02 1.35–3.26	14.61	0.00001	**	**	

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TABLE 3 (continued)

	I. cyreni (1) (n=49)	*I. martinezricai* (2) (n=17)	*I. monticola* (3) (n=181)	*F*	*P*	1-2	1-3	2-3
GrS r	10.73±0.27 6–18	11.41±0.47 6–14	9.96±0.14 5–15	6.54	0.0017		*	**
GrS l	10.61±0.27 7–16	10±0.46 6–13	9.96±0.14 5–15	2.51	0.08			
GUL	24.69±0.30 21–31	25.23±0.51 23–28	24.19±0.15 19–31	2.66	0.07			
COLL	10.57±0.17 7–13	11.07±0.29 8–13	10.76±0.09 8–14	1.08	0.34			
DORS	50.69±0.51 45–59	53.35±0.86 50–58	52.31±0.26 44–62	5.12	0.006	*	*	
VENT	25.30±0.14 23–29	25.41±0.24 24–27	26.28±0.07 24–29	20.91	0.00000		**	**
FEM r	18.63±0.23 15–24	18.94±0.39 15–24	17.72±0.12 15–23	9.11	0.00015		**	**
FEM l	18.40±0.23 16–24	19.23±0.40 16–25	17.81±0.12 14–24	7.33	0.0008			**
LAM	25.28±0.24 21–29	24.29±0.41 21–28	25.13±0.12 21–31	2.21	0.11			
CircA	7.41±0.14 5–12	6.7±0.24 5–8	6.86±0.07 5–9	6.16	0.002	*	**	
R-I	1.87±0.11 0–2	0.70±0.20 0–2	1.33±0.06 0–2	14.34	0.00000	**	**	*
Po-Pa	0.19±0.06 0–2	0.05±0.10 0–0.5)	0.22±0.03 0–2	1.10	0.33			
PV	0.79±0.10 0–3	1.41±0.17 1–3	1.91±0.05 1–3	46.61	0.00000	**	**	*
BO	0.04±0.02 0–1	0.88±0.04 0–1	0.97±0.013 0–1	498.61	0.00000	**	**	
FLL/SVL	35.8±0.003 29.22–42.37	34.8±0.005 31.65–38.70	35.4±0.001 30.14–43.06	2	0.1372			
HLL/SVL	51.0±0.008 43.93–56.34	50.2±0.01 45.79–53.27	50.7±0.004 44.4–84.54	0.85	0.42			
PL/PW	214.1±0.01 120.16–231.34	223.1±0.02 200.46–280.72	210.9±0.008 191.05–232.43	10.38	0.00004	**		**
DM/PaL	40.1±0.009 30.90–53.84	29.7±0.001 13.93–44.13	39.6±0.005 28.43–57.14	17.13	0.00000	*		*
DT/PaL	35.6±0.009 21.81–49.18	36.6±0.015 26.96–43.95	35.3±0.004 17.59–68.57	0.33	0.71			
AL/AW	56.6±0.01 40.81–81.48	53±0.01 35.38–73.87	55.8±0.005 37.44–77.77	1.49	0.22			
AS/SVL	547.4±0.07 439–689	494±0.11 417.18–556.89	496±0.03 372.98–611.49	21.74	0.00000	**	**	

TABLE 4.— ANOVA/ANCOVA results of the morphometric, scalation and biometric indexes from FEMALES of ***I. monticola, I. martinezricai*** and ***I. cyreni***.

	I. cyreni (1) (n=79)	*I. martinezricai* (2) (n=18)	*I. monticola* (3) (n=161)	*F*	*P*	1-2	1-3	2-3
SVL	65.29±0.79 48.02–80.14	59.58±1.66 46.45–68.86	60.61±0.55 45.88–79.81	12.71	0.00000	**	**	
FLL	20.98±0.13 15.56–25.96	18.84±0.27 16.27–20.7	19.53±0.09 14.82–23.78	44.95	0.00000	**	**	*
HLL	29.26±0.16 23.2–36.76	26.97±0.35 23.18–28.17	27.56±0.11 21.18–33.75	35.80	0.00000	**	**	
PL	13.58±0.09 11–18.26	13.29±0.19 10.89–14.53	13.26±0.06 3.66–17.28	3.75	0.024		*	
PW	6.47±0.04 5.1–10.8	6.33±0.09 5.15–6.88	6.53±0.03 5.1–8.1	2.3	0.099			
PaL	4.40±0.03 3.4–6.32	4.38±6.76 3.49–5	4.51±2.26 3.37–6.28	3.84	0.37			
DM	1.67±0.03 1.16–2.5	1.35±0.07 0.68–1.84	1.76±0.02 0.8–2.5	14.08	0.00001	**		**
DT	1.54±0.03 0.9–2.64	1.65±0.06 1.1–2.12	1.74±0.02 0.83–2.5	13.97	0.00001		**	
AW	4.43±0.05 3–6.3	3.85±0.10 2.55–4.59	3.86±0.03 0.4–5.26	41.71	0.00000	**	**	
AL	2.55±0.03 1.8–3.82	2.18±0.07 1.25–2.79	2.23±0.02 1.3–3.11	29.67	0.00000	**	**	
GrS r	10.31±0.21 5–17	10.83±0.44 5–15	9.79±0.14 1–18	3.87	0.022			
GrS l	10.50±0.20 5–16	10.83±0.43 6–15	9.66±0.14 2–16	7.45	0.0007		**	*
GUL	24.55±0.24 19–33	24.88±0.52 22–27	23.65±0.17 18–31	5.93	0.003		**	
COLL	10.37±0.12 8–14	10.22±0.26 9–12	10.16±0.08 7–14	0.95	0.38			
DORS	49.88±0.39 43–59	51.38±0.83 46–56	50.18±0.27 43–59	1.33	0.26			
VENT	28.41±0.13 25–31	28.77±0.27 27–30	29.48±0.09	22.5	0.00000		**	*
FEM r	17.92±0.17 14–22	17.33±0.36 16–20	16.59±0.12 13–21	20.33	0.00000		**	
FEM l	17.78±0.17 15–22	17.38±0.37 15–19	16.71±0.12 12–25	12.55	0.00001		**	

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TABLE 4 (continued)

	I. cyreni (1) (n=79)	*I. martinezricai* (2) (n=18)	*I. monticola* (3) (n=161)	*F*	*P*	1-2	1-3	2-3
LAM	24.25±0.18 20–29	22.88±0.38 20.26	24.44±0.13 21–28	7.19	0.00083	**		**
CircA	7.79±0.11 5–11	6.38±0.24 5–8	7±0.08 4–9	21.20	0.00000	**	**	*
R-I	1.65±0.09 0–2	0.55±0.19 0–2	1.29±0.06 0–2	13.98	0.00001	**	**	**
Po-Pa	0.40±0.07 0–2	0.22±0.16 0–1	0.56±0.55 0–2	2.95	0.0541			
PV	0.40±0.08 0–3	0.61±0.18 0–2	1.37±0.06 0–3	43.53	0.00000		**	**
BO	0.012±0.04 0–1	0.66±0.09 0–1	0.68±0.03 0–1	79.54	0.00000	**	**	
FLL/SVL	33.32±0.002 27.73–39.68	31.10±0.005 25.64–36.13	31.92±0.002 25.82–38.63	12.22	0.00001	**	**	
HLL/ SVL	46.50±0.003 37.82–52.75	44.52±0.007 39.41–51.76	45.06±0.002 36.97–55.15	6.53	0.0017	**	**	
PL/PW	210.80±0.01 132.40–232.75	210.10±0.03 195.35–222.24	202±0.01 50.94–227.53	9.57	0.00009		**	
DM/PaL	37.80±0.008 28–50.1	30.94±0.001 16.83–43.49	39.22±0.006 18.43–56.81	10.45	0.00004	**		**
DT/PaL	34.60±0.007 4.2–47.21	38.19±0.01 24.4–54.04	38.95±0.005 17.58–56.09	12.79	0.00001		**	
AL/AW	57.69±0.03 37.25–101.88	56.91±0.06 43.06–63.86	60.73±0.02 40–74.3	0.38	0.6822			
AS/SVL	5.407±0.07 4.45–7.40	4.643±0.11 3.66–5.44	4.706±0.03 1.43–5.94	53.46	0.00000	**	**	

Iberolacerta martinezricai differs statistically from both *I. cyreni* and *I. monticola* in FLL (in females: abb. f), PL (in males: abb. m), DM (m, f), LAM (f), CircA(f), R-I (m, f), PV (m) and Masseteric Index (m, f).

Iberolacerta martinezricai differs only from *I. cyreni* in SVL (f), FLL (m), HLL (f), PL (m), AW (f), AL (m,f), DORS (m), CircA (m), BO (m, f) FLL index (f), HLL index (f) and "Anal size index" (m, f); and only from *I. monticola* in GrSl (m, f), VENT (m, f), FEMr (m) and PV(f).

Oromediterranean (Peña de Francia) and Mesomediterranean (Batuecas valley, at lower altitude) populations of *I. c. martinezricai* are nearly identical in coloration and scalation characters. Only the number of 4th toe subdigital lamellae is significantly greater in

the Peña de Francia population than in lower altitude one. Males from Peña de Francia have a mean±stdD (range;n) of 24.57±0.38 (22–28; n=14), whereas males from Batuecas have 22.6±0.76 (21–25; n=6) lamellae (T-test: T=2.47, *P*=0.02). Females from Peña de Francia have 23.7±0.41 (22-26; n=13) lamellae, whereas females from Batuecas have 21.9±0.54 (20-26; n=11) (T-test: T=2.64, *P*=0.014).

Discussion

The molecular and morphological results presented above clearly show that *I. cyreni martinezricai* deserves full species status. Therefore, we will refer to it hereafter as *I. martinezricai*. Other similar genetic studies (Mayer & Arribas, 2003; Crochet *et al*. 2004; Carranza *et al*., *in press*) also discuss the systematic status of *I. martinezricai*, but all three works are based solely on DNA sequences and therefore are not so useful for extracting taxonomic conclusions.

Both morphological (CDA, MST, UPGMA, ANOVA/ANCOVA) and molecular (mitochondrial and nuclear DNA) analyses carried out in this study demonstrate that *I. martinezricai* is closely related to *I. monticola* but, at the same time, well differentiated from it. These results are also supported by differences in the karyotype of both species (Arribas and Odierna, *in press*). In *I. martinezricai* the NOR is situated in the middle of a medium-large (M-type) chromosome, while in *I. monticola* the NOR is situated in a terminal position of a long chromosome (L-type). Interestingly, *I. cyreni* presents a similar karyotype to *I. martinezricai*, with the NOR situated in the same position. However, the karyotypes of these two species differ in some details of centromeric heterochromatine and in the presence of heterochromatic W sex-chromosomes in *I. cyreni*. The L-NOR type chromosome of *I. monticola* is also present in *I. horvathi* and in all three species of Pyrenean Rock lizards, although in the latter, the L-NOR is situated in one of the extremes of a biarmed chromosome as a result of a Robertsonian fusion. Considering the phylogenetic position of *I. horvathi* (basal to all Iberian *Iberolacerta* in Arribas, 1997, 1999a; Mayer and Arribas, 1996, 2003; Carranza *et al*., *in press*) and all the rest of species of *Iberolacerta* (see Fig. 2), it seems clear that the L-NOR bearing chromosome is the ancestral situation in *Iberolacerta*, with two independent (convergent) mutations producing the secondarily derived M-type NOR-bearing chromosomes observed in *I. martinezricai* and *I. cyreni.*

According to the phylogeny presented in Fig. 2 (and also in Mayer & Arribas, 2003; and Carranza *et al.*, *in press*), the ancestor of *I. martinezricai* and *I. monticola* split from *I. cyreni* approximately 6.1 Mya (5.5% genetic divergence with the dataset including the three genes). This date coincides with the beginning of the Messinian salinity crisis when, as a result of the almost complete desiccation of the Mediterranean Sea, there was a general increase in aridity around the Mediterranean basin. This increased aridity produced the desiccation of the internal lakes present in many Iberian depressions, forcing mesic

species to retreat to the moister Atlantic-influenced areas and up into the mountains (García-Antón *et al*., 2002). This phenomenon might have played an important role in the speciation process of *Iberolacerta*. An alternative scenario suggests *Iberolacerta* was restricted by competition with *Podarcis* lacertid lizards (Arnold, 1981, 1987; Carranza *et al*., *in press*). Diversification in *Podarcis* took place around the time that *Iberolacerta* seems to have fragmented, including diversification of the Iberian *P. hispanica* clade, which occurred some 7.5 ±1.2 Mya (Carranza *et al*., *in press*). Probably both phenomena, the mountains retreat of *Iberolacerta* to shady, fresh or even cold conditions of the mountains and more oceanic localities, and the diversification of Iberian *Podarcis* are parallel phenomena linked to the environmental changes and climatic deterioration developed during the upper Miocene.

The morphological analysis presented here (see results) is congruent with the molecular analysis and indicates both males and females of *I. martinezricai* are well differentiated from *I. monticola* and *I. cyreni*. The MST analysis joins together all populations of *I. cyreni* and *I. monticola* indicating these are homogeneous taxa. In the same analysis, males of *I. martinezricai* cluster with populations CCA (*I. monticola*) and GUA (*I. cyreni*), but the reciprocal distances are greater than the observed intraspecific distances within *I. cyreni* and *I. monticola* (see MST results above and Table 2). Females, however, are less differentiated: distances between *I. martinezricai* and its closest population (GAL, from *I. monticola*), are similar to distances between populations of *I. cyreni* or between *I. m. monticola* (EST) and all *I. m. cantabrica* populations.

The phylogenetic tree presented in Fig. 2 suggests speciation between *I. martinezricai* and *I. monticola* occurred approximately 2 Mya. This speciation event may have been triggered by competition with *Podarcis* (Arnold, 1981, 1987; Carranza *et al*., *in press*), by a geographical barrier or may have resulted from one or more of the climatic fluctuations frequent in the western Paleartic during the Late Pliocene and Pleistocene. These promoted genetic and morphological differentiation by changing the distribution and demography of many species (Hewitt, 1996, 2000; Veith, Kosuch and Vences, 2003). According to the tree topology and the actual distribution ranges of *I. monticola* and *I. martinezricai*, several alternative biogeographical scenarios are possible to explain the past and present distribution of these two species. One possibility considers speciation between *I. monticola* and *I. martinezricai* occurred around the western Sistema Central, approximately in the same general area of Serra da Estrela and Peña de Francia. According to this hypothesis, Serra da Estrela would have acted as a Pleistocene refuge from which a rapid range expansion of *I. monticola* would have probably occurred after the end of the Würm glacial period. This interpretation is coherent with the climatic conditions during the Quaternary and is also in agreement with biogeographical scenarios proposed for other reptiles and amphibians around the same general area: *Chioglossa lusitanica* (Alexandrino et al. 1997, 2000); *Lacerta schreiberi* (Gordinho et al. 2002, 2003; Paulo et al. 2001, 2002) and *Podarcis* (Pinho et al. 2002).

An alternative scenario, considers speciation between *I. monticola* and *I. martinezricai* occurred as a result of the formation of the current Duero river drainage. The Duero river watershed stopped being an endorreic drainage between the Middle and the Upper Pliocene, appearing as presently known only from the Villafranchian (Lower Pleistocene). During this period, the Duero river cut its own path across the Arribes del Duero area towards the ocean, and was probably captured by the upper reaches of a small Portuguese river, cutting Portugal across from East to West and acting as a biogeographical barrier until today (Jimenez-Fuentes, 1983; Perez-Gonzalez *et al.*, 1994). These dates fit well with the inferred speciation event between *I. monticola* and *I. martinezricai* (2 Mya) and, if true, would imply that the split between these two species would have occurred around the general area of the Duero river. This scenario also implies *I. moticola* would have originated north of the Duero river, being the Estrela population a recent southern isolate. The phylogeny presented in Fig. 2, suggests the north-south range expansion of *I. monticola* from Galicia and Cantabria to Serra da Estrela and subsequent area fragmentation would have happened very recently, probably after the last glaciations. This hypothesis of a northern origin of *I. moticola* is supported by the existence of greater morphological variability in the north, and the fact that Estrela recognized haplotype is nested within the Gallaecian and Cantabrian ones. The northern (Cantabrian) populations are more variable than *I. monticola* from the Serra da Estrela in pattern and coloration, as well as in scalation (as measured by the variation coefficients of several characters, in which the lowland Coruña sample is the most variable one; see Arribas, 1996). The presence of a supernumerary scale among prefrontals is a rare circumstance but widespread among lacertid lizard species. In the three Pyrenean species, *I. cyreni* and *I. martinezricai* this character is exceptional and appears in less than a 5% of specimens, but it is more abundant in *I. monticola*. The presence of a supernumerary scale among prefrontals is common among *I. monticola* from Galicia and the westernmost extreme of Cordillera Cantabrica, (GAL: 58.8 %; WCA: 46.8 %), it is less common towards the east (CCA: 14.5 %) and it is really rare in localities situated in the extreme east (PIC: 6.5 %) and in Portugal (EST: 7.14 %). However, the greater morphological variability found in *I. m. cantabrica* may be the consequence of the existence of higher habitat diversity in the north, and the fact that *I. m. cantabrica* presents a much larger distributional range than *I. m. monticola* with many more individuals.

In order to clarify the biogeography of *I. monticola* and decide between these two competing scenarios, a much more thorough molecular and morphological analysis is needed, including many more populations from both the northern and the southern limits of *I. monticola*, as well as from all intermediate areas where this species also occurs.

Despite a very recent origin of all populations of *I. monticola* is supported by identical karyology and extremely small genetic distances both in DNA sequences and allozyme electrophoresis (Arribas, 1996; Mayer and Arribas, 1996, 2003; Almeida *et al*. 2002; Carranza *et al*., *in press*), populations outside Serra da Estrela (*I. m. monticola*) have been historically recognized as a different subspecies (*I. monticola cantabrica*). Moreover, our

analysis presented in Fig. 2 and similar analyses in Mayer and Arribas, (2003); Crochet *et al*. (2004) and Carranza *et al*. (*in press*) show *I. m. monticola* is paraphyletic. As a result of that, Mayer & Arribas, (2003) have suggested *I. m. monticola* and *I. m. cantabrica* should be synonymized. Only morphology, especially scalation, supports the existence of differences between *I. m. monticola* from Serra da Estrela and all the remaining *I. monticola* populations (see Fig. 3 and 4).

Conservation

Despite being one of the lizards with the smallest distribution area in the world (at the moment it is only known from an area of 12–15 km^2 of discontinuous habitat), the discovery of new populations of *I. martinezricai* at lower altitudes, in Mesomediterranean and Supramediterranean habitats (Arribas, *in press*), suggests it might inhabit rock boulders among Mediterranean-type environments more to the west, in the Sierra de Gata, where its presence was discarded before due the absence of high mountains. Until very recently (see for instance Perez Mellado, 1983; Arribas, 1996, 1999b) *I. martinezricai* was thought to live only in the summit (the last 100 m) of the Peña de Francia mountain, an increasingly humanized and touristically occupied small plateau where the numbers of lizards have been decreasing during the last three decades (Pérez-Mellado, 1983; Arribas, 1999b). A visual estimation a decade ago indicated the number of adults was probably lower than 50 specimens. The entire distributional range of *I. martinezricai* remains inside the "Parque Natural de las Batuecas y Sierra de Francia", a protected natural space. *Iberolacerta martinezricai* should be considered as Critically Endangered, as its currently known area is extremely small, it lives in climatically extreme areas and is probably very sensible to changes in the microthermic conditions of habitat, which might be affected by frequent fires at short term, and by global climatic change at middle and long term.

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