

Karyological and osteological data supporting the specific status of *Iberolacerta (cyreni) martinezricai* (Arribas, 1996)

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Abstract. *Iberolacerta (cyreni) martinezricai* has a karyotype of $2n = 36$, all acrocentric macrochromosomes. Nucleolar Organizer Regions (NORs) are interstitially situated on a medium small (MS-type) chromosome, a derived character shared with *I. cyreni*, which differs in details of W-sex chromosome: W is heteromorphic and heterochromatic in *I. cyreni*, while it is homomorphic and euchromatic in *I. (c.) martinezricai*. Osteologically, it is characterized by the presence of a triangular-shaped expansion of the squamosal towards the supratemporal fenestra, and by the presence of seven (instead of six) posterior short ribbed presacral vertebrae. These odd characters probably became fixed in this relic taxon by inbreeding. Its differences and affinities are discussed, proposing a specific status for this taxon.

Introduction

The Peña de Francia lizard was first regarded as coming from the Cordillera Cantabrica by Klemmer (1957) and was later correctly situated in the Spanish Sistema Central by Martinez-Rica (1979) (see Arribas, 1996; 1999a for more details). The last population was described as *Lacerta cyreni martinezricai* owing to its *cyreni*-like overall habit and in spite of remarkable differences from other *cyreni* populations in scalation (lack of rostral-internasal scale contact) and coloration (blue ocelli in the shoulder) (Arribas, 1996). Although the overall aspect of this lacertid is different from that of the other populations from Sistema Central (Spain), its validity has been put in doubt (see for instance Perez-Mellado, 1997; Barbadillo et al., 1998). A possible separation of *I. (c.) martinezricai* from *I. cyreni* was suggested by mtDNA analysis (Mayer and Arribas, 2003), which grouped

it in a distinctive and well differentiated branch close to *I. monticola*. In this paper we provide new evidence from different data-sets (karyological and osteological) supporting the separation of *I. (c.) martinezricai* from *I. cyreni*. For practical reasons, along this paper we provisionally call this lizard with its original denomination, *I. (c.) martinezricai*, before to propose its elevation to species status.

Material and methods

Specimens studied

For chromosome work, we examined two young females of *I. (c.) martinezricai* captured in Puerto del Portillo, near La Alberca (Salamanca, Spain). For osteological work, we used the previous two specimens plus another young female from the same locality and a juvenile male from Peña de Francia (Salamanca, Spain). Currently, these specimens are deposited in the personal collection of an author (OJA).

Chromosomes

The specimens were injected with a dose (0.1 ml/10 g body weight) of a colchicine solution (0.5 mg/ml). One hour later, they were deeply anaesthetized. Chromosomes were obtained from the intestine, spleen, lungs and ovaries using the scraping and air drying method described by Odierna et al. (1994), employing a 0.7% sodium citrate solution as hypotonic solution. Conventional chromosome staining were performed using a 5% Giemsa solution at pH 7. The nucleolus organizer regions (NORs) were detected using the Ag-NOR banding method reported by Howell and Black (1980). C-banding stainings were performed according to the Sumner's (1972) method, but incubating the slides for 5 min at 45°C in Ba(OH)₂. Sequential staining of Cb banding+CMA₃+DAPI was performed as reported in Odierna et al. (1999).

Osteology

The four specimens considered in this study were cleared and stained. The clearing has been performed by means of KOH at 1% in previously fixed and alcohol preserved specimens. Bones were stained with alizarine red and permanently conserved in glycerine (Taylor, 1967) (nomenclature is as in Arribas, 1998).

Results

Chromosomes

The two examined females of *I. (c.) martinezricai* displayed a chromosome complement of $2n = 36$ unarmed elements gradually decreasing in length. Ag-NOR banding localized

NORs interstitially on a medium-large uniarmed pair (tentatively 11th chromosome pair) (fig. 1A). Heterochromatin was distributed in the pericentromeric regions of near all the chromosome pairs (fig. 1B) and resulted CMA₃ negative (fig. 1C) and DAPI positive (fig. 1D).

Osteology

All the studied specimens bore 9 premaxillary teeth and a slender processus nasalis, 15 to 17 maxillary teeth-positions, and from 19 to 21 dentary ones, near half of them more or less bicuspid, the remaining monocuspid. Maxillo-jugal suture (margo ocularis) smooth, not stepped. Postorbital and postfrontal separated and subequal in length. Anteromedial process of postorbital and anterodistal process of postfrontal present. Squamosal in contact with (or juxtaposed to) postocular along near a third of the length of the latter. In three of the four studied specimens this squamosal had an unusual triangular-shaped medial process just behind the posterior tip of postocular and postfrontal bones, with a border perpendicular or even concave in front of this tip (fig. 3A). There are no ribs associated to the third vertebra. Sternal-xiphisternal costal formula (3 + 2) and sternal fontanelle oval-shaped. Claviculae closed (emarginated) (Fig. 3B). Interclavicular cross shaped (anterior/posterior branches relation close to 0.39). The three studied females presented 28 presacral vertebrae (26 in the male, confirmed by radiography of other specimens), the last seven associated to short ribs. The fifth pre-autotomic vertebrae was of A-type after Arnold (1973).

Discussion

Chromosomes

Iberolacerta (c.) martinezricai and all the previously studied *Iberolacerta* taxa (Odierna et al., 1996) display a basic karyotype of $2n = 36$ uniarmed macrochromosomes or a one derived from it by a number of centric fusions (see fig. 2). This chromosome formula differs from the standard lacertid karyotype in that it lacks the pair of microchromosomes, probably due to their tandem translocation on macrochromosomes (Olmo et al., 1991, 1993; Odierna et al., 1996).

The loss of microchromosomes is an autapomorphy of *Iberolacerta* among all the Eurasian Lacertid Radiation group (sensu Mayer and Benyr, 1994) and must have occurred before the early separation of the Ibero-Pyrenean species from their more closer relative, i.e. the Alpine-Dinaric *I. horvathi* (Arribas, 1999b; Harris et al., 1998), which also possesses a karyotype of $2n = 36$ uniarmed macrochromosomes (De Luca and Dulic, 1988; Capula et al., 1989).

However, the loss of microchromosomes did not occur exclusively in the *Iberolacerta* clade, as it occurred independently in *Zootoca vivipara* (Chevalier et al., 1979; Odierna

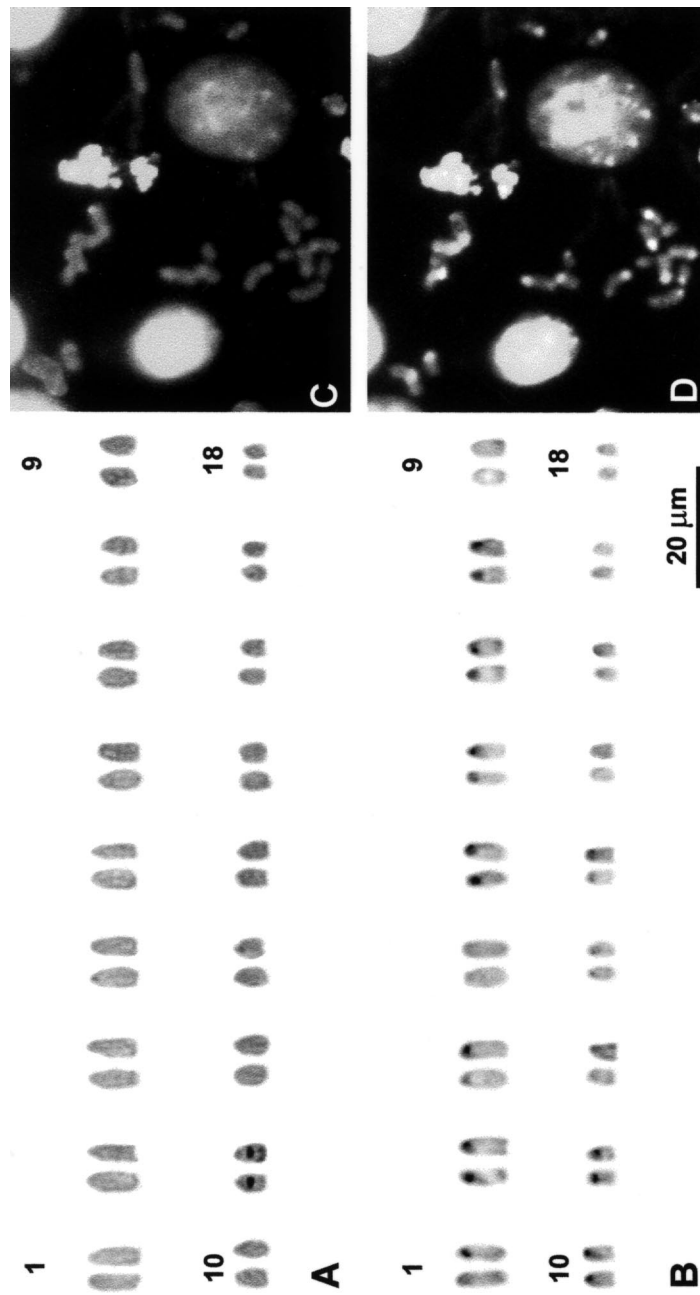


Figure 1. Ag-NOR banded (A) and C-banded (B) karyotypes and a metaphase plate, sequentially stained by C-banding CMA3 (C) + DAPI (D), of a female of *I. (c.) martinezricai*.

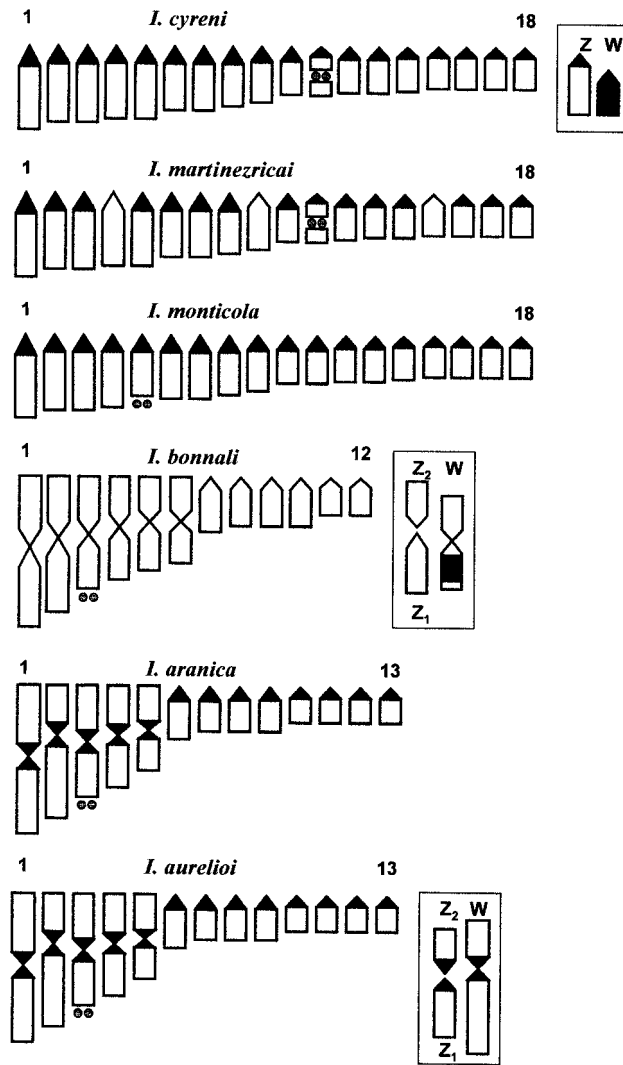


Figure 2. Idiogram summarizing the karyological characters of *I. (c.) martinezricai* and of the other *Iberolacerta* taxa (modified from Odierna et al., 1996). Heterochromatin = black areas; NORs = gray circles. The boxes include the sex chromosomes.

et al., 1998), which anyway belongs to a different and distant clade (Mayer and Benyr, 1994; Harris et al., 1998).

Ag-NOR staining indicates that *I. (c.) martinezricai* is more related to *I. cyreni* than to the other *Iberolacerta*, as was previously stated by morphological grounds (Arribas, 1996). *Iberolacerta (c.) martinezricai* and *I. cyreni* share NORs interstitially localized on a medium chromosome pair (M-type after Olmo et al., 1993), while in *I. monticola* and in

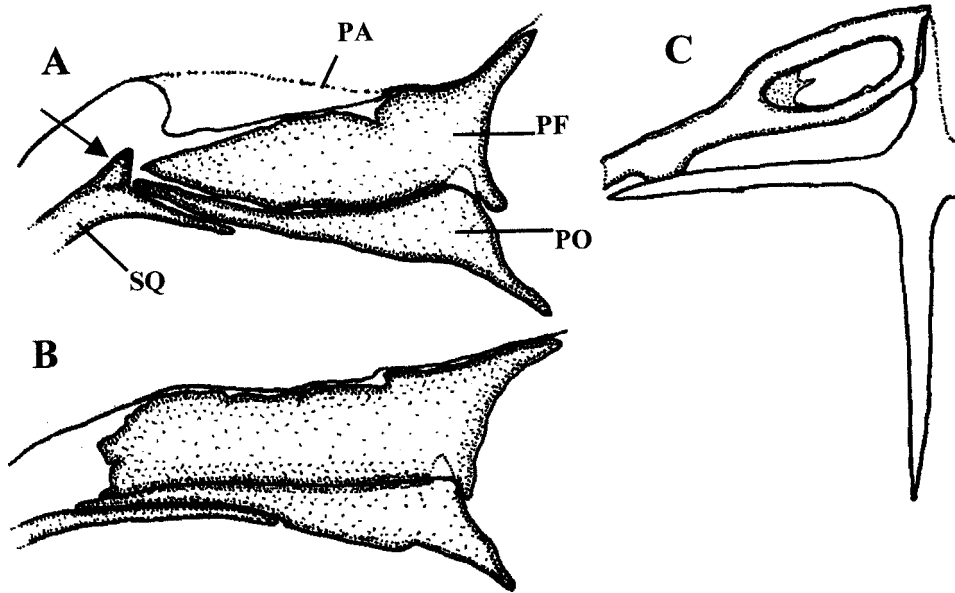


Figure 3. Representation of several skull and thoracic girdle bones. **A)** Postfrontal (PF), Postorbital (PO) and Squamosal (SQ) from *I. (c.) martinezricai* from Puerto del Portillo (1000 m a.s.l., Salamanca, Spain). Note (arrow) the triangular shaped process from Squamosal (see arrow), in part obliterating the Supratemporal Fenestra (PA: Parietal). **B)** The same bones from *I. cyreni* from Circo de Gredos (2200 m a.s.l., Avila, Spain) for comparison. **C)** Clavicle and interclavicle (only the right half represented) from the same specimen of *I. (c.) martinezricai*.

the three Pyrenean *Iberolacerta* species (*I. aranica*, *I. aurelioi* and *I. bonnali*), NORs are localized peritelomerically on a large chromosome pair (L-type after Olmo et al., 1993) (fig. 2). The last localization of NORs is plesiomorphic, since also *I. horvathi* displays NOR loci on the telomeres of a L-type chromosome pair. It is noteworthy to stress that a L-type localization of NORs is very widespread among lacertids (Olmo et al., 1993, 2002). In contrast, the interstitial NOR localisation on M-type chromosome, is exclusive (a synapomorphy) for *I. (c.) martinezricai* and *I. cyreni*, apart from two populations of *Timon lepidus ibericus*, showing a NOR polymorphism with a single L-type NOR pair in some specimens, and with a single M-type NOR pair in other specimens and with two NOR pairs (L and M-types) in other specimens (Mateo et al., 1999).

A very similar heterochromatin pattern has been displayed by both *I. monticola* and *I. cyreni* (Odierna et al., 1996). However, even if with slight interspecific difference in the distributional pattern, a DAPI positive, pericentromeric heterochromatin is quite common among lacertids (Olmo et al., 1993; Odierna et al., 1996, 2001 and unpublished data) and its presence might be related to a putative role in structuring chromosome centromeres (see Capriglione et al., 1989 and 1994).

Concerning the sex chromosomes, our previous studies have shown that all the lacertid species possess a female (ZW or Z_1Z_2W) sex chromosome system, even if in some instances sex chromosomes are not obvious and result evidents are only by means of adequate methods of investigation (Olmo et al., 1993; Odierna et al., 1993). The morphology of the W chromosome displays an interspecific variability resulting heteromorphic or homomorphic to Z chromosome, and could also be completely heterochromatic or euhromatic. Among *Iberolacerta*, in *I. (c.) martinezricai*, *I. monticola* and *I. aranica*, both conventional and C-banding staining methods were inadequate to evidence differentiated sex chromosomes. In contrast, in *I. cyreni* C-banding staining revealed Z and W chromosomes: W was homomorphic to Z and differed in being nearly all heterochromatic (Odierna et al., 1996) (fig. 2).

Osteology

Iberolacerta (c.) martinezricai presents a unique character (autapomorphy) among the other Iberian species studied, i.e. the frequent presence (three of the four studied specimens) of a triangular-shaped medial process in the squamosal.

The presence of seven vertebrae with short ribs may be occasionally found also among specimens of other taxa which usually bear six ribs. However, in small populations these odd characters frequently become fixed, and therefore are found in most or in all the specimens. This could be the case of the relictual *I. (c.) martinezricai*. A similar case occurs in Bigorre specimens of *I. bonnali* which have fixed the number of five instead of six short ribs (Arribas, 1998).

Iberolacerta (c.) martinezricai shares with *I. monticola* (especially with the Cordillera Cantabrica populations) the processus nasalis slender (more arrow shaped in *I. cyreni*), whereas it shares with *I. cyreni* the number of premaxillary teeth (nine, frequently seven in *I. monticola*), and the presence of closed (emarginated) clavicles, dominant character in *I. cyreni*, and less evenly represented in *I. monticola*. However, apart from the above mentioned odd characters which appear scattered as individual variation among species but fixed and dominant in very small populations, as in *I. (c.) martinezricai* (Arribas, 1998), in general there are no striking qualitative osteological differences among closely related species of the *I. monticola* group.

Taxonomic conclusions

A clear and sharp differentiation of *I. (c.) martinezricai* from *I. cyreni* and *I. monticola* is apparent. However, their relationships are not clear and it is not easy to interpret the contrasting results from the different approaches. Morphological data (Arribas, 1999b and unpublished) point to a relationship between *I. (c.) martinezricai* and *I. monticola*, whereas chromosomal data indicate that *I. (c.) martinezricai* is related to *I. cyreni*. Alternatively, mtDNA analysis (Mayer and Arribas, 2003) mirrors morphological data, while preliminary results of a microsatellite analysis (Fulgione et al., pers. comm.) supports chromosomal

evidence. Osteological data are unable to show affinities to one or another species. In summary, mitochondrial (only maternal inheritance) and morphological data suggest a closer relationship of *I. (c.) martinezricai* to *I. monticola* than to *I. cyreni*, whereas karyological analysis suggests a closer relationship of *I. (c.) martinezricai* to *I. cyreni* than to *I. monticola*. Though relationships among them remain unsolved, the deeply rooted differences of *I. (c.) martinezricai* in respect to *I. cyreni* and *I. monticola* permit us to suggest it is treated as a separate species: *Iberolacerta martinezricai* **stat. nov.**

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