

Bonn zoological Bulletin	Volume 57	Issue 2	pp. 197–210	Bonn, November 2010
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Intraspecific variability of the Carpetane Lizard (*Iberolacerta cyreni* [Müller & Hellmich, 1937]) (Squamata: Lacertidae), with special reference to the unstudied peripheral populations from the Sierras de Avila (Paramera, Serrota and Villafranca)

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Abstract. Canonical Discriminant (CDA), ANOVA and ANOSIM analyses were calculated for all recently known distribution areas of *Iberolacerta cyreni* including several small and unstudied peripheral populations. The only differentiated sample is Guadarrama (the nominate subspecies), with very limited overlap in the CDA (correct classification > 70%) and different from nearly all the other samples in ANOSIM. Guadarrama is a recently differentiated but well diagnosable (morpho)subspecies (with lower values of dorsalia, ventralia and greater values of circumanalia). Despite the mtDNA differences of the Béjar specimens, their morphology is largely equivalent to that of *I. cyreni castiliana* (Gredos), but clearly differ in their female body elongation (near 1 cm) with shorter limbs, a possible strategy to increase clutch size. Populations from the Sierras de Avila (Villafranca, Serrota and Paramera) are very similar among them. Villafranca (in males) together with Béjar (in females) are the most connected samples in MST, and the root of the species differentiation from a morphological point of view, once discarded geographical and climatic influence on morphology. All populations except Guadarrama shall be considered as *I. c. castiliana* by their morphological identity with Gredos. These morphological similarities probably are the reflect of extensive gene flow among them, responsible of maintaining their morphology largely equivalent.

Key words. Lacertidae, *Iberolacerta cyreni*, Intraspecific variability, subspecies, Geographical variation, Iberian Peninsula.

Resumen. Se ha calculado un Análisis Discriminante Canónico, ANOVA y ANOSIM con toda el área de distribución de *Iberolacerta cyreni*, incluyendo varias poblaciones periféricas no estudiadas hasta la fecha. La única muestra diferenciada es Guadarrama (la subespecie nominal), con muy poco solapamiento en el CDA (clasificación correcta > 70%) que difiere de prácticamente todas las demás muestras en el ANOSIM. Guadarrama es una población recientemente diferenciada, pero bien diagnosticable como (morfo)subespecie (valores bajos de dorsalia y ventralia, y altos de circumanalia). A pesar de las diferencias mitocondriales de Béjar, su morfología es ampliamente asimilable a *I. c. castiliana* (Gredos), siendo destacable el relativo elongamiento corporal de las hembras (casi 1 cm) con miembros proporcionalmente cortos, una posible estrategia para incrementar el tamaño de puesta. Las poblaciones de las Sierras de Ávila (Villafranca, Serrota y Paramera) son muy similares entre sí. Villafranca junto con Béjar (en machos y hembras respectivamente) están morfológicamente en la raíz de la diferenciación de la especie (MST), una vez descartada cualquier influencia climática o de distancia geográfica. Excepto Guadarrama, todas deben considerarse como *I. c. castiliana* por su identidad morfológica con Gredos, lo que refleja la probable presencia de un flujo genético extensivo y reciente entre ellas.

INTRODUCTION

The Spanish Sistema Central consists of a series of Sierras, more or less aligned in a ENE-WSW direction, which separate the Duero (to the North) and Tago (to the South) river drainages, or what is the same, the Old and New Castile plateauxes. It runs from the Portuguese Serra da Estrela (inhabited by a relict population of *Iberolacerta monticola* [Boulenger, 1905]), across the Spanish Sierra de Gata (apparently too low and dry for *Iberolacerta*), the Sierra de Francia with *Iberolacerta martinezricai* (Arribas, 1996) and the main part of the Spanish portion of its range

(over 240 km in length), which is inhabited in several points by populations of the Carpetane Lizard (*Iberolacerta cyreni* [Müller & Hellmich, 1937]).

The Carpetane Lizard is widespread through the main parts of the Sistema Central and is mainly known from Sierras de Béjar, Gredos and Guadarrama (Fig. 1). It was raised to species level (Arribas 1996) based on allozymes (Mayer & Arribas 1996), karyology (heterochromatinization of sex-chromosome and localization of the NORs;

Odierna et al. 1996) and adult and hatchlings pattern and coloration. Two subspecies were defined, the nominal *I. c. cyreni* from Guadarrama (type locality: Puerto de Navacerrada), and *I. cyreni castiliana* from Gredos (type locality: Circo de Gredos, Avila) to which frequently are assimilated Béjar specimens. This latter subspecies differs from the nominate one by a reduced dark pattern, more dorsalia, ventralia, slightly larger diameter of the masseteric and hindlimb length, and lower circumanalia (Arribas 1996).

The degree of genetic differentiation between *I. c. cyreni* (Guadarrama) and *I. c. castiliana* (Gredos) was analyzed by Mayer & Arribas (2003), who found a mtDNA sequence divergence of 0.6 % in the 12s rRNA (12s) and 16s rRNA (16s) mitochondrial genes, which corresponds to approximately 0.6 MY BP. Carranza et al. (2004) suggested that both subspecies diverged approximately 0.8 ± 0.2 MY BP, an estimation mainly based on the Cytochrome b (Cyt b) mitochondrial coding gene (the 12s and the nuclear gene *C-mos* were uninformative at this level), a divergence time almost identical to the one calculated by Crochet et al. (2004) using also the Cytb gene [1.6 % genetic divergence, which roughly corresponds to 0.6 to 1, with a mean of 0.8 MY BP). These two values were very similar to the above-mentioned ciphers. The inferred divergence time increased up to 1.2 (Cyt b) or 1.6 MY BP (Cyt b+12s) when different terminal taxa evolutionary models and phylogenetic methods were used (Arribas et al. 2006; Arnold et al. 2007; respectively).

On the other hand, specimens from Sierra de Béjar branched at the base of the *I. cyreni* clade in some mtDNA analyses (Carranza et al. 2004; Arribas & Carranza 2004). It was suggested that the split of this populations occurred approximately 1.7 ± 0.3 MY BP (Carranza et al. 2004). However, in analyses using the same mtDNA regions but different taxa and other evolutionary models and phylogenetic methods than above (Arribas et al. 2006; Arnold et al. 2007), the specimens from Béjar formed a trichotomy with *I. c. castiliana* from Sierra de Gredos and *I. c. cyreni* from Navacerrada.

Apart from the uninformative *C-mos* nuclear gene fragment analyzed by Carranza et al. (2004) there is only one other information about differences at the nuclear level, the analysis of allozyme data by Almeida et al. (2002), which showed a Nei's distance of 0.002 between specimens from Gredos and Guadarrama.

From West to East, the distribution of *I. cyreni* can be divided into two axes connected by low mountain valleys (see appendix II), but not clearly interrupted by clear cut barriers as river valleys. One axis runs across Sierra de Béjar (summit in La Ceja, 2,425 m) and Gredos (Alman-

zor, 2,592 m), whereas the other axis is constituted by the Sierras de Villafranca (Moros, 2,065 m), La Serrota (Serrota, 2,294 m), La Paramera (Zapatero, 2,160 m) and slightly separated by lower areas, Guadarrama (Peñalara, 2,430 m). The two axes greatly overlap longitudinally leaving the Villafranca, Serrota and Parameras just to the North of the Sierra de Gredos, but at their orographic shadow for rains, and climatically more continentalised. This explains the botanical similarities between the Paramera-Serrota-Villafranca axis and the Sierra de Guadarrama (Luceño and Vargas 1991).

In Guadarrama (where *Podarcis muralis* also exists), *I. cyreni* occurs only at the highest areas, from 1,760 m (Puerto de Cotos, Puerto de Navacerrada) up to the peaks (2,340 m in Peñalara). In Gredos it lives almost from 1,700 to 2,500 m. It was seen in 17. VII. 1986 (own data) in Puerto del Pico (at 1,352 m close to one of the fountains of the pass) but recent research in this area has been totally unfruitful. It is possible that these lower stations favoured by accelerated cold winds in the mountain passes (Venturi effect) had disappeared by climatic or best, by habitat degradation due to human over-frequentation during the last 20 years. In Béjar it has been found between 1,837 m (own data) and 2,443 m (see Lizana et al. 1988, 1992, 1993; and Martin 2005 for general data; own data corrections for the confirmed lower limits).

Apart from the better known Sierras, the presence of the Carpetane Lizard in the small parallel mountain ranges called "Sierras de Avila" or "Parameras" (composed by three Sierras: Villafranca, La Serrota, and La Paramera) was first discovered by the mountaineering group "Valle de Ambles" (Lizana et al. 1993), but no specimens have been studied so far. All aspects of morphology, status and relationships of these small and isolated populations from the Sierras de Avila are totally unknown. In these Sierras the species is extremely localized, especially in La Serrota and Paramera. In Sierra de Villafranca, the area with a relatively more extended suitable area, I have found it from 1,850 m probably up to the highest areas (Pico Moros, 2,065 m). In La Serrota it is extremely rare and localized, also cornered in the highest parts, from 2,284 m (perhaps 1,935 m where excrements, possibly of this species, were seen; pers. obs.) to the very summit (Pico Serrota, 2,294 m); and in La Paramera from 1,700 m in the northern slopes to the summit (Pico Zapatero, 2,160 m) (own data).

After a three-year prospection of these parallel ranges, I gathered data from these localized and barely known populations in order to check the relationships of all the Carpetane Lizards throughout its range. My aim is: a) to reassess differences between *I. c. cyreni* and *I. c. castiliana* in the light of the existence of other small and isolated pop-

ulations; b) to ascertain the taxonomic status of the Béjar populations and to check if these represent a further subspecies; c) to study both the relationships among the samples from the Sierras de Avila (=Parameras) massifs, as well as their similitude and differences with their neighbouring and well known populations from Gredos, or the more distant populations from Guadarrama and Béjar; and d) as the type series of *I. cyreni* was destroyed during the Second World War (SWW), to choose a Neotype for the species (see appendix I) in order to fix unequivocally the type locality (although apparently all lost, there were also specimens from Gredos in the original type series).

MATERIAL & METHODS

Morphology

A total of 106/92 male specimens, and 136/135 female specimens of *I. cyreni* with a complete measurements dataset and snout-vent length greater than 45 mm, were included in the univariate (ANOVA) and multivariate (discriminant) analyses, respectively. Given that these populations present sexual dimorphism (Arribas 1996, 1999a; Arribas et al. 2006), analyses were carried out for males and females separately. All material is from Oscar Arribas (OA) database.

OTUs names, localities and specimens included in the morphological multivariate analysis were as follows (Fig. 1):

GUADARRAMA: Sierra de Guadarrama (Madrid and Segovia provinces, Spain). 25 males and 36 females [*I. cyreni cyreni*]. MIJARES: Puerto de Mijares (Sierra del

Cabezo, Gredos Oriental Massif, Avila province, Spain). 6 males and 7 females. GREDOS: Circo de Gredos (Gredos Central Massif, Avila province, Spain). 23 males and 46 females [*I. cyreni castiliana*]. BÉJAR: Sierra de Béjar (Gredos Occidental Massif, also known as Sierra de Candelario, Salamanca province, Spain). 11 males and 28 females. VILLAFRANCA: Sierra de Villafranca (Avila province, Spain). 20 males and 14 females. SERROTA: La Serrota (Avila province, Spain). 3 males and 2 females. PARAMERA: Sierra de La Paramera (Avila province, Spain). 4 males and 1 female.

These populations are discontinuous among them (Appendix II) and constitute discrete geographical OTUs (Fig. 1).

Due to lower sample, it was necessary to cluster the Serrota and Paramera specimens in the male discriminant analysis, and these two plus Villafranca (all of them “Sierras de Avila”) in the female one. However, reciprocal distances between each one of these poorly represented samples to the best represented ones were carefully checked and commented in the results section. As the three populations from Sierras de Avila seemed to be largely equivalent in the multivariate analyses, a posteriori, all of them were treated as a single OTU (S^a AVILA) in ANOVA.

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. These measurements were transformed to the following more informative and not dimensional-depending ratios: FLL/SVL (relative forelimb length, “FLL index”); HLL/SVL (relative hindlimb 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 $\sqrt{(AL \cdot AW)} \cdot 100 / SVL$, relative anal plate size with respect to the total length, “Anal size index”) (Arribas 1996, 2001). The results of the linear measurements and indexes yielded largely similar results. All ratios were given multiplied by 100 to avoid excessive decimal scores.

Scalation characters. Supraciliar Granula (GrS) for the right and left sides; Gularia (GUL); Collaria (COLL); Dorsalia (DORS); Ventralia (VENT); Femoralia right (FEMr) and left (FEMl); 4th. digit Lamellae (LAM); and Circum-analia (CIRCA).

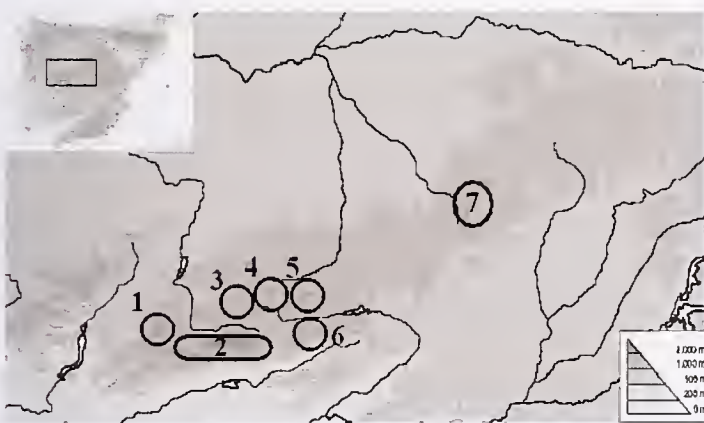


Fig. 1. Schematic representation of the distribution of *Iberolacerta cyreni* in the Spanish Sistema Central. The different localities (OTUs) cited in the text are represented. 1: Béjar; 2: Gredos; 3: Villafranca; 4: Serrota; 5: Paramera; 6: Mijares; 7: Guadarrama.

Statistical Procedures

Statistical analyses used in the morphological study included both Univariate (ANOVA for SVL, scalation characters and indexes, with *post-hoc* Tukey-Kramer tests at $P < 0.05$ and $P < 0.01$ to detect differences among samples) as well as Multivariate techniques (Canonical Discriminant Analysis, CDA). In this later analysis, each population is represented by a centroid (a hypothetical middle individual). Minimum-length spanning tree (MST) was computed from the Mahalanobis' distance matrix to detect the nearest neighbours based on their position in the multidimensional space. MST representation also avoids distortion of UPGMA trees by the reciprocal pairwise distances recalculation in every step during their construction. UPGMA frequently clusters samples reflecting sample sizes than their true relationships. Distances of small samples or isolated specimens appear greatly exaggerated with respect to the well represented ones. As a result of that, the small-sized samples appear ever as the most external or differentiated in UPGMA derived trees (Kunkel et al. 1980; Cherry et al. 1982; Arribas 1997). Moreover, the UPGMA trees based in very unevenly sized samples also gave very poor Cophenetic Correlation Indexes between the tree-derived ultrametric distances matrix and the original Mahalanobis distance matrix and therefore we have not used them (Arribas et al. 2006).

To test the significance of the differences among pre-established groups for the Discriminant Analysis (based in a geographical origin), we carried out an Analysis of Similarity (ANOSIM) (Clark 1988, 1993) that tests if the assigned groups are meaningful, this is, more similar within groups than with samples from different groups. The method uses the Bray-Curtis measure of similarity to construct clusters of specimens. The null hypothesis is therefore that there are no differences between the members of the compared groups (they are randomly blended). R-statistic scales from +1 to -1. Values closer +1 correspond to a perfect case in which all groups were completely different (all specimens of the same group are more similar among them than to any specimens of the other groups). $R = 0$ occurs if the high and low similarities are perfectly mixed and bear no relationship to the group, a common situation if some of the groups are largely equivalent. A value of -1 indicates that the most similar samples are all outside of the groups (all groups largely equivalent and randomly formed). To check for significance, pseudoreplication tests (1000 randomizations) were run to test if the given results can occur by chance. If the value of R is significant, there is evidence that the samples within groups are more similar than would be expected by random chance. Even more important, pairwise tests among

populations permit to test significance of the differences among the concerned groups and to detect which ones are really different from the others.

Mahalanobis' (squared) distance matrices were compared by means of Mantel Test (with 1000 permutations) with matrices composed by Euclidean (squared) distances for the climatic characteristics of localities: a) Precipitation (mm) during the incubation months (July and August, as scalation is invariant during lizard's life); b) Annual precipitation (mm), c) Temperature ($^{\circ}\text{C}$) (July and August); d) Annual average temperature e) Sun radiation ($\text{n } 10 \text{ kJ}/(\text{m}^2 \cdot \text{day} \cdot \text{micrometer})$) (July and August), and f) Annual Sun radiation. Data were extracted from Ninyerola et al. (2005). Also, these Mahalanobis' distances were compared with (d) the aerial (straight) geographical distances among the sampling localities.

Multivariate (Discriminant and ANOSIM) analyses were performed with Community Analysis Package 4.0 (Henderson & Seaby 2007). MST trees and Mantel tests were calculated with NTSYS 2.1[©] (Rohlf 2000). Univariate statistics were processed with NCSS 2001[©] package (Hintze 2001).

RESULTS

Males

Canonical Discriminant Analysis. The CDA conducted with 106 male specimens shows three significant axes that explain an 85 % of the total intersample variation. The two first axes together explain the main part (70.6 %) and discriminate fairly well the samples, especially the first one, the unique with an eigenvalue > 1 . The first discriminant axis has an Eigenvalue of 1.54 (51.2 % of variance explained; Chi-Sq. with 85 df = 200.71, $P < 0.0001$) and distributes the samples with fairly overlap among them, except Guadarrama, that has only a small coincidence with the other ones (Fig. 2 A). Guadarrama appears in the negative part of the axis, characterised by the lower values for DORS (0.441553) and VENT (0.560765) and greater values of CIRCA (-0.572683). Second and third axes (eigenvalues < 1) present a considerable overlap among the samples and do not discriminate populations.

This discriminant analysis applied to the samples reached a 72.6% of correct classification among the specimens. The Guadarrama sample (*I. cyreni cyreni*) reaches a 71.9 % of correct classification in respect to all the other samples (*I. c. castiliana*).

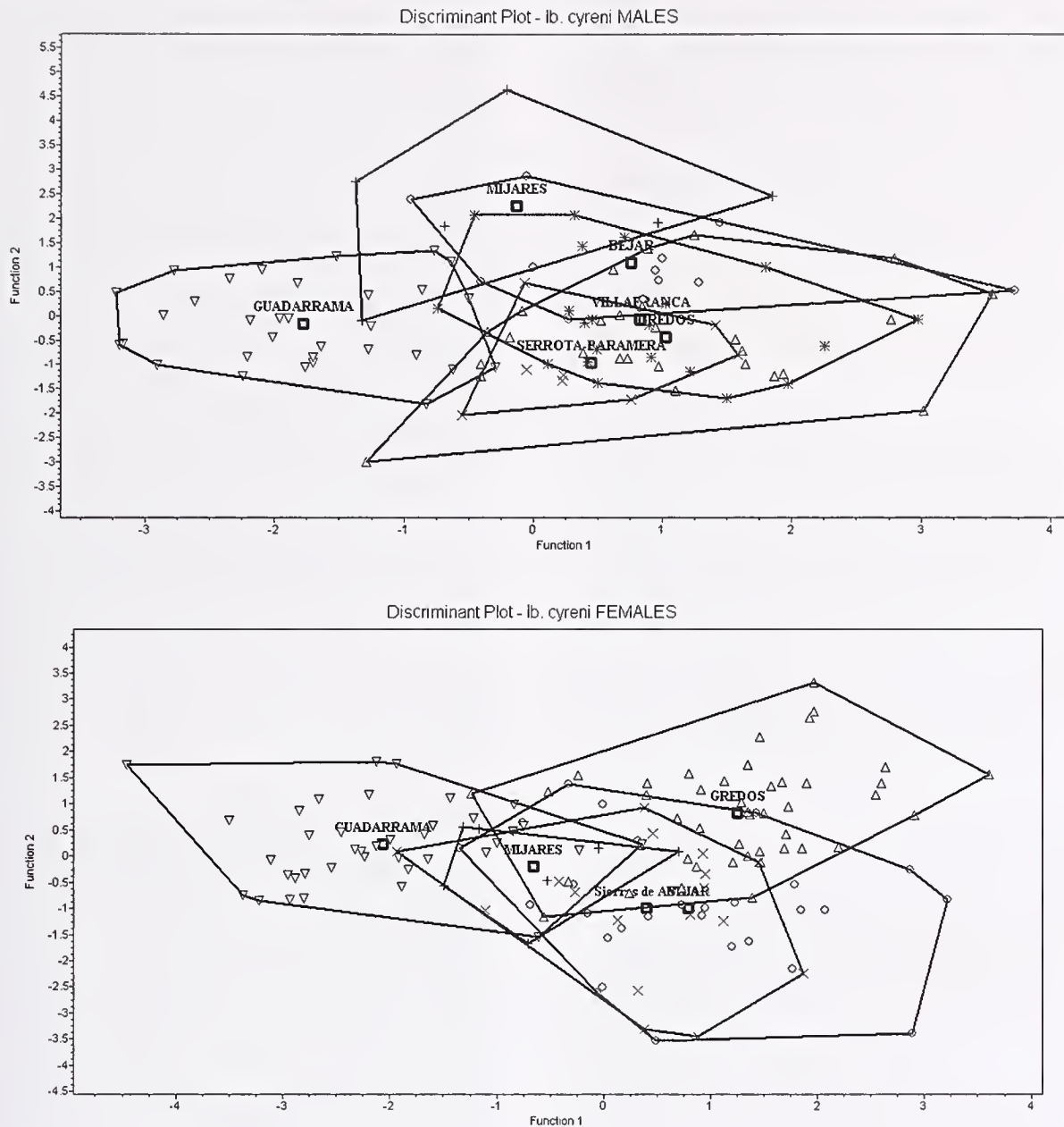


Fig. 2. Canonical Discriminant Analysis (CDA) plots for a) males (above) and b) females (below). Specimens, sample centroids and group perimeters are represented. Guadarrama (inverted triangles), Gredos (triangles), Béjar (irregular circles), Mijares (cross), Villafranca (asterisk) and Serrota-Paramera (sail). In females, the three last samples are grouped as Sierras de Avila (sail). Sample centroids are represented by a square. See text for axis characteristics and results.

Minimum-length spanning tree (not represented) connecting the centroid (hypothetical middle specimens) of each sample is fairly congruent with their geographical position, connecting in general neighbouring samples. The most “central” (most connected) population is Villafranca that connects with Gredos (at Mahalanobis Distance of 3.1870), Béjar (3.2199), Paramera (4.8042) and finally, to the most isolated one, Guadarrama (6.8412). Two populations show overestimated distances due to their small sample sizes: Mijares (East Gredos) that connects with Béjar (7.0951), and Serrota with their neighbouring Paramera (9.1822).

Analysis of Similarity (ANOSIM) (Table 3) shows that there is a considerable overlap among samples (R -statistic = 0.122088, $P < 0.005$; 1000 randomizations) as our value (that can range from 1 to -1), although positive, is very small. Very significant differences among the (geographically) assigned groups, appear only among Guadarrama and Béjar, Gredos and Villafranca ($P < 0.01$) but do not reach significance with Mijares and Serrota + Paramera (both with small samples). The other populations are not differentiated among them ($P > 0.01$).



Fig. 3. *Iberolacerta cyreni castiliana*. a) La Covatilla Sky resort (Sierra de Béjar), July 2007, Male ; b) El Travieso (S^a de Béjar), July 2004, Female ; c) El Calvitero (Sierra de Béjar), July 2004, Female (atypical pattern, with diffumination and coalescence in a unique vertebral line); d) Puerto de Mijares (Gredos Oriental Massif), July 2006, Female.

The Analysis of Variance (ANOVA) (Appendix III, Table 1) indicate that Guadarrama differs from all or nearly all the other populations in VENT and CIRCA (with the smaller and greater values for these parameters, respectively, in the former population), but also appeared differences between Guadarrama and Béjar in Dors (smaller in the former), and with Gredos in PV (greater in the former). An interesting and significant difference appears in DORS among Gredos and Béjar samples (clearly greater in the later).

There is no significant correlation among Mahalanobis' distances and any of the geographic and climatic parameters analyzed (all Mantel Tests $P > 0.05$).

Females

Canonical Discriminant Analysis: The CDA conducted with 136 female specimens shows three significant axes that explain a 93.8 % of the total intersample variation. The two first axes together explain a large part of the variance (85.7 %), and especially along the first one, that accounts itself for 62.7 % of the total variation and is the unique with an eigenvalue >1 (1.77), discriminating Guadarrama specimens from the other neighbour samples only with a small overlap (Fig. 2B). The other samples show a considerable overlap among them. Guadarrama discriminates towards the negative part of the axis, characterised by the lower values of DORS (0.62) and VENT (0.66) and greater ones of CIRCA (-0.38). Second and third axes (eigenvalues < 1) present a considerable overlap among the samples and do not discriminate populations.

The discriminant analysis applied to the samples reached a 74.26% of correct classification among the specimens. Guadarrama sample (*I. c. cyreni*) reaches an 87.2 % of correct classification with respect all the other samples (*I. c. castiliana*).

Minimum-length spanning tree (not represented) connecting centroids is very similar to the male one. The most connected sample is Béjar, which clusters with Villafranca (at 2.9), Gredos (3.5) and Mijares (7.38, but here exaggerated by the scarce sample of the later). Guadarrama connects with the scarcely represented (and geographically intermediate) Mijares (East Gredos) (at 6.09), and all the Sierras de Avila samples cluster together (Villafranca with Serrota + Paramera at 8.66).

Analysis of Similarity (ANOSIM) (Appendix III, Table 3) shows that there is a considerable overlap among samples (R -statistic = 0.162588, $P < 0.001$; 1000 randomizations), but the results are slightly best than for the male



Fig. 4. *Iberolacerta cyreni castiliana*. a) Pico Zapatero (Sierra de la Paramera), July 2005, Male; b) Puerto de Peña Negra (Sierra de Villafranca), July 2006, Male; c) Pico Serrota (La Serrota Massif), July 2005, Female; d) Pico Serrota (La Serrota Massif), July 2006, Male.

analysis. Very significant differences ($P < 0.01$) appear among Guadarrama and all the other samples except with Mijares. The other populations are not differentiated among them ($P > 0.01$).

The Analysis of Variance (ANOVA) (Appendix III, Table 2), as in the male analysis, it shows that Guadarrama is the most different one, especially in DORS, VENT and CIRCA (the first two characters smaller, and the third one greater in the former population). Guadarrama also differs from Gredos by its lower GUL, from Béjar by its greater FLL, HLL, a lower SVL; and from Sierras de Avila by its greater relative anal scale surface.

Also, significant differences appear in SVL between Béjar (clearly the great sized female population) and Gredos, and among this latter (with relative greater FLL and HLL) with Béjar and Villafranca.

As in male analysis, there is no significant correlation among Mahalanobis' distances and the geographic and climatic parameters analyzed (all Mantel Tests $P > 0.05$).

DISCUSSION

From the Discriminant and ANOVA analyses it appears that the only differentiated sample is Guadarrama. It appears with very limited overlap with the other samples in the CDA graphs (Figs 2 A and B). Diagnostic characters for this population (nominate subspecies: *I. c. cyreni*) are the lower values of DORS (difference more marked in females), lower values of VENT and greater CIRCA. Moreover, ANOSIM analyses show that Guadarrama is the unique OTU that is significantly different from nearly all the other samples, except from the close population of Mijares (in both sexes) and Serrota + Paramera (but these exceptions occur only in the males and probably due to their scarce sample size).

Mijares sample (very small) seems in some aspects approaching to Guadarrama (specially in DORS and VENT values) but globally are clearly closer to *I. c. castiliana* (specially to Béjar in male and female MST).

Populations West from Guadarrama show a great overlap in CDA and lack differences in ANOSIM, being morphologically fairly equivalent and all of them assimilable to *I. c. castiliana*. There are only a few scattered very significant differences among them ($P < 0.01$) in ANOVA, as for instance among Gredos and Béjar (this latter has greater DORS and a strikingly greater SVL and proportionally shorter limbs than Gredos, but only in female specimens). The reason of the longer SVL in Béjar females (from 8 mm to 1 cm greater than in the other populations)

which leaves proportionately shorter limbs, can be a consequence of body elongation that in lacertids appears linked to a greater clutch size (Braña 1996). This is an interesting question for future study: if Béjar specimens effectively have greater clutch size than other *I. cyreni* populations.

Both the MST results (in which Villafranca and Béjar are the most connected samples) as well as the presence of related species further West (*I. martinezricai* and *I. monticola*), suggest an origin of the species towards the western extreme of their current distribution area. From these westernmost parts, where it also occurs the higher haplotype diversity (see below), *I. cyreni* spread towards the East. Despite that the Sierras de Avila (Villafranca, Serrota and Paramera) are slightly more aligned with the Guadarrama axis than with Gredos one, we cannot be sure from which of these two mountain ranges the former was colonized, as MST results in males and females are contradictory. According to the male analysis Guadarrama is more related to Villafranca, whereas in the female analyses, it is Mijares (Eastern Gredos) the most related one.

The results of the mitochondrial analyses of these samples (Cyt B and 12s) (unpublished, Carranza, pers. com.) indicates that the interruption of gene flow is fairly recent, as a common haplotype appears in all populations except Villafranca and Béjar. All Gredos, Guadarrama and La Serrota specimens are identical for these two mitochondrial fragments. Independent changes in one nucleotide with respect to the common haplotype appear in Villafranca (the unique change is different in two specimens), Paramera, Mijares and Béjar specimens, and two changes accumulate in one Béjar and one Mijares specimen (others have only one).

The current morphological differences of Guadarrama specimens seem to be relatively recent, and are possibly the result of bottleneck effects during the colonization process, or alternatively of strong selective pressures (or a combination of both causes). Conversely, the absence of marked differences among the other populations (more or less with a similar age) could be due to the maintenance of a more continuous gene flow among them, responsible of maintaining their morphology largely equivalent (nuclear genes remain unstudied). The current larger geographical gap in the distribution of *I. cyreni* occurs precisely between Guadarrama and the remaining populations to the West.

Despite the presumably short isolation time, as commented above, a considerable selection pressure or a genetic bottleneck in the expanding populations might have promoted and fixed the morphological differences now seen in Guadarrama specimens. These factors do not seem to

be due to isolation-by-distance processes but by historical vicariant events (cf. Irwin 2002) as there is no relationship between morphological differentiation and geographic distances. Also there is no relationship among the more obvious climatic parameters (precipitation, temperature and sun radiation) and these differences.

Concerning the position of Béjar populations, only specimens from the west-facing slopes of the massif have been studied, and therefore it is possible that in other parts of the massif other haplotypes (the common one with Gredos) could be present. The species was cited from "Puerto de Tornavacas, SA" (for Salamanca, sic.!: a mistake as this locality is in Avila) (Lizana et al. 1992). This is the natural pass between Béjar and Gredos, but I have been unable to find it there. This place is a sub-Mediterranean environment with Pyrenean Oak open forest inhabiting populations of *Timon lepidus*, *Psanmodromus algirus* and even *Buthus occitanus*, all thermophilous species typical from dry conditions. The lower height of Puerto de Tornavacas (1010m) makes me to suspect that *I. cyreni* is not there and the record is possibly a mistake. One possibility is that it was from the higher neighboring mountains.

An account about the pattern and coloration of *I. cyreni* is in Arribas (1996) and it is especially detailed for the main Sistema Central massifs in Perez-Mellado et al. (1993). Both colour as well as the dark pattern, seem to be selected in accordance to the substrate characteristics. Overall, the background colour is brown in juveniles and subadult specimens, changing in different percentages to green in adult specimens (more frequently in males and becoming more vividly linked to reproductive processes). In populations inhabiting rocks (plenty of *Rhizocarpon* gr. *geographicum* lichens) as in Gredos and the upper parts of Guadarrama (Peñalara) green adults are more frequent (both males and big females). When living in rocky talus with sands and bare ground (as in Navacerrada area) brownish adult specimens are more frequent (Arribas 1999b: Figs 9 and 10).

Concerning the reticulate pattern, it also varies in a different degree among the different populations depending on the substrates inhabited. Juveniles and subadults have temporal uniform or reticulate bands that coalesce during growth with dorsal spots (more frequently in males) giving reticulated-like patterns. Usually, there is a relationship between the size of the granite phanocrystals (the granite-rock spotting) and the habitus of the lizards living on it. Lizards living on rocks that present large crystals (as for instance the Béjar ones) are more reticulated than specimens living in places in which the rocks present smaller crystals (and thus finely spotted). Coloration accounts described in Perez Mellado et al. (1993) are fairly precise, especially for Gredos specimens. Concerning

Béjar (=Candelario in Perez Mellado et al. [1993]) the description should be corrected as, although it is true that very old specimens are fairly reticulated, especially males, females more frequently have two paravertebral rows of distinctive spots (photo 2), as in females of other populations and in mid-grow specimens of both sexes in all localities. The statement that “the most common background colour of the back and flanks is greenish or bluish” probably is true for fully adult specimens during the breeding period, but in July and August only some big males conserve greenish tones, appearing even fully adult females more or less brownish.

Concluding: a) *I. c. cyreni* is a recently differentiated but well diagnosable (morpho)subspecies, a case paralleling the relationship of *I. monticola monticola* from Serra da Estrela (Portugal) with respect to *I. m. cantabrica* (from Galicia and Cantabrian Mts.), in this later case, with no genetic differences, but with singular morphological traits that distinguishes it from other *I. monticola* in a multivariate analysis (Arribas & Carranza 2004; Arribas et al. 2006). Highly variable nuclear markers as for instance introns or microsatellites may help to clarify definitively the status of these well diagnosable (morpho)subspecies.

b) Despite the mtDNA differences of the Béjar specimens (one or two nucleotides), their morphology is largely equivalent to *I. c. castiliana*. Lacking data from other Béjar populations and genetic nuclear markers, I assume that these morphological similarities reflect the presence of a very recent gene flow with other neighbouring populations. The Béjar populations are however outstanding by their female body elongation (up to near 1 cm larger), conserving proportionately shorter limbs, which can be a strategy to increase clutch size.

c) The Sierras de Avila populations are very similar; the closer among all the populations compared. One of them, the Sierra de Villafranca, is together with Béjar, the most connected sample, and it is, from a morphological point of view, at the root of the species expansion. Both also present the unique slightly variant haplotypes. All they should be considered as *I. c. castiliana* by their closer identity with Gredos.

Acknowledgements. Dr. Sergi Pla (Barcelona, Spain), Jesús García (Huesca, Spain) and Dr. Vicente M. Ortuño (Madrid, Spain) helped in some prospections. Dr. Salvador Carranza (Barcelona, Spain) corrected and improved parts of the manuscript. This study was financed completely by the author and therefore it did not cost any Euro to the public arks. Prospections of the small Sierras de Avila and Béjar populations were carried out under permissions nº 20051630007003 (2005), 20061630024599(2006), 2007167004130 (2007), 20081630020386 (2008), 20092390004760 (2009), issued by the competent organisms in charge of the wildlife protection (Junta de Castilla y León, Spain)

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Fig. 5. *Iberolacerta cyreni* NEOTYPE (here designed). MNCN n. 39934.

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Received: 29.VI.2010

Accepted: 15.X.2010

APPENDIX I

Neotype designation for *Iberolacerta cyreni* (Müller & Hellmich, 1937)

The type series of *Iberolacerta cyreni* (*Lacerta monticola cyreni* Müller & Hellmich, 1937) included 66 specimens (not only from Guadarrama, the species' type locality, but also some paratypes from Gredos) formerly deposited in the Zool. Staatssammlung (Herpet. Samml.) München. In fact, two syntype specimens, male and female, respectively numbered "ZSM (SLM) 2329 a" and "ZSM (SLM) 2329 b", were originally considered as types labelled "Guadarrama, Puerto de Navacerrada. W. Hellmich". (Müller & Hellmich, 1937).

Although it seems that all the original type series was destroyed during the Second World War (Franzen & Glaw, 2007), and due to the fact that in this type series there were included some Gredos specimens (today part of another subspecies; *I. c. castiliana*), and also that there was early confusion about the *I. monticola* type locality which lead to the description of a new taxon as *Lacerta estrellensis* Cyren, 1928 (Arribas 2008), I design a new type specimen (neotype) to fix unequivocally the type locality against any contingency (as could be the highly improbable apparition of any "surviving" original Gredos paratype).

I designate here as NEOTYPE for the species a specimen from the Museo Nacional de Ciencias Naturales (Madrid) (MNCN n. 39934) (Fig. 5).

A male labelled as follows:

Left hindleg: (white label, Typewriter letter) MNCN (anverse), 39934 (reverse).

Right hindleg: (white label, pencil handwritten) "Pto. de Cotos-Pto de Navacerrada. Srra. de Guadarrama () [blank inside parenthesis], 21-IV-84, 18,15 h. Sol. Pedriza en pinar con nieve. Ps=8.5 gr." (no collector's data).

Left foreleg: (White label, ink handwritten) Neotypus. O. Arribas designatio (anverse), "*Lacerta monticola cyreni* Müller & Hellmich, 1937" (= *Iberolacerta cyreni*) (reverse).

Right foreleg: (Red plastic label, Dymo® lettering) NEOTYPUS.

Neotype description (Fig. 5):

Biometry: Adult male with snout-vent length of 66.85 mm. Tail 126 mm (intact). Forelimb length 23.34 mm. Hindlimb length 34.52 mm. Pileus length 16.4 mm. Pileus width 8.2 mm. Parietal length 5.5 mm. Masseteric widest diameter 2.71 mm. Tympanic widest diameter 1.94 mm. Anal plate width 5.04 mm. Anal length 3.19 mm. FLL/SVL (relative forelimb length): 0.349. HLL/SVL (relative hindlimb length): 0.5163. PL/PW (pileus

shape): 2.003. DM/PaL (relative masseteric plate size): 0.491. DT/PaL (relative tympanic size): 0.352. AL/AW (anal plate surface): 0.6329. AS/SVL (relative anal plate size in respect to total legth): 5.998.

Scalation: Number of supraciliary granules: 9 (right) and 11 (left). Supralabials: 5 (both sides). Sublabials: 6 (right side) and 7 (left side). Submaxillars: 6 (both sides). Gularia: 25. Collaria: 9. Dorsalia: 53. Ventralia: 26. Femoral pores 19 (right) and 18 (left). Lamellae: 25. Circumanal Plates: 8. Rostral in full contact with frontonasal. Supranasal separated from first loreal. One postnasal (in both sides). First Postocular separated from Parietal plate. Alternate wide and narrow scale rings in the tail. Twenty-six scales across one of these rings.

Coloration: (in alcohol). Dorsal tract and pileus brown (probably also in life), densely spotted of medium-sized black spots that in the middle of the dorsum nearly form transverse bands and connect the two temporal bands. Temporal bands reticulated fairly dark (black-brown) with traces of clear ocelli (barely visible) inside, also connecting with more light reticulated with infratemporal band (barely discernible). No blue axillar ocelli. Traces of blue points in the outermost ventral scales. Only the outermost ventral scale ranges are clearly black spotted. Belly light bluish or white-bluish.

APPENDIX II

Barriers and high mountain passes (among parentheses) between the different *I. cyreni* populations. All these intermediate areas are at present apparently devoid of *I. cyreni*, thus constituting these OTUs discrete populations:

BÉJAR-GREDOS: no barrier (Puerto de Tornavacas, 1275m).

BÉJAR-VILLAFRANCA: Tormes River Valley (no pass).

GREDOS-VILLAFRANCA: Tormes River Valley (Collado de Cepegato, 1550m).

GREDOS-S^a AVILA [Serrota+Paramera]: Alberche River Valley (no passes).

SERROTA-PARAMERA: no barrier (Puerto de Menga, 1566m).

VILLAFRANCA-SERROTA: no barrier (Puerto de Chia, 1663m).

S^a AVILA (as a whole) – GUADARRAMA: no barrier (Puerto del Boquerón, 1315m).

GREDOS-GUADARRAMA: Alberche River Valley (no pass)

APPENDIX III

Table 1. ANOVA and descriptive statistics (mean, standard error, minimum and maximum) from morphometric, scalation and biometrical indexes of *I. cyreni* male samples. See Material and Methods text for abbreviations of characters and indexes used in the morphometric analysis.

MALES	GUADARR. (1) (n=25)	MIJARES (2) (n=6)	GREDOS (3) (n=23)	BEJAR (4) (n=11)	S ^a AVILA (5) (n=27)	F _{4,87}	p	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5
SVL	61.85±1.76 45.1-74.5	56.09±4.12 48.17-71.37	66.34±1.37 49.9-72.4	64.23±3.21 48.3-77.38	63.43±1.79 45.12-74.91	1.90	0.118272										
GrS r	10.4±0.37 6-13	10.83±0.79 9-14	11.26±0.55 7-18	11.36±0.85 6-15	11.22±0.37 8-16	0.67 1.29	0.616208 0.279781										
GrS l	10.52±0.36 7-15	11±0.85 9-15	10.43±0.46 7-16	11.63±0.66 8-15	11.44±2.15 8-17												
GUL	24.04±0.36 21-27	23.66±0.42 22-25	25.30±0.44 23-31	25.36±0.62 23-29	23.81±0.33 20-28	3.14	0.018460										
COLL	10.44±0.20 8-12	12.16±0.40 11-13	10.73±0.26 7-13	10.45±0.28 9-12	10.25±0.19 8-13			**	*			*	*	**			
DORS	49.64±0.55 45-56	52.5±1.28 50-57	51.21±0.70 46-57	55.18±1.22 50-64	52±0.57 46-57	4.03 6.15	0.004827 0.000209		**	**					**		*
VENT	24.6±0.2 23-26	25.66±0.33 25-27	26.08±0.22 24-29	25.9±0.36 24-28	26.14±0.22 24-29	8.28	0.000010		**	*	**						
FEM r	18.72±0.31 16-22	18.83±0.30 18-20	18.3±0.34 15-22	19.18±0.55 17-24	18.07±0.29 15-22	1.27	0.287378										
FEM l	18.56±0.28 16-21	18.5±0.34 18-20	18.13±0.36 14-22	18.72±0.70 16-24	18.07±0.26 16-22	0.58	0.678824										
LAM	25.2±0.33 22-29	26±0.96 23-29	25.13±0.43 21-29	26.27±0.4 24-29	24.85±0.36 21-30	1.40	0.241656										
CircA	8.2±0.20 6-12	6.83±0.30 6-8	6.63±0.21 5-9	7±0.26 6-9	7.4±0.17 6-9	8.85	0.000005	*	**	**	*					*	
R-I	1.88±0.06 1-2	2±0 2-2	1.82±0.10 0-2	1.90±0.09 1-2	1.88±0.06 1-2	0.31	0.869978										
Po-Pa	0.2±0.08 0-1	0±0 0-0	0.15±0.09 0-2	0.13±0.09 0-1	0.24±0.06 0-1	0.58	0.680982										
Sn-Lor	0±0 0-0	0.16±0.16 0-1	0±0 0-0	0.04±0.04 0-0.5	0.07±0.04 0-1	1.84	0.127894					*					
PV	1.12±0.17 0-3	1.33±0.42 0-3	0.43±0.12 0-2	0.72±0.19 0-2	0.85±0.07 0-1	4.07	0.004509	**	**		*						
FLL/SVL	36.24±0.003 30.81±39.91	34.54±0.004 32.83-35.66	35.46±0.006 29.22-42.37	35.03±0.004 32.77-37.01	34.55±0.003 31.95-38.94	2.52	0.046941			*							
HLL/SVL	51.46±0.004 46.39-56.34	50.27±0.004 48.49-51.20	50.96±0.005 43.93-55.19	50.62±0.004 49.01-53.15	50.87±0.003 47.89-54.59	0.69	0.602439										
PL/PW	216.03±0.02 195.45-231.34	214.28±0.04 199.14-228.20	211.42±0.04 120.16-226.31	225.32±0.11 197.48-338.30	214.47±0.02 191.53-235.88												
DM/PaL	38.33±0.009 30.90-50.36	37.88±0.02 30.97-46.21	42.12±0.01 32.30-53.84	39.97±0.03 16.57-55.86	44.11±0.01 29.49-53.78	1.06 3.24	0.380913 0.015768										
DT/PaL	35.25±0.01 21.81-48.83	33.22±0.02 22.52-39.36	36.09±0.01 27.5-49.18	37.97±0.02 29.1-56.1	35.37±0.009 28.61-47.2	0.77	0.550028										
AL/AW	55.22±0.01 45.83-66.66	62.54±0.03 55.01-71.61	59.59±0.01 40.81-81.48	58.33±0.02 45.23-68.55	59.41±0.009 49.71-71.54												
AS/SVL	566.31±0.11 436.00-689.68	509.68±0.28 449.53-639.73	546.24±0.11 449.13-662.44	522.42±0.08 474.43-566.42	563.25±0.08 473.26-639.11	2.36 2.74	0.059363 0.033479										

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Table 3. Analysis of Similarity (ANOSIM) results (with 1000 randomizations). Males above diagonal and females below. The number is the test probability results among each two concerned populations (significant results in bold).

FEM \ MAL	BÉJAR	GREDOS	GUAD.	MIJARES	SERR-PAR	VILLAFR.
BÉJAR	—	0.058	0.001	0.644	0.138	0.064
GREDOS	0.021	—	0.001	0.691	0.867	0.198
GUAD.	0.001	0.001	—	0.097	0.128	0.001
MIJARES	0.53	0.039	0.327	—	0.224	0.513
SERR-PAR	0.127	0.025	0.001	0.217	—	0.86

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Zeitschrift/Journal: [Bonn zoological Bulletin - früher Bonner Zoologische Beiträge.](#)

Jahr/Year: 2010

Band/Volume: [57](#)

Autor(en)/Author(s): Arribas Oscar J.

Artikel/Article: [Intraspecific variability of the Carpetane Lizard \(*Iberolacerta cyreni* \[Müller & Hellmich, 1937\]\) \(Squamata: Lacertidae\), with special reference to the unstudied peripheral populations from the Sierras de Avila \(Paramera, Serrota and Villafranca\) 197-210](#)