A new mountain lizard from Montes de León (NW Iberian Peninsula): *Iberolacerta monticola astur* ssp. nov. (Squamata: Lacertidae)

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Abstract

*Iberolacerta* populations from the Northern Montes de León (NML) were studied by means of external morphology (sca-lation and biometry), osteology and genetics (mtDNA and microsatellites), searching for their homogeneity (“intrazonal analysis”) and, once verified, comparing them with *Iberolacerta monticola* s. str. (from Central Cantabrian Mountains) and *I. galani* (from Southern Montes de León) (“extrazonal analysis”) from neighboring areas.

Our “intrazonal analysis” revealed discordances between the different approaches, especially the patterns of variation of nuclear microsatellites (congruent with external morphology) and mtDNA, namely a very low nuclear differentiation between relatively highly differentiated mtDNA lineages. The morphological approach was unable to discriminate any of the populations as significantly different from the others in the NML. Mitochondrial DNA revealed a haplotype lineage closely related to *I. galani* (MNL-II in our text) in some specimens of Sierra de Villabandin and Suspirón, but these populations are morphologically indistinguishable from the main part of the other populations that belong to lineage NML-I, phylogenetically closer to *I. monticola*. After a separation from *I. monticola* ca. 1.8 Mya, the populations in this geographic region must have suffered at least two different waves of gene flow from *I. galani*, the second one not much later than 0.5 Mya. Microsatellite results indicate that all the NML populations are genetically similar in terms of their nuclear genomes, independently of their mitochondrial differentiation (NML-I vs. NML-II haplotype groups). Since all the morphological and microsatellite evidences point towards the fact that, independently of the mitochondrial haplotypes that they bear (NML-I or NML-II), there is only one taxon in the area, we describe it as: *Iberolacerta monticola astur* ssp. nov.

Concerning the relationships of *I. m. astur* ssp. nov. with *I. monticola* s. str. and *I. galani* (“extrazonal analysis”), in the female analyses the new taxon centroid is closer to *I. monticola* s. str. than to *I. galani* (more similarity with *I. monticola* s. str.), whereas in the male analyses the relationship is just the contrary (closer to *I. galani*, paralleling the direction of the hypothesized past hybridization). Moreover, in both sexes’ ANOVA, *I. m. astur* ssp. nov. results more similar (less P<0.05 differences) to *I. galani* than to *I. monticola* s. str. Osteologically, *I. m. astur* ssp. nov. is slightly more similar to *I. monticola* s. str. than to *I. galani*, especially in the squamosal bone, which is regularly arched (primitive shape). Genetically, as indicated above, the NML populations can be subdivided in two groups according to their mitochondrial DNA, namely NML-I (bearing clearly differentiated haplotypes, phylogenetically closer to I. monticola) and NML-II (whose haplotypes could have been mistaken for those of an *I. galani* population). This mitochondrial subdivision has at most a subtle nuclear correlate, however. According to the nuclear microsatellite markers, all the NML populations belong to a single group (*I. m. astur* ssp. nov.), which would be more similar to *I. galani* than to *I. monticola*, with NML-II populations lying closer to *I. galani* than those from the NML-I group and, correspondingly, more distant from *I. monticola*. The discordant phylogenetic signal of mitochondrial and nuclear markers is discussed in terms of past introgression events and sex-biases in phylopatry and dispersion in these species.

*Iberolacerta monticola astur* ssp. nov., inhabits the Northern Montes de León (Sierra de Gistreo sensu latissimo): Gistredo, Catoute, Tambarón, Nevadín, Villabandin (or Macizo del Alto de la Cañada), Arceos del Agua (or Fernán Pérez), Tiendas and Suspirón, mainly in quartzite and slate rock substrates. Its current distribution, cornered in the NW of the Northern part of the Montes de León, suggests a possible competitive exclusion between this taxon and *I. galani*, as the *galani* haplotypes (NML-II) appear cornered in the most harsh and continental areas, speaking also about a, even in the past, very limited presence of this species in the area that probably was soon absorbed by *I. m. astur* ssp. nov. (with NML-
I haplotypes). Variation in watershed limits (especially with *I. monticola* s. str. in the North) and Pleistocene climatic oscillations (with *I. galani* in the South) probably played a crucial role in isolation of the different *Iberolacerta* colonization waves in this zone. These changes in the boundaries among watersheds limited the contact between the NML and the main Cantabrian Mountains, restricting to narrow points (different along time) the contact between the two ranges, and thus, the areas for possible contact between *I. m. astur* ssp. nov. and *I. monticola* s. str. (see Fig. 1B). The origin of this taxon dates back to the end of Pliocene or Lower Pleistocene (around 1.8 Mya), according to mtDNA divergence. On the other side, climatic oscillations allowed expansion and contact with the more continental harsh climate-dwelling *I. galani*.

**Key words:** Reptiles, *Iberolacerta*, *Iberolacerta monticola*, *Iberolacerta galani*, *Iberolacerta monticola astur* ssp. nov., morphology, osteology, mtDNA, microsatellites, speciation, introgression.

**Resumen**

Se han estudiado las poblaciones de *Iberolacerta* del Norte de los Montes de León (NML) mediante morfología externa (folidosis y biometría), osteología y genética (ADNmt y microsatélites), probando su homogeneidad (“análisis intrazonal”) y una vez demostrada, comparándolas con *Iberolacerta monticola* s. str. (de la Cordillera Cantábrica central) e *I. galani* (del Sur de los Montes de León) (“análisis extrazonal”) de áreas cercanas.

Nuestro “análisis intrazonal” mostró diferencias entre los distintos enfoques, especialmente entre los patrones de variación de los microsatélites nucleares (congruentes con la morfología externa) y los del ADNmt; a saber: una diferenciación nuclear muy baja entre linajes muy diferenciados en su ADNmt. El estudio morfológico fue incapaz de distinguir ninguna de las poblaciones de NML como significativamente diferente de las otras. El ADN mitocondrial reveló la existencia de un linaje haplotípico cerVECamente emparentado a *I. galani* (NML-II en el texto) en algunos especies de la Sierra de Villabandín y el Suspirón, pero estas poblaciones son morfológicamente indistinguibles de la mayoría de las otras poblaciones que pertenecen al linaje NML-I, filogenéticamente más cercano a *I. monticola*. Tras su separación de *I. monticola* hace cerca de 1.8 millones de años, las poblaciones de esta región (NML) deben haber sufrido al menos dos oleadas diferentes de flujo genético procedente de *I. galani*, la segunda no muy posterior a hace 0.5 millones de años. El resultado de los microsatélites indica que todas las poblaciones de NML son genéticamente similares en sus genomas nucleares, independientemente de sus diferencias mitocondriales (haplotipos de los grupos NML-I y NML-II). Así pues, las evidencias morfológicas y de microsatélites indican que, independientemente de los haplotipos mitocondriales que portan (NML-I o NML-II), sólo existe un taxón en el área que describimos como *Iberolacerta monticola astur* ssp. nov.

En referencia a las relaciones de *I. m. astur* ssp. nov. con *I. monticola* s. str. e *I. galani* (“análisis extrazonal”), en el análisis morfológico de las hembras el centroide del nuevo taxón es más cercano a *I. monticola* s. str. (portadora de haplotipos claramente diferenciados, filogenéticamente cercanos a *I. monticola* s. str.) mientras que en el análisis de los machos la relación es justa la contraria (más cercano a *I. galani*, en paralelo con la dirección de la hipotética hibridación en el pasado). Además, en el ANOVA de ambos sexos, *I. m. astur* ssp. nov. es más similar (menos diferencias *P<0.05*) a *I. galani* que a *I. monticola* s. str. Osteológicamente, *I. m. astur* ssp. nov. es ligeramente más similar a *I. monticola* s. str. que a *I. galani*, especialmente en la forma del hueso escamoso, que es uniformemente arqueado (forma primitiva). Genéticamente, tal como se ha indicado arriba, las poblaciones de NML pueden ser divididas en dos grupos de acuerdo con su ADN mitocondrial: NML-I (portadora de haplotipos claramente diferenciados, filogenéticamente cercanos a *I. monticola*) y NML-II (cuyos haplotipos podrían confundirse con los de una población de *I. galani*). Esta subdivisión mitocondrial tiene, no obstante, una sutil correlación nuclear. De acuerdo con los marcadores nucleares (microsatélites), todas las poblaciones de NML pertenecen a un único grupo (*I. m. astur* ssp. nov.), que sería algo más similar a *I. galani* que a *I. monticola*, con los ejemplares que portan haplotipos NML-II algo más cercanos a *I. galani* que los que portan haplotipos NML-I, y por lo tanto algo más lejanos a *I. monticola*. La señal filogenética discordante entre los marcadores nucleares y mitocondriales es discutida en términos de una pasada introgresión y del sesgo por sexos en la filopatría y dispersión en estas especies.

*Iberolacerta monticola astur* ssp. nov. habita el Norte de los Montes de León (Sierra Gístreo sensu latissimo): Gistredo, Catoute, Tambarón, Nevadín, Villabandín (o Macizo del Alto de la Cañada), Arcos del Água (o Fernán Pérez), Tiendas y Suspirón; principalmente sobre sustratos de cuarcitas y pizarras. Su distribución actual, arrinconada en el NW de la parte Norte de los Montes de León, sugiere una posible exclusión competitiva entre este taxón y *I. galani*, ya que las poblaciones con haplotipos *galani* (NML-II) aparecen arrinconadas en las partes climáticamente más duras y continentales, abogando por, incluso en el pasado, una presencia muy limitada de esta especie en el área que probablemente fue pronto absorbida por *I. m. astur* ssp. nov. (con haplotipos NML-I). La variación en las divisorias de aguas (especialmente con *I. monticola* s. str. al Norte) y las oscilaciones climáticas durante el Pleistoceno (con *I. galani* al Sur) probablemente jugaron un papel crucial en el aislamiento entre las diferentes oleadas de colonización de *Iberolacerta* en esta zona. Estos cambios en la divisoria de aguas limitaron el contacto entre NML y la parte principal de la Cordillera Cantábrica, restringiendo a puntos estrechos (diferentes a lo largo del tiempo) en contacto entre ambas áreas, y por tanto, las áreas para un posible contacto entre *I. m. astur* ssp. nov. e *I. monticola* s. str. (ver Fig. 1B). El origen del
new taxon remonta al final del Plioceno o Pleistoceno Inferior (hace alrededor de 1.8 millones de años), de acuerdo con su divergencia en el ADNmt. Por otro lado, las oscilaciones climáticas propiciaron la expansión y contactos con la habitante de zonas climáticamente más duras y continentales, *I. galani*.

**Palabras clave:** Reptiles, *Iberolacerta, Iberolacerta monticola, Iberolacerta galani, Iberolacerta monticola astur ssp. nov.** morfología, osteología, ADNmt, microsatélites, especiación, introgresión

**Introduction**

Until very recently (Salvador 1974, 1984; Arnold & Burton 1987; Barbadillo 1987; Pérez-Mellado 1997, 1998, 2002) all mountain lizard populations of the genus *Iberolacerta* Arribas, 1997 were considered to belong to *Iberolacerta monticola* (Boulenger, 1905). Thorough investigations in the last 20 years including advanced morphological and osteological studies (Arribas 1996 and 1998), karyotypes (Odierna et al. 1996; Arribas et al. 2006), allozyme-electrophoresis studies (Mayer & Arribas 1996; Almeida et al. 2002), and phylogenetic analyses including DNA sequences (Mayer & Arribas 2003; Crochet et al. 2004; Carranza et al. 2004; Arribas et al. 2006; Galán et al. 2007) indicated that *I. monticola* was, in fact, a species complex. As a result of all these analyses *Iberolacerta cyreni* (Müller & Hellmich, 1937) and *I. bonnali* (Lantz, 1927) were upgraded to the species level (Arribas 1993a, 1996; Perez-Mellado et al. 1993), and four new species were described: *I. aranica* (Arribas, 1993), *I. aurelloii* (Arribas, 1994), *I. martinezricai* (Arribas, 1996), and *I. galani* Arribas, Carranza & Odierna, 2006 (Arribas 1993b, 1994, 1996; Mayer & Arribas 1996; Arribas & Odierna 2004; Arribas & Odierna 2005; Arribas et al. 2006). These six species and *I. horvathi* (Mehely, 1904), from the Western Alps and northern Dinaric Chains (Arribas 1997, 1999), constitute the genus *Iberolacerta*.

The most widely distributed species is *Iberolacerta monticola*. During most of the XXth century, this taxon was considered a strictly mountain species, distributed across the main Cantabrian Mountains, from Picos de Europa (Asturias) to Os Ancares (Lugo), with an isolated population in Serra da Estrela (Portugal). As a result of surveys carried out in the 1980’s, the range of *I. monticola* was extended to other areas, such as inland and Western Galicia (NW Spain), and low altitude sites outside the main mountain range (Galán 1982; Elvira & Vigal 1982; Curt & Galán 1982; Bas 1983). More recently, its area was extended to the east into Santander and Northern Palencia provinces (Fuentes Carrionas, Peña Prieta Massif) (Arribas 2002) (see Fig. 1A). The presence of *Iberolacerta* in the southern part of the Montes de León was first noted by Elvira & Vigal (1982), in the Sierra de La Cabrera, and then in this same area and Sanabria by others (Brown & Pérez-Mellado 1993; Arribas 1996; Carranza et al. 2004; Arribas et al. 2006). The morphological and molecular study of lizards from the southern part of the Montes de León led to the description of a new species, *Iberolacerta galani* (Arribas et al. 2006), distributed across the Sierras de Peña Trevínca, Segundera, Cabrera Baja, Teleno, and the high Sanabria plateaus (see insert in Fig.1A). However, the presence of *Iberolacerta* in the northern part of the Montes de León (considered Pre-Cantabrian Mountains; NML hereinafter) was known only by a relatively old reference from El Tamborón (Salvador 1984). In 2006, the first author (OA) confirmed the existence of this population of *I. monticola*, when specimens very near to El Tamborón (in the Molar de Montrando mountain) were found.

A genetic analysis of *I. monticola* populations across its entire distribution range, including both mitochondrial and nuclear markers, has been carried out by Remón (2011) and Remón et al. (2013). As already found by previous authors, there is no conspicuous genetic differentiation between the nominate subspecies from the Serra da Estrela and *I. m. cantabrica* (Mertens, 1929) from Galicia and the Cantabrian Mountains. In general, *I. monticola* shows a low level of intraspecific genetic variability (Mayer & Arribas 1996; Almeida et al. 2002; Mayer & Arribas 2003; Crochet et al. 2004; Carranza et al. 2004; Arribas et al. 2006; Galán et al. 2007). However, according to Remón (2011) and Remón et al. (2013) (also Carranza, unpublished), the population from NML (“Sierra de Gistredo” sensu lato), which had never been analyzed before, is well differentiated from all the remaining populations of *I. monticola* and also from its related species *I. galani* and *I. martinezricai* (Fig.1A). This population from NML (“Gistredo”) apparently differs less from *I. monticola* (time to the most recent common ancestor estimated at 1.8 Ma, according to Remón et al. 2013) than from *I. galani* and *I. martinezricai* (2.4 to 2.8 Ma respectively; Remón et al. 2013).

In the present manuscript, we analyze morphologically the populations from the NML and combine this evidence with new and previously reported results from molecular markers (Remón 2011; Remón et al. 2013), so that we finally revise the taxonomy of *I. monticola* and describe a new subspecies from this area.
FIGURE 1. A) General situation and localities of *Iberolacerta monticola* in the study area. *Iberolacerta monticola* s. str. occupies the main axis of the Cantabrian Range, north to the Luna and Sil rivers (inverted triangles), and *I. m. astur* ssp. nov. (round symbols) the Northern Montes de León, south to the abovementioned rivers. Localities of *I. m. astur* ssp. nov. are: 1.—Pico Nevadín and Braña de Peña Vendimia. 2.—La Poza del Puerto, Puerto del Portillín (=Alto de Vivero). 3.—Vivero. 4.—El Molar de Montrando, El Tambarón. 5.—Braña La Forcada, Pico de la Robeza, Peña Carnicera, Cerneya and Peñona de Brañalibrán. 6.—Peña de Valdiglesia. 7.—Alto de los Grillos. 8.—Catoute. 9.—Collado de Ocidiello and Pico Arcos del Agua (=Fernán Pérez). 10.—Sierra de las Tiendas. 11.—El Suspirón and Mortera de la Vieja. 12.—Los Bayos. 13.—Valdeloso Valley. 14.—Villabandin (Sierra de Villabandin, Southern Slopes) and 15.—Riolago de Babia (Sierra de Villabandin, northern slopes).

B) Watershed evolution along the End-Miocene to Plio-Pleistocene transition (left) (datations are uncertain; García de Celis 1997; Alfonso Gómez 2003), to be compared with the current situation (right) (dashed, fossil river paleocourses). A, B and C are the watershed divisory (suitable corridor for lizards) along successive moments of the capture of part of the Luna river watershed by the Sil river, from where possibly the ancestors of *I. m. astur* ssp. nov. crossed to our study area. A.—A wide divisory pass at 1600 m (end Miocene?), from Cueto del Oso (1904 m) to Cuerno peak (1932 m). B.—Posteriorly divisory was at 1450–1500 m (Pliocene?), and finally C.—the current situation with contact area situated in Puente de las Palomas (1220 m), too low and dry, and nowadays devoid of *Iberolacerta*.

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Material and methods

Morphology. Samples. A total of 105 male specimens and 113 female specimens of *Iberolacerta* with a snout-vent length greater than 50 mm were included in the morphological analyses: 55 males and 38 females of *I. monticola s. str.* ("cantabrica"); 26 males and 31 females of *I. galani*; and 24 males and 44 females of the new undescribed taxon from the NLM. All this study material is from Oscar Arribas’ (OA) scientific collection and database except for types deposited in the Museo Nacional de Ciencias Naturales (MNCN) (Madrid, Spain) and Naturhistorisches Museum Wien (Wien, Austria).

Characters studied. Biometric characters: The following linear measurements were taken with a digital caliper to the nearest 0.01 mm: Snout-vent length (SVL), measured from tip of the snout to the vent slit; Forelimb length (FLL), from the insertion of the limb up to the tip of the longest toe; Hindlimb length (HLL), from the insertion of the limb up to the fourth (the longest) toe; Pileus length (PL), from the tip of the snout up to the postriormost points of the parietal plates; Pileus width (PW), measured at its widest part (temporal area); Parietal length (PaL), in sagittal direction; Masseteric scale diameter (DM), measured at its largest diameter; Tymanic scale diameter (DT), measured at its largest diameter; Anal plate width (AW), measured at its widest part; and Anal plate length (AL), length of the plate in sagittal direction. These measurements were transformed to the following more informative and not dimensional-depending ratios: FLL/SVL (relative forelimb length; "FLL index"); HLL/SVL (relative hind limb 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 (√(AL*AW)*100/SVL, relative anal plate size with respect to the total length, "Anal size index") (see Arribas 1996, 2001). The results of the linear measurements and indexes yielded largely similar results. In addition, we run analyses with transformed (log [x+1] for measurement and scalation characters, and arc sine [SqRoot] for indexes) and untransformed data, and results were equivalent. All ratios were given multiplied by 100 to avoid excessive decimal scores.

Scalation characters: Supraciliar Granula (GrS) for the right and left sides, counted between the supracoaculilar and supraciliar scales; Gularia (GUL), from the submaxillary symphysis up to the collar scales in straight line; Collaria (COLL), transverse collar scales bigger (and distinguishable) from the gular scales; Dorsalia (DORS), counted from one side to the other side of the ventral scales across the body at the height reached by the tip of the forelimb; Ventralia (VENT), from the first scale larger than the small subcollars along the body just before the femoral and preanal area; Femoralia right (FEMr) and left (FEMl), number of scales with developed femoral pores; 4th. Digit Lamellae (LAM), from the insertion of the longest toe of the posterior limb up to the subungual pad included; and Circumanalia (CIRCA), number of large scales that surround the preanal plate. The full presence (2), contact at one point (1) or absence (0) of contact between Rostral-Internasal (R-I), Supranasal-first Loreal contact (Sn-Lor), and Postocular-Parietal contact (Po-Pa) was also studied.

Pattern and coloration: The pairs of ventral plates ranges (symmetric) with black dots were recorded (PV), as well as the number of blue ocelli on the shoulders (BO). Coloration in life for the description was standardized with a color code (Kornerup & Wanscher 1967), and given with their official name, Methuen codification among parentheses, and their Munsell Notation equivalent among brackets [Hue_Value/Chroma]. The ratio of green and black scales in the new taxon (Salientes sample) was estimated for a ring of dorsal scales in the middle of the body. Only full adult males were considered (SVL > 68 mm) and during the reproductive period (April-June), when this coloration is fully developed (methodology follows Galán 2008). Ultraviolet photographs were done according to Arribas (2001, 2012).

Statistical procedures: As a result of the sexual dimorphism presented by the different species of the genus *Iberolacerta* (Arribas 1996, 1999 a; Arribas et al. 2006), morphological analyses were carried out for males and females separately.

Statistical analyses used in the morphological study included both Multivariate techniques (Principal Components Analysis, PCA; and Canonical Discriminant Analysis, CDA) as well as univariate (ANOVA for SVL, scalation characters and indexes, with post-hoc Scheffé and Tukey-Kramer tests at \( p < 0.05 \) and \( p < 0.01 \) to detect differences among samples). Chi-square and Wilks’ Lambda were used to test the significance of each axis of the PCA or CDA axes. If the groups have different scores, then the model is discriminating between the groups and axes are significant (Sokal & Rohlí 1969; Blackith & Reymen 1971; Legendre & Legendre 1998, and online help in the statistic programs utilized, see below).
To test the significance of the differences between the NML samples as well as among this latter and the pre-established groups (*I. monticola*, *I. galani*), we carried out an Analysis of Similarity (ANOSIM) (Clarke 1988, 1993) that tests if the assigned groups are meaningful, this is, more similar within groups than with samples from different groups (see more details in Arribas 2010). To check for significance, pseudo replication 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. The most useful feature of this test is that pair wise 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. Multivariate (PCA, CDA—called CVA, Canonical Variate Analysis in the software package used- and ANOSIM) analyses were performed with Community Analysis Package 4.0° (Henderson & Seaby 2007). Univariate statistics and some partial (not represented) CDA were processed with NCSS 2001° package (Hintze 2001).

Morphological study phases and samples grouping

**Two successive analyses were performed:** A first one (“Intrazonal Analysis”), among the NML populations (the subject of this study) to check their degree of homogeneity and to trace the limits of the new taxon identified previously by its divergent mtDNA.

The second analysis (“Extrazonal Analysis”) was done among the new taxon, after demonstration of its homogeneity, and two samples of the neighboring *Iberolacerta* species: *I. monticola* from the Central and Western Cantabrian Mountains—mainly Ancares and Somiedo areas- (55 males and 38 females) and *I. galani* from the Southern Montes de León (Sierras del Teleno, Cabrera Baja, Segundera and Peña Trevinca) (26 males and 31 females), all of them in the León province and neighboring areas of Zamora and Ourense provinces.

In parallel to these two analyses, some explorative CDA were done to allocate isolated specimens or poor-represented populations. In these analyses (not included but commented in the results and discussion section) the small populations were plotted in a CDA against two of the three main ones (*galani* and NML new taxon, *monticola* and NML new taxon, or *monticola* and *galani*) and their graphic position in the first discriminant axis (the main variation one) was used for its assignation to one or to the other taxa—or eventually as possible hybrids if clearly intermediate.

From the NML area, OTUs names, localities and specimens included in these intrazonal analyses were as follows (Fig. 1A):

- ARCOS DEL AGUA (9. Collado de Ocidiello and Pico Arcos del Agua): one male and three females.
- TIENDAS (10. Sierra de las Tiendas): one male and three females.
- VILLABANDÍN (14. Sierra de Villabandín or del Alto de la Cañada, southern slopes): five males and six females.
- BABIA: (15. Riolago de Babia, Sierra de Villabandín, northern slopes): Two males and two females.

These different samples were pooled in some cases for the partial intrazonal analysis: Los Bayos was pooled together with Salientes (identity confirmed by mtDNA analyses; Remón 2011 and present paper). Arcos del Agua, Sierra de las Tiendas and Suspirón represent three samples situated along a narrow mountain ridge of 4.5 km roughly running from WNW to ESE, and were pooled together (in a collective sample named “Sierra de las Tiendas”).

**Osteological study.** Previously fixed and alcohol preserved specimens were cleared by means of 1 % KOH in deionized water, and bones stained with alizarin red, being posteriorly differentiated and the excess of pigment eliminated with Mall solution (80% of the previous clearing solution plus 20% of glycerol), and preserved permanently in glycerol following procedures by Taylor (1967) and Durfort (1978). Osteological nomenclature is as in Arribas (1998). Specimens studied of the new taxon described herein (6 in total) were from Salientes (1 male and 2 females from Puerto del Portillín and 1 male from Peña Valdiglesia, all them with NML-I haplotype; one
male from Sierra de Villabandín and one female from el Suspirón, with NML-II haplotype) for comparison. These specimens were compared with 3 typical I. galani (male, female and juvenile) and 13 I. monticola specimens (7 males and 6 females), covering its entire distribution range, from Serra da Estrela to Picos de Europa (Portugal and Spain).

**Genetics.** Genetic samples (tail tip biopsies preserved in 96% ethanol) were quickly (< 5 min) processed at the capture site and specimens immediately released afterwards. Genetic variation was analyzed at seven nuclear microsatellite loci (B107, B114, B135, C9, C113, C118, and D115, see Remon et al. 2008 for PCR details), and two mitochondrial loci (Cyt b cytochrome b, 572 bp; CR control region, 411 bp, after deleting gaps; see Crochet et al. 2004 for PCR details), corresponding to position intervals 14,193–14,763 and 16,310–16,752, respectively, of the *Lacerta viridis* (Laurenti, 1768) mitochondrial genome (GenBank acc. no. AM176577).

For mitochondrial loci, both markers were bidirectionally sequenced for 23 specimens from the Northern Montes de León (NML), together with 12 of *I. galani* and three of *I. martinezricai*. Electropherograms were visualized and aligned using CodonCode Aligner v. 3.5.7 (CodonCode Corporation). Sequences thus obtained were compared with former reports for these loci, and have been deposited in GenBank (acc. nos. HQ234877–82, EF121835–36 and JN048497, for CR, and HQ234900–03 and JN048500–02, for Cyt b; see Table 1 for detailed information). Maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods were used for reconstruction of mitochondrial phylogenetic trees. Homologous sequences from the other species of the genus *Iberolacerta*, available in GenBank (see Table 1 for accessions), were included in the analyses, namely *I. aurelioi*, *I. aranica*, *I. bonnali*, *I. horvathi*, *I. cyreni cyreni*, *I. cyreni castilliana*, *I. martinezricai*, and finally *I. monticola*. A species of another genus of Lacertini, *Lacerta agilis* Linné, 1758 was used as outgroup to root the phylogenies. Conversion among appropriate input data formats was carried using ALTER (Gonzalez-Peña et al. 2010), which also provided several descriptive statistics (number of variable and parsimony informative sites, p-distances between sequences). MP trees were obtained using the Close-Neighbor-Interchange algorithm, with search level 3, and 10 random addition sequence replicates. A ML test of the molecular clock hypothesis for the Cyt b. Analysis parameters of MetaPIGA were set to heuristic search using stochastic consensus pruning (metaGA), with consensus tree only intra-step optimization, and automatic selection of the best model of evolution for each partition. MP analyses of the concatenated sequences (no partition) were conducted in Mega 5 (Tamura et al. 2011), which also provided several descriptive statistics (number of variable and parsimony informative sites, p-distances between sequences). MP trees were obtained using the Close-Neighbor-Interchange algorithm, with search level 3, and 10 random addition sequence replicates. A ML test of the molecular clock hypothesis for the consensus topology obtained with MetaPIGA was also carried out with Mega 5. As for the Bayesian phylogenetic inference, we used MrBayes v. 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), specifying a consensus topology obtained with MetaPIGA was also carried out with Mega 5 for testing the molecular clock hypothesis, provided the priors for clockrange and treeage. The output cladogram summarizing the trees was visualized with FigTree v. 1.3.1 (Rambaut 2009).
**TABLE 1.** Geographic origin and data availability of the different mitochondrial and microsatellite samples used in this work (see also Figure 1A for NML localities). Microsatellite data have been deposited at Dryad: doi:10.5061/dryad.rh05d. Abbreviations: *n* ¾ sample size; a hyphen is used to indicate either sequences obtained directly from the Genbank (mtDNA) or populations/species not analysed in this work (microsatellites). *cytb* ¾ cytochrome b. *CR* ¾ control region.

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>mtDNA</th>
<th>micros</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region and/or species</strong></td>
<td><strong>Site</strong></td>
<td><strong>n</strong></td>
</tr>
</tbody>
</table>
| **Northern Montes de León (NML)** | (A) Salientes | 5 | Hap_1  
Hap_2 | HQ234900 | HQ234877 | 9 |  
HQ234878 |
| | (B) Los Bayos | 5 | Hap_1  
Hap_3 | HQ234900 | HQ234877 | 6 |  
HQ234879 |
| | (C) Babia | 3 | Hap_4 | JN048500 | EF121835 | 2 |  
| | (D) La Cañada | 5 | Hap_4  
Hap_5 | JN048500 | EF121835 | 8 |  
JN048502 |
| | (E) Tiendas | 4 | Hap_4 | JN048500 | EF121835 | 4 |  
| | (F) Suspirón | 1 | Hap_6 | JN048501 | EF121835 | 1 |  
| **I. galani** | (A) Trevinca | 4 | Hap_7 | HQ234901 | EF121835 | 4 |  
| | (B) Sanabria | 5 | Hap_7  
Hap_8 | HQ234901 | EF121835 | 5 |  
HQ234902 | EF121836 |
| | (C) Teleno | 3 | Hap_9  
Hap_10 | HQ234902 | HQ234881 | 3 |  
JN048497 |
| **I. martinezricai** | Peña de Francia | 3 | Hap_11  
Hap_12 | AY267237 | AY267250 | - |  
HQ234903 |
| **I. monticola** | (A) Somiedo | - | Hap_13 | HQ234897 | EF121832 | - |  
| | (B) Ancares | - | Hap_14 | AY267235 | EF121828 | - |  
| | (C) Queixa | - | Hap_15 | HQ234885 | EF121829 | 11 |  
| | (D) Capelada | - | Hap_16 | HQ234883 | EF121828 | - |  
| | (E) Estrela | - | Hap_17 | AY267234 | AY267247 | - |  
| **I. cyreni cyreni** | - | Hap_18 | AY267232 | AY267245 | - |  
| **I. cyreni castiliana** | - | Hap_19 | AY267233 | AY267246 | - |  
| **I. aurelioi** | Pyrenees | - | Hap_20 | AY267238 | AY267251 | - |  
| **I. aranica** | Pyrenees | - | Hap_21 | AY267239 | AY267252 | - |  
| **I. bonnali** | Pyrenees | - | Hap_22 | AY267240 | AY267253 | - |  
| **I. horvathi** | Croatia | - | Hap_23 | GQ142125 | AY267244 | - |  
| **Lacerta agilis** | - | Hap_24 | GQ142118 | EU497975 | - |  

As for microsatellites, 30 lizards from five sites at the Northern Montes de León (NML) were genotyped. In addition, 12 individuals identified as *I. galani* (from three localities at the Southern Montes de León), and 11 of *I. monticola* (from Serra da Queixa), were also genotyped (see Table 1 and Figure 1A for further details of the sampling sites). The performance of a small battery of microsatellite loci (Remón *et al.* 2008) on these samples was assessed in terms of the success of the PCR amplifications, the segregation of null alleles, and the goodness of fit of observed frequencies to gametic equilibria expectations, using the tests implemented in Genepop v. 4.0 (Rousset 2008). The significance level of all these tests was adjusted by the sequential Bonferroni procedure (Rice 1989). Only seven loci were finally retained for further analyses, namely B107, B114, B135, C9, C113, C118, and D115 (see Remón *et al.* 2008 for PCR details). Individuals were first assigned to different populations by the Bayesian procedures implemented in the software Structure v. 2.1 (Pritchard *et al.* 2000). To identify the most likely number of populations (*k*) in our dataset, we used both the method suggested in the original Structure paper, based on
scoring mean log likelihoods penalized by one-half of their variance (estimated “log probability of data”, \( L(k) \)), and the approach developed by Evanno et al. (2005), based on the rate of change in the log probability of data between successive \( k \) values. We used an admixture model of genetic clustering with correlated allele frequencies, run for 900,000 generations after a burn-in of 100,000 generations, assuming that there were up to 4 clusters (\( k = 2 \) to \( k = 4 \)), and ran 10 parallel chains to estimate what number of genetic clusters had the highest probability. Membership coefficients of individuals in each of the clusters were plotted with Distruct v. 1.1 (Rosenberg 2004). The similarities among multilocus genotypes were further investigated by performing a factorial correspondence analysis (FCA), carried out with Genetix v. 4.05.2 (Belkhir et al. 2004). This program also provided estimates of Nei's genetic distance, whose significance was assessed by 1000 permutations of individuals between groups. Nei’s distances were also calculated for a set of 1000 bootstrap samples produced by Phylip v. 3.6 (Felsenstein 2005), so as to obtain variance estimates and statistical support for different planned comparisons using sign tests.

Results

External morphology

Variation in Northern Montes de León (NML) [“Intrazonal analysis”]

(Canonical) Discriminant Analyses (CDA): To study the relationships among the different intrazonal samples (NML) we first ran a Discriminant Analysis (data not shown). This kind of analysis maximizes the separation among the samples (pre-defined groups), even if the results are not significant, as is the case here. In males, almost all the variability appears concentrated in the first axis (Eigenvalue of 26.31 and 64 % of variance explained), whereas the second axis is less variable (Eigenvalue 11.26, and 27.4 % of variance explained). The two first axes reach in total 91.4 % of variability. However, even the first axis is not significant (\( F_{85,28.6}=1.4; \ P > 0.05, \ NS \)) as is obviously the case also for the second one (\( F_{64,25.8}=0.9; \ P > 0.5, \ NS \)) and the following ones (not shown).

In females, the situation is very similar but even more confusing, with a first axis (Eigenvalue of 1.69) concentrating 36.3% of variance, whereas the second one (Eigenvalue of 1.25) with only 26.9 % of the variance (63.2 % accumulated among the two first axes). As occurs in the male analysis, none of these axes were significant: first axis (\( F_{102, 132.4}=1.0; \ P > 0.5, \ NS \)); second (\( F_{80, 115}=0.8; \ P > 0.5, \ NS \)) as in the following ones (three axes were necessary to obtain a 77.9 % of variability).

Principal Components Analysis (PCA): Since these Discriminant Analyses (CDA) in which the computation units are the pre-defined groups resulted not significant, we ran a PCA in which each specimen was represented on its own and was not forced to any a priori defined group, resulting significant and thus more reliable with respect to the relationships among the specimens of the different populations. This analysis was significant for both sexes. The results show that there are no clearly differentiated specimens among the populations analyzed.

In males (Fig. 2) (Bartlett Test=193.82; DF=136; \( P < 0.001 \)) the two first axes have a very poor discriminative power, with only 33.8 % of variance explained. This can be explained because the variability is uniformly spread in the sample and the axes are not able to discriminate some specimens from others. The first axis presents an Eigenvalue of 3.06 (18.01 % variance), and the second one of 2.68 (and 5.81 % of the variance). The first axis has higher loadings of COLL (0.30), CIRCA (0.28), GUL (0.24) and AS/SVL (0.23) in their positive part and FEMI (-0.46), FEMr (-0.37), LAM (-0.32), DM/PaL (-0.32) and AL/AW (-0.28), without any clear geographical interpretation. The second axis has positive loadings for DT/PaL (0.39) and negatives for VENT (-0.42), LAM (-0.33), AS/SVL (-0.32), FEMr (-0.30), GUL (-0.29), DORS (-0.26) and FEMI (-0.24), and has a geo-climatic interpretation, separating the northernmost (oceanic influenced) samples of BABIA (“Riolago de Babia”), situated in the northern slopes of the Sierra de Villabandín (loc. 15 in Fig. 1A) from populations situated in the more continental and climatically harsher areas [VILLABANDIN -the southern slopes- (loc. 14 in Fig. 1A), BAYOS (loc. 12 and 13 in Fig. 1A) and TIENDAS-SUSPIRON (loc. 10 and 11 in Fig. 1A)], in the positive and negative parts of the PCA, respectively. The situation in the PCA graph is independent from the haplotype present in the sample: just in the extremes of the second axis are the populations from the northern slopes of the Sierra de Villabandín (“Riolago de Babia”, more oceanic; loc. 15 in Fig. 1A) and the southern slopes (“Villabandín”, more continental; loc. 14 in Fig. 1A), both with the same NML-II haplotype. The more continental populations show the highest values for the negative loaded characters (most scalation characteristics) and the smaller ones for the
FIGURE 2. Principal Components Analysis. Males. Bidimensional plot of the specimens scores. Triangle: BABIA (Northern slopes of Sierra de Villabandín; NML-II haplotype). Inverted triangles: SALIENTES (Nevadín-Tambarón-Catoute populations; NML-I haplotype). Crosses: TIENDAS & SUSPIRON (Sierra de las Tiendas and Suspiron peak; NML-II haplotype). Blades: VILLABANDIN (Sierra de Villabandín or La Cañada; NML-II haplotype). Despite to be significant, the two first axes had a very poor discriminative power, with only a 33.8 % of variance explained (see text for details) and show a considerable overlap of the NML-I specimens that are distributed overlapping with the main part of the other samples. The second axis seems to have a geo-climatic interpretation, separating in the positive part the most northern (oceanic influenced) samples (BABIA) and in the negative part the ones inhabiting the more continental and harsh climate areas facing to the Spanish Northern Plateau (Meseta) (VILLABANDIN -Southern slopes of the same Sierra de Villabandín-), BAYOS, TIENDAS and SUSPIRON. Note that BABIA (triangle symbol) specimens are geographical neighbors (in the northern slopes) of VILLABANDIN (blades symbol) (in the southern slopes), but appear totally opposite in the PCA, in accord with this bioclimatic influence in the scalation. These more continental climate populations show the greater values for most scalation characteristics and the smaller ones for tympanic plate size. Specimens from SALIENTES (loc. 1–8 in Fig. 1A), which include samples from all the mountains surrounding Salientes and Salentinos valleys, are spread over the graphic and overlapping with the main part of the other specimens (Fig. 2). As mentioned above, the unique specimens slightly deviating from the common pattern are the ones from the more continental areas (facing to the Spanish Plateau or Meseta).

In females (Fig. 3) the PCA is significant but also has a poor discrimination of the specimens into differentiated samples (Bartlett Test=234.23; DF=136; \( P < 0.000001 \)). The fist axis has an Eigenvalue of 2.91 (17.14% all intersample variability explained) and the second one of 2.57 (15.14% of variability). The two axes together include 32.3 % of variability explained, which is a very poor discriminative result (even if the third axis was added, the sum of the three axes was 44.38%). The first axis has positive loadings for GRSr (0.36), GRSI (0.32), COLL (0.20), DORS (0.36), FEMr (0.23), FLL/SVL (0.29), and negative for CIRCA (-0.36), DM/PaL (-0.30), DT/PaL (-0.23) and AS/SVL (-0.22). The second axis has positive values of COLL (0.30), HLL/SVL (0.30), FLL/SVL (0.23) and negative of FEMr (-0.43), FEMI (-0.41), and VENT (-0.39). As in the male analysis, the samples from SALIENTES overlap with all the other ones and the PCA does not discriminated among the samples or allows any geographic or climatic interpretation of the axes (see Fig.3).

ANOSIM tests: Since the results from the previous CDA showed no statistical support for the predefined intrazonal structure, ANOSIM tests were also not significant and confirmed again that there are no differences among the studied populations (R-statistic = 0.00801645, \( P > 0.1 \), NS; with 1000 randomizations, all the intersample comparisons \( P > 0.09 \)). In females, ANOSIM tests were also not significant (R-statistic = -0.044265, \( P > 0.5 \), NS; with 1000 randomizations) and unable to find differences among any of the samples (all the intersample comparisons \( P > 0.28 \)).
Differences between specimens from the Northern Montes de León and *Iberolacerta monticola* s. str. and *I. galani* [“Extrazonal analysis”]

Once demonstrated in the intrazonal multivariate analyses that all the populations from NML are homogeneous and morphologically indistinguishable (independently of their mitochondrial haplotype), we proceeded to check the differences of these populations as a whole with respect to *Iberolacerta monticola* s. str. (from Central Cantabrian Mountains) and *I. galani* (from Southern Montes de León).

(Canonical) Discriminant Analyses (CDA): To study the relationships among the different samples, the intrazonal (NML) and two extrazonal (*I. monticola* and *I. galani*) we ran a Discriminant Analysis for males and females separately (Figures 4 and 5, for males and females respectively).

The results of the male analysis are shown in Fig. 4. A large amount of the variability is concentrated in the first axis (Eigenvalue of 0.958 and 70.6% of variance explained; Canonical Correlation is 0.70) whereas the second axis presents a much lower value (Eigenvalue 0.39, and 29.4 % of variance explained; Canonical Correlation 0.53). In this case, both axes are significant (First axis: Chi-sq. \( \chi^2=94.72; P < 0.000001 \); Second axis: Chi sq. \( \chi^2=31.55; P < 0.02 \)). There are significant differences among centroids (Wilks’ Lambda=0.365062; \( F= 3.12979, 36 \) d.f., \( P < 0.0000001 \)). The two axes together configured a bidimensional space with moderate overlap among the three taxa. The first axis separates *I. monticola* in its negative part from the NML-taxon and *I. galani* in the positive one. *Iberolacerta monticola* is characterized by the greater values of AS/SVL (-0.53) and HLL/SVL (-0.39), and smaller number of VENT (0.43), DORS (0.38) and BO (0.37) (NML-taxon and *I. galani* have smaller anal plates and shorter hindlimbs, but higher ventralia, dorsalia and more blue ocelli in the shoulder). The second axis (fairly less informative, see above) separates the NML-taxon from *I. monticola* and *I. galani*. The NML-taxon specimens are differentiated towards the negative part of the axis, with smaller values of LAM (0.76), COLL (0.41) and relatively higher values of VENT (-0.37) (*I. monticola* and *I. galani* have more lamellae and collaria, and smaller ventralia). These discriminant axes obtain a 78.3% of correct reclassification. The NML-taxon samples are the ones with the higher percentage of correct classification (84.6%), followed by *I. monticola* (77.7%) and *I. galani* (73.1%). The centroids of *Iberolacerta galani* and the NML-taxon in the bidimensional space are more similar to each other than to *I. monticola*.

---

**FIGURE 3.** Principal Components Analysis. Females. Symbols as in Fig. 2. As in males, it is significant but also has a poor discrimination of the specimens into differentiated samples. The two axes together reach 32.3 % of variability explained (First axis: Eigenvalue of 2.91 and 17.14 % variance; Second axis 2.57 (15.14 % of variability), which is a very poor discriminative result. As in the male analysis, SALIENTES samples overlap with all the other ones, not allowing discriminating among groups or any geographic or climatic interpretation of the axes.
FIGURE 4. Canonical Discriminant Analysis. Males. Projection of the first two axes. First axis: Eigenvalue=0.958; 70.6 % variance explained; Canonical Correlation 0.70. Second axis: Eigenvalue=0.39; 29.4 % variance explained; Canonical Correlation 0.53. Both axes are significant and configure a bidimensional space with moderate overlap among the three taxa. The first axis separates *I. monticola* s. str. in its negative part from *I. m. astur* ssp. nov. and *I. galani* in the positive one. The second axis separates *I. m. astur* ssp. nov. from the other two taxa. These discriminant axes obtain a 78.3 % of correct reclassification. *Iberolacerta galani* and *I. m. astur* ssp. nov. centroids are the closest in the males bidimensional space, thus, their males are more similar in our analysis.

The results of the female analysis are shown in Fig.5. Like in the males, two thirds of the variability is concentrated in the first axis (Eigenvalue of 1.3796 and 69% of variance explained; Canonical Correlation 0.76) whereas the second axis presents the remaining third of variability (Eigenvalue 0.6169, and 31% of variance explained; Canonical Correlation 0.61). In this case, both axes were significant (First axis: Chi sq. $\chi^2=137.4; P < 0.000001$; Second axis: Chi sq. $\chi^2=49.01; P < 0.000005$). There are significant differences among centroids (Wilks’ Lambda=0.259893; F= 5.0215; 36 d.f., $P < 0.000001$; Bartlett’s Test$= 138.117, 36$ d.f., $P < 0.000001$). Females present less overlap among samples than males. The first axis separates *I. galani* in its positive side from *I. monticola* and the NML-taxon in the negative side. *Iberolacerta galani* is characterized by higher values of COLL (0.60), VENT (0.43), BO (0.41) and DORS (0.36), and lower values of FLL/SVL (-0.28) and PL/PW (-0.20) (NML-taxon and *I. monticola* have lower collaria, ventralia, number of blue ocelli, dorsalia, and comparatively longer forelimbs and pilei). The second axis separates the NML-taxon in its positive part from the other two taxa in the negative part. Specimens of the NML-taxon have higher values of GrS l (0.61) and PL/PW (0.34) and present the lowest scores for LAM (-0.65), AS/SVL (-0.39) and GrS r (*I. monticola* and *I. galani* have more lamellae, right side supraciliaria, greater anal plate and smaller left side supraciliaria and shorter pilei). The Discriminant axes obtained 80.7% of correct reclassification in the NML-taxon. *Iberolacerta galani* females are the ones with the highest percentage of correct classification (87.09%), followed by *I. monticola* (78.9%) and very closely by the NML-taxon (77.7%). Contrary to what happens in males, in the females of the NML-taxon the centroid is closer to *I. monticola* than to *I. galani*.

**Analysis of Similarity (ANOSIM):** The results of the ANOSIM analysis of males showed that, despite the considerable overlap among samples, significant and highly significant differences exist among all the three taxa: *I. galani-I. monticola* ($P < 0.001$), NML taxon- *I. galani* ($P < 0.01$) and NML taxon- *I. monticola* ($P < 0.05$) (R-statistic = 0.124398, $P < 0.01$; 1000 randomizations). The best a priori assignation was between *I. galani* and *I. monticola* (partial R-statistic 0.17), and the worse between the NML-taxon and *I. galani* (0.07), being the comparison between the NML-taxon and *I. monticola* intermediate (0.09). However, it is remarkable that the NML-taxon is statistically more different to *I. galani* ($P < 0.01$) than to *I. monticola* ($P < 0.05$).
FIGURE 5. Canonical Discriminant Analysis. Females. First axis has two thirds of the total variability (Eigenvalue=1.3796; 69 % variance; Canonical Correlation 0.76) whereas the second one had the remaining one third (eigenvalue 0.6169, 31 % variance; Canonical Correlation 0.61). The female analysis presents less overlap among samples than the male analysis. The first axis separates I. galani in its positive part from I. monticola s. str. and I.m. astur ssp. nov. in its negative part. The second axis separates I.m. astur ssp. nov. in its positive part from the other two taxa. Discriminant axes obtained an 80.7 % of correct reclassification. Contrary to the male analysis, the I. m. astur ssp. nov. centroid is closer to the centroid of I. monticola than to the centroid of I. galani.

The results of the ANOSIM analysis of females were better than the results obtained with males (R-statistic = 0.213456, P < 0.001; 1000 randomizations). Very significant differences among the (geographically) assigned groups appear among all of them. The best a priori assignation was between I. galani and I. monticola (partial R-statistic 0.34), and the worse assignation between NML-taxon and I. monticola (0.08), being the assignation between the NML-taxon and I. galani intermediate (0.25) (all three intersample comparisons P<0.001).

Analysis of Variance (ANOVA): Results of the ANOVA analyses are shown in Tables 2 (males) and 3 (females).

The NML-samples differ significantly (p<0.05; if underlined, p<0.01) from its most closely related taxa in the following characters, analyzed for males (m) and females (f) independently (Tables 2 and 3): from I. monticola in GrS l (f), VENT (m, f), CIRCA (f), LAM (m, f), PV (m, f), FLL/SVL (m), HLL/SVL (m), PL/PW (f), AL/AW (m), AS/SVL (m, f), BO (m), R-I (f), Po-Pa (f) and SVL (f). From I. galani differs in COLL (m, f), VENT (f), DORS (f), LAM (f), R-I (f) and FEM r (f), Sn-Lor (m, f) and PV (m).

In summary, and including both sexes, the NML-taxon also differs from I. monticola in eight characters for p<0.01, and in 18 characters for p<0.05. The NML-taxon also, differs from I. galani in 6 characters at p<0.01, and in 10 characters at p<0.05. The NML-taxon differs significantly in more characters from I. monticola than from I. galani.

The evaluation of the number of dorsal green scales in adult males of the NML-taxon, counted across a ring in the center of the body, was (average ± StdE): 11.86 ± 1.88, with a range of 2–34 scales (n=22) and the number of black scales of 18.91 ± 1.05 (range: 12–30, n=22). These numbers are not significantly different from I. monticola males from Puerto de Leitariegos and Ancares (neighboring areas of the Cantabrian Mountains). The number of green dorsal scales of the NML-taxon is not significantly different from I. galani from Trevinca (green dorsal scales: 16.94 ± 1.78, range 4–29 scales, n=18), however, the number of black dorsal scales is significantly different from I. galani, which has a higher number: 29.89 ± 1.08, range: 25–40, n=18. (NML- I. galani: ANOVA, Scheffé a posteriori test: F = 8.53, P < 0.001).
TABLE 2. ANOVA results of the morphometric, scalation and biometric indexes from males of *I. monticola* s. str., *I. m. astur* ssp. nov. and *I. galani*. See text for abbreviations of characters and indexes used in the morphometric analysis.

<table>
<thead>
<tr>
<th>MALES</th>
<th><em>Ib. monticola</em> s. str. (1) (n=55)</th>
<th><em>Ib. monticola</em> astur ssp.nov. (2) (n=24)</th>
<th><em>Ib. galani</em> (3) (n=26)</th>
<th>F&lt;sub&gt;2,104&lt;/sub&gt;</th>
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<th>1–3</th>
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<td>10.26±0.22 8–12</td>
<td>11.34±0.26 9–13</td>
<td>5.22</td>
<td>0.006928</td>
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<tr>
<td>DORS</td>
<td>51.05±0.41 45–60</td>
<td>51.26±0.37 46–57</td>
<td>53.26±0.70 47–59</td>
<td>4.32</td>
<td>0.015790</td>
<td>*</td>
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<tr>
<td>VENT</td>
<td>25.4±0.13 24–28</td>
<td>26.34±0.22 24–28</td>
<td>26.34±0.22 24–28</td>
<td>7.23</td>
<td>0.001147</td>
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<tr>
<td>FEM r</td>
<td>17.30±0.20 15–21</td>
<td>17.65±0.26 16–20</td>
<td>17.65±0.26 16–20</td>
<td>1.81</td>
<td>0.168688</td>
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<tr>
<td>FEM l</td>
<td>17.41±0.18 14–21</td>
<td>17.61±0.26 15–21</td>
<td>17.61±0.26 15–21</td>
<td>0.18</td>
<td>0.834973</td>
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<tr>
<td>LAM</td>
<td>24.74±0.25 21–30</td>
<td>25.38±0.33 23–30</td>
<td>25.38±0.33 23–30</td>
<td>6.50</td>
<td>0.002188</td>
<td>*</td>
<td>**</td>
<td></td>
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<tr>
<td>CircA</td>
<td>7.01±0.11 5–9</td>
<td>6.53±0.21 5–8</td>
<td>6.5±0.94 5–8</td>
<td>3.69</td>
<td>0.028195</td>
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<tr>
<td>R-I</td>
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<td>0.76±0.17 0–2</td>
<td>2.49</td>
<td>0.087997</td>
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<tr>
<td>Po-Pa</td>
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<td>0.11±0.05 0–1</td>
<td>0.04±0.03 0–1</td>
<td>3.20</td>
<td>0.044893</td>
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<tr>
<td>Sn-Lor</td>
<td>0±0</td>
<td>0±0</td>
<td>0.15±0.06 0±0</td>
<td>8.40</td>
<td>0.000416</td>
<td>**</td>
<td>**</td>
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</tr>
<tr>
<td>BO</td>
<td>1.85±0.10 0–5</td>
<td>2.84±0.43 1–11</td>
<td>2.84±0.43 1–11</td>
<td>6.93</td>
<td>0.001498</td>
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<td>**</td>
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</tr>
<tr>
<td>PV</td>
<td>1.7±0.08 1–3</td>
<td>1.73±0.13 1–3</td>
<td>1.73±0.13 1–3</td>
<td>8.57</td>
<td>0.000357</td>
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<td>**</td>
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<tr>
<td>FLL/SVL</td>
<td>35.21±0.002 30.15–43.06</td>
<td>33.81±0.003 30.36–37.57</td>
<td>33.98±0.004 27.64–37.16</td>
<td>5.39</td>
<td>0.005955</td>
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<tr>
<td>HLL/SVL</td>
<td>50.61±0.003 44.76–63.13</td>
<td>49.10±0.003 45.86–51.97</td>
<td>49.83±0.003 45.25–53.46</td>
<td>3.91</td>
<td>0.023138</td>
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</tr>
<tr>
<td>PL/PW</td>
<td>212.12±0.01 197.56–232.46</td>
<td>215.16±0.02 199.20–255.28</td>
<td>216.57±0.01 198.48–230.98</td>
<td>2.26</td>
<td>0.109104</td>
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<tr>
<td>DM/PaL</td>
<td>40.84±0.007 25.48–53.48</td>
<td>41.61±0.01 28.11–54.24</td>
<td>41.34±0.01 31.18–54.54</td>
<td>0.17</td>
<td>0.840998</td>
<td></td>
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<tr>
<td>DT/PaL</td>
<td>35.92±0.07 24.56–46.55</td>
<td>37.16±0.09 30.75–45.12</td>
<td>38.92±0.01 29.53–50.59</td>
<td>2.97</td>
<td>0.055715</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL/AW</td>
<td>56.81±0.08 41.46–74.34</td>
<td>56.25±0.01 41.77–68.84</td>
<td>52.16±0.01 43.6–64.11</td>
<td>5.28</td>
<td>0.006551</td>
<td>*</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>AS/SVL</td>
<td>517.48±0.05 430.04–611.49</td>
<td>486.7±0.07 427.28–576.37</td>
<td>479.56±0.06 404.31–545.57</td>
<td>11.93</td>
<td>0.000022</td>
<td>**</td>
<td>**</td>
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</tbody>
</table>
TABLE 3. ANOVA results of the morphometric, scalation and biometric indexes from females of *I. monticola* s. str., *I. m. astur* ssp. nov. and *I. galani*. See text for abbreviations of characters and indexes used in the morphometric analysis.

<table>
<thead>
<tr>
<th>FEMALES</th>
<th><em>lb. monticola</em> s. str.</th>
<th><em>lb. monticola</em> astur ssp. nov.</th>
<th><em>lb. galani</em></th>
<th>F_{2,110}</th>
<th>P 1–2</th>
<th>1–3</th>
<th>2–3</th>
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<tbody>
<tr>
<td>SVL</td>
<td>62.5±0.76, 51.7–74.08</td>
<td>66.05±0.96, 54.5–78.12</td>
<td>64.66±1.6, 50.7–84.42</td>
<td>2.85</td>
<td>0.062389 *</td>
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<td></td>
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<tr>
<td>GrS r</td>
<td>9.23±0.31, 4–13</td>
<td>9.88±0.21, 6–13</td>
<td>9.35±0.37, 5–13</td>
<td>1.50</td>
<td>0.227606</td>
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<td></td>
</tr>
<tr>
<td>GrS I</td>
<td>9.13±0.35, 3–15</td>
<td>10.22±0.20, 7–13</td>
<td>9.25±0.32, 5–12</td>
<td>4.53</td>
<td>0.012857 *</td>
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<tr>
<td>GUL</td>
<td>22.94±0.32, 19–29</td>
<td>23.35±0.31, 19–29</td>
<td>23.8±0.4, 16–29</td>
<td>1.44</td>
<td>0.241949</td>
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<tr>
<td>COLL</td>
<td>9.78±0.24, 6–14</td>
<td>9.68±0.15, 8–12</td>
<td>11.1±0.26, 9–14</td>
<td>18.4</td>
<td>0.000000 **</td>
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</tr>
<tr>
<td>DORS</td>
<td>48.5±0.42, 42–51</td>
<td>50±0.54, 43–59</td>
<td>53.45±0.64, 48–62</td>
<td>19.55</td>
<td>0.000000 **</td>
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<tr>
<td>VENT</td>
<td>29.02±0.17, 26–31</td>
<td>29.93±0.18, 25–32</td>
<td>30.71±0.19, 28–33</td>
<td>18.35</td>
<td>0.000000 **</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>FEM r</td>
<td>15.89±0.21, 13–19</td>
<td>16.51±0.2, 14–19</td>
<td>17.45±0.3, 14–21</td>
<td>10.20</td>
<td>0.000086 **</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>FEM l</td>
<td>16.10±0.26, 13–23</td>
<td>16.51±0.21, 14–20</td>
<td>17.22±0.3, 13–21</td>
<td>4.15</td>
<td>0.018299 *</td>
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</tr>
<tr>
<td>LAM</td>
<td>23.76±0.22, 21–27</td>
<td>22.88±0.24, 18–26</td>
<td>25±0.32, 22–30</td>
<td>15.69</td>
<td>0.000001 *</td>
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<td>**</td>
</tr>
<tr>
<td>CircA</td>
<td>7.28±0.17, 6–9</td>
<td>6.55±0.14, 4–9</td>
<td>6.22±0.12, 5–8</td>
<td>11.91</td>
<td>0.000021 **</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>R-I</td>
<td>1.07±0.15, 0–2</td>
<td>0.6±0.13, 0–2</td>
<td>1.22±0.13, 0–2</td>
<td>5.40</td>
<td>0.005805 *</td>
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<tr>
<td>Po-Pa</td>
<td>0.40±0.1, 0–2</td>
<td>0.15±0.05, 0–1</td>
<td>0.11±0.03, 0–1</td>
<td>4.40</td>
<td>0.014547 *</td>
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<tr>
<td>Sn-Lor</td>
<td>0±0, 0–0</td>
<td>0.04±0.02, 0–1</td>
<td>0.25±0.07, 0–1</td>
<td>11.16</td>
<td>0.000038 **</td>
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<tr>
<td>BO</td>
<td>1.07±0.13, 0–2</td>
<td>1.4±0.14, 0–4</td>
<td>1.93±0.21, 0–6</td>
<td>6.41</td>
<td>0.002324 **</td>
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<tr>
<td>PV</td>
<td>1.34±0.13, 0–3</td>
<td>0.57±0.08, 0–2</td>
<td>0.9±0.12, 0–3</td>
<td>13.50</td>
<td>0.000006 **</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>FLL/SVL</td>
<td>30.65±0.003, 26.78–34.81</td>
<td>30.22±0.003, 26.51–36.78</td>
<td>30.29±0.003, 26.75–34.62</td>
<td>0.46</td>
<td>0.633760</td>
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<td></td>
</tr>
<tr>
<td>HLL/SVL</td>
<td>43.75±0.0004, 37.57–50.48</td>
<td>43.29±0.004, 37.18–50.45</td>
<td>42.98±0.004, 37.06–47.76</td>
<td>0.67</td>
<td>0.511574</td>
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<tr>
<td>PL/PW</td>
<td>198.56±0.04, 150.97–227.53</td>
<td>210.49±0.01, 194.07–233.11</td>
<td>204.71±0.01, 196.05–217.64</td>
<td>5.31</td>
<td>0.006290 **</td>
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<tr>
<td>DM/PaL</td>
<td>42.52±0.01, 41.75±0.01</td>
<td>41.75±0.01, 39.13±0.01</td>
<td>39.13±0.01, 19.54–50.4</td>
<td>1.98</td>
<td>0.143529</td>
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<tr>
<td>DT/Pal</td>
<td>41.70±0.007, 30.95–49.11</td>
<td>39.72±0.009, 25.89–54.22</td>
<td>42.32±0.01, 32.1–59.52</td>
<td>2.09</td>
<td>0.128191</td>
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</tr>
<tr>
<td>AL/AW</td>
<td>57.25±0.009, 45.09–70.00</td>
<td>57.37±0.008, 43.96–71.20</td>
<td>57.96±0.01, 48.12–71.31</td>
<td>0.14</td>
<td>0.868380</td>
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</tr>
<tr>
<td>AS/SVL</td>
<td>492.83±0.06, 430.31–566.82</td>
<td>461.55±0.06, 398.16–564.53</td>
<td>444.34±0.06, 360.15–529.88</td>
<td>14.54</td>
<td>0.000002 **</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>
Osteology of the NML-taxon. Skull: Processus nasalis variable: elongated and undifferentiated or slightly widened and leaf-shaped (spathulated), never arrow-shaped (one Salientes male shows a double expanded one–two expansions with a constriction in the middle). Seven to nine premaxillary teeth in males and females (nine is the most common number). From 15 to 20 maxillary teeth-positions (average 17.2) and from 20 to 23 dentary teeth-positions (average 21.5). Teeth mainly bicuspid. Margo ocularis (maxillo-jugal suture) smooth. Postocular and postfrontal separated, subequal in length, but in males postocular can be slightly longer than postfrontal, whereas in females postfrontal is equal or slightly longer than postocular. Anteromedial process of postocular and anterodistal process of postfrontal, both present. Squamosal bone more or less regularly arched as is typical in other Iberolacerta (similar to I. galani in one of the sides of a Suspirón female, but clearly different in the other side of the same specimen and different from the rest of specimens), with 1/2 to 1/3 of lateral overlap with the postocular bone.

Postcranial skeleton: Males with 26 (rarely 25) and females with 27, 28 or 29 presacral vertebrae (pv). Males with six short lumbar ribs (five in the case to have 25 pv), and females with six (in the case of 28 pv) or seven (if 27 & 29 pv), independently of their total presacral vertebral number. There are no ribs associated to the third presacral vertebrae (a Salientes female presents them as an atavistic character, one out of six specimens). Preautotomic vertebrae with A-type processes (the male specimen from Villabandín and a female from El Suspirón show, apart from the A type, some similar to B-type). Clavicles variable (open -marginated- or closed -emarginated-) but when open, are near to the closed state. One specimen presents one clavicle of each type. Interclavicle cruciform with anterior/posterior branches ratio from 0.20 to 0.38 (average 0.28). Sternal-xiphisternal costal formula (3+2), but in one specimen 4+2 in one side and 3+2 on the other. Sternal fontanel round or oval (more or less cordiform in two females from Salientes and Suspirón).

Genetic results. The sequences of the two mitochondrial markers (CR and Cyt b) were concatenated to produce a two-gene data set (983 bp, containing 101 singletons and 213 parsimony informative sites). The null hypothesis of equal evolutionary rate throughout the ML tree was not rejected at a 5% significance level ($P = 0.209$), and Bayes factor comparisons rendered strong evidence in favor of a strict molecular clock (log-difference of 5.3 units), which was accordingly assumed for all the phylogenetic reconstructions. As shown in Fig.6, the samples from the Northern Montes de León are grouped in two different lineages, closely related either to I. monticola (NML-I) or I. galani (NML-II), with very strong statistical support in both cases. The split between NML-I and I. monticola took place ca. 1.8 Million years ago (Mya; 95% confidence interval 1.36–2.36), whereas the most recent common ancestor (MRCA) of NML-II and I. galani haplotypes dates back to ca. 0.5 Mya (0.29–0.83). Haplotype sequences within NML-I and NML-II clades are scarcely differentiated, their divergence having been initiated not sooner than 0.4 Mya in both cases (95% confidence intervals of 0.039–0.360 and 0.041–0.393, respectively). The two clades show a clear geographic segregation. All the samples so far examined contain haplotypes entirely from one or the other clade, with NML-I sites lying to the north and NML-II to the south of an imaginary SW-NE line that crossed the Sierra de Gistredo (between localities 1–9 and 10–11 in Fig. 1A, respectively) and the Sierra de Villabandín (between 12–13 and 14–15 in Fig.1A). This phylogeographic pattern is not entirely concordant with the results offered by the analyses of microsatellite nuclear markers, though. According to the Bayesian inference, all the samples from the Northern Montes de León should be included in a single cluster, distinct from both I. galani and I. monticola (see Structure results in Fig. 7). The factorial correspondence analysis confirms the genetic differentiation between these three groups, but also reveals a subtle differentiation between NML-I and NML-II sites. Altogether, the three main axes of variation explain (“trace") 97.73% of the observed variation. Most of this trace is accounted for by axis 1 (47.33%) and axis 2 (39.02%), corresponding, respectively, to the separation of the clouds of I. monticola and I. galani individuals. But there is still axis 3, accounting for 13.64% of the trace, which separates NML-I and NML-II (see Fig. 8 for a 3D representation of the results of the factorial correspondence analysis). Nei’s distance between them is relatively small ($D = 0.243$), and non-significant according to the permutations test ($P = 0.128$), although bootstrap sampling strongly supports the existence of consistent genetic differences between these two kinds of NML populations. NML-II is closer to I. galani than NML-I (average $D$ scores of 0.648 and 0.826, respectively, with $P <0.001$ in the signs test), and correspondingly more distant from I. monticola ($D = 1.201$, as compared to $D = 1.078$ between this species and NML-I, again with $P<0.001$ in the signs test). The two reference species, I. galani and I. monticola, are separated by a $D$ score of 1.270, significantly higher than the distance between either of them and the NML populations ($P<0.001$). Table 4 displays in matrix form these distances and their sampling error analogs (above
diagonal), as well as rough estimates of divergence times drawn from them (below diagonal), assuming that the separation of *I. monticola* and *I. galani* took place 2.5 Mya (see Fig. 6).

**FIGURE 6.** Phylogenetic tree of the mitochondrial haplotypes of *Iberolacerta* from the Northern Montes de León (NML), based on a strict-clock bayesian reconstruction. The tree is rooted using *Lacerta agilis*, and it includes representative sequences from the different *Iberolacerta* species. Statistical support values for the major clades, obtained by three different methods of analysis, are shown on each node; namely, from left to right, Bayes posterior probability (x100), ML metaGA best trees in consensus (%) and equally MP trees (%). A hyphen is inserted instead of a numerical value whenever a particular method did not support the Bayesian topology. Node bars indicate 95% credibility intervals (regions of highest posterior density) for the corresponding divergence time (in million years). Among brackets the localities of *I. m. astur* ssp. nov. referred in Fig. 1A.

**FIGURE 7.** Summary plot of estimated membership coefficients for each individual, in each cluster (Q). Each individual is represented by a single vertical line broken into k colored segments, with lengths proportional to each of the k inferred clusters. The numbers (1–4) correspond to the predefined populations.
FIGURE 8. Three-dimensional representation of a Factorial Correspondence Analysis based on microsatellite genotypes of Iberolacerta monticola astur ssp. nov. individuals from the Northern Montes de León. The figure includes individuals of I. galani and I. monticola s. str. to be used as reference.

TABLE 4. Genetic divergence between the operational taxonomic units of this study, based on the scores of Nei’s distance in 1000 bootstrap samples of the real microsatellite data. Above the diagonal: average scores; in parenthesis, intervals covering 68% of the bootstrap distances, formally equivalent to 1 standard deviation. Below the diagonal: estimated time of divergence (Mya), using the estimate of 2.5 Mya for the separation of the mitochondrial lineages of I. galani and I. monticola as calibration point.

| Taxonomic conclusions. Due to the differences of the NLM populations, especially genetic, we describe them as a new taxon. In spite of the relatively striking mitochondrial differences that can suggest it to be a different (phylogenetic) species, with other data sources in our hands (morphology and osteology): a) we cannot find heterogeneity among the different populations inhabiting the area, a question corroborated by microsatellite analyses, so that we consider all NML populations as belonging to the same taxon, independently of their mtDNA haplotype; b) the original haplotype of this NML populations is NML-I with a deep divergence from I. monticola, being NML-II a posteriorly galani-introgressed one; c) also we cannot reject the null-hypothesis and assure that they constitute a different species from I. monticola because despite the marked difference, the genetic distance is lower than in other recognized lacertid species. As a result of that, we describe it as a subspecies of I. monticola (in fact, the unique subspecies genetically differentiated within this species). |
**Iberolacerta monticola astur** Arribas & Galán ssp. nov. (Fig. 9–12)

**Synonymy/Chresonymy:**


**Holotype.** (MNCN 44652) (ex OA09081509). An adult male (Fig. 9 E) from Salientes (Palacios del Sil, León, Spain). White cardboard label attached to the right forelimb (hand-written): La Poza del Puerto. Salientes (Le.). 15-VIII-2009. 09. A red plastic label (Dymo ®) attached to left hindlimb (in white letters and relief) "HOLOTYPUS". Oscar Arribas leg. In the MNCN collection (Museo Nacional de Ciencias Naturales. Madrid, Spain).

**Paratypes:** (23 males and 43 females). Ten males and ten females from Salientes and El Tambarón (Palacios del Sil, León, Spain), 7-VIII-2008 (OA08080701-10, 03, 10-19). Three males and six females from Salientes (Palacios del Sil, León, Spain) 15-VIII-2009 (OA09081502-08,10). One male and three females from Molar de Monrando (Palacios del Sil, León, Spain), 5-VIII-2006 (OA06080501-04). Two females from El Tambarón (Palacios del Sil, León, Spain), 9-VII-2007 and 17-VIII-2008 (OA07070901, OA08081707). One male and four females from Braña de Peña Vendimia (Palacios del Sil, León, Spain), 14-VIII-2009 (OA09081401-05). One male and two females from Collado de Ocidiello-Arcos del Agua (Igüeña, León, Spain), 14-VII-2009 (OA09071401-03). One male and two females from Los Bayos-Valdeloso (Murias de Paredes, León, Spain), 12-VII-2010 (OA10071201-03). One male and two females from Riolago de Babia (San Emiliano de Babia, León, Spain), 28-VII-2010 (OA10072801-03). One male and three females from Sierra de las Tiendas, Posada de Omaña (Murias de Paredes, León, Spain), 10 & 27-VII-2010 (OA10071001-03; OA10072901). Three females from Mortera de la Vieja-El Suspíron (Murias de Paredes, León, Spain), 9-VIII-2008 (OA08080901-03). Five males and six females from Sierra de Villabandín (Murias de Paredes, León, Spain), 8-VIII-2008 (OA08080801-11).

All paratypes with red plastic labels (Dymo ®) with white letters in relief "PARATYPUS" attached to their left hindlimbs. Six paratypes are cleared and alizarin-stained for bone study. Eight paratypes in the MNCN (Madrid) (MNCN 44653-44660; ex-OA08080708-10,14-16, 18 and 19, from Salientes, León; four males and four females), four in NHMW (Vienna) (NHMW 39207-39210; ex-OA09081501-04; from Salientes, León; two males and two females), and the remaining in Oscar Arribas study collection, that will be deposited in future in a Spanish public collection.

**Topogenetypes (sensu Chakrabarty 2010):** Sequences of Genbank are expressed in Table 1. Cyt b: HQ234900 (Salientes and Los Bayos), JN048500 (Riolago de Babia, La Cañada=Villabandín and Tiendas), JN048502 (La Cañada=Villabandín). CR: HQ234877-79 (Salientes and Los Bayos), EF121835 (Riolago de Babia, La Cañada = Villabandín, and Suspíron). Microsatellite data are in Dryad: doi:10.5061/dryad.rh05d.

**Diagnosis.** A relatively large *Iberolacerta*, especially characterized among NW Iberian *Iberolacerta* by the following characters: Low number of collar scales, high number of transversal ventral plate rows, lower counts of 4th-toe subdigital lamellae, relative low frequency of contact among rostral and internasal plates (only in near a third of specimens contact clearly). Postocular usually separated from parietal plate. No contact between supranasal and loreal plates. High number of axillary blue ocelli. Dark pigmentation in ventral plates less extended. Males with shorter hind and forelegs. Relatively longer pilei (especially in males). Greater maseteric and smaller tympanic plates. Less transverse ventral scale rows (in males) and smaller anal plate. High counts of premaxillary, maxillary and dentary teeth. Squamosal bone regularly arched. Males with 26 (rarely 25) and females with 27, 28 or 29 presacral vertebrae.

Partial mitochondrial DNA sequences for the Cyt b gene (398 bp) present a genetic distance of 5% to *I. galani*, 5.2–5.3% to *I. martinezricai* and 3.5% (14 mutations) to *I. monticola* s. str. Nuclear (Microsatellites) distances to *I. galani* are 0.64 (NML-II) to 0.82 (NML-I), and to *I. monticola* s. str. 1.07 (NML-I) to 1.2 (NML-II), being smaller to the former due to the past introgression. Internal Nei’s distance (microsatellites) between NML-I and NML-II is small (*D* = 0.243).

**Description of holotype.** An adult male with regenerated tail and a very distinctive bifurcate tail tip (Fig. 9 E). Biometry: Snout-vent length (SVL): 65.11 mm; Forelimb length (FLL): 20.56 mm; Hindlimb length (HLL): 31.57 mm; Pileus length (PL): 15.94 mm; Pileus width (PW): 7.51 mm; Parietal length (PaL): 5.77 mm; Masseteric scale diameter (DM): 3.13 mm; Tympanic scale diameter (DT): 1.79 mm; Anal width (AW): 4.24 mm, and Anal length (AL): 2.24 mm.
FIGURE 9. Specimens of *I. monticola* astur ssp. nov. from several localities across its distribution range: A.—Adult male in breeding period, with the typical green coloration. Note a typical disposition of scales in front of the masseteric plate. Salientes (Palacios del Sil, León)[loc. 2 in Fig. 1A]. B.—Ventral view of the same specimen of Fig. 9 A. Throat and belly show greenish tinges, being in this later in a gradation from darker toward the sides to clearer in the middle. C.—A young male specimen of the vermiculated morph in breeding period. Greenish to brownish gray above. Salientes (Palacios del Sil, León) [loc. 2 in Fig. 1A]. D.—Ventral view of the same specimen as in C. E.—HOLOTYPUS (MNCN 44652). A brown colored male with eclipsed coloration in summer (probably also brown during the breeding period). Salientes (Palacios del Sil, León) [loc. 2 in Fig. 1A]. F.—Male from Sierra de las Tiendas (Murias de Paredes, León) [loc. 10 in Fig. 1A]. Eclipsed coloration still showing green coloration. G.—Male from Sierra de Villabandin (Murias de Paredes, León) [loc. 14 in Fig. 1A]. Summer (eclipsed) coloration. Moreover the animal is close to the molt and shows a very dark appearance due to the old skin. In this locality, NML-II haplotypes have been identified. However, these animals fall perfectly inside the morphological variability of the NML-I haplotype populations. H.—Hatchlings from Salientes (Palacios del Sil, León) [parents from loc. 2 in Fig. 1A]. Tail tips cut for genetic analysis.
FIGURE 10. Specimens of *I. monticola astur* ssp. nov. from several localities across its distribution range: A.—Adult female from Salientes (Palacios del Sil, León) [loc. 2 in Fig. 1A]. Note the more typical disposition of temporal scales in front the masseteric plate. B.—Young female from Los Bayos (Murias de Paredes, Leon) [loc. 12 in Fig. 1A]. In the foothills of the Sierra de Villabandin (where NML-II haplotype was identified) these specimens show NML-I haplotypes and are morphologically indistinguishable from all the other *I. m. astur* ssp. nov., including the Villabandin ones with NML-II haplotype. C.—An old female from El Tambarón, near Salientes (Palacios del Sil, León) [loc. 4 in Fig. 1A]. Very clear and contrasted pattern appears in these specimens living over quartzites instead of slates. This animal was the first NML-I specimen sequenced and identified. D.—The same specimen of Fig. 10K, in ventral view. E.—Female from El Molar de Montrando, near Salientes (Palacios del Sil, León) [loc. 4 in Fig. 1A]. Also clear colored and living over quartzites. F.—Young male from Sierra de Villabandin (Murias de Paredes, León) [loc. 14 in Fig. 1A]. Summer (eclipsed) coloration. See also fig. 9G. G.—Old female from Mortera de la Vieja, near El Supirón summit (Murias de Paredes, León) [loc. 11 in Fig. 1A]. This is the second area where NML-II haplotypes have been found. H.—Same specimen of Fig.10G in ventral view. Note the bluish tinge of the lateral ventral scale rows, found in other female specimens from this area and Sierra de las Tiendas.
FIGURE 11. Head of a male *I. monticola astur* ssp. nov. from Alto de la Poza del Puerto, Salientes, Palacios del Sil (León prov.) (Paratype NHMW39210 [ex-OA09081504]) showing the characteristic temporal disposition with a big masseteric and two enlarged scales ahead.

Ratios: FLL/SVL (relative forelimb length, "FLL index"): 0.31577; HLL/SVL (relative hindlimb length, "HLL index"): 0.48487; PL/PW (pileus shape, "Pileus index"): 2.1225; DM/PaL (relative masseteric plate size, "Masseteric index"): 0.54246; DT/PaL (relative tympanic size, "Tympanic index"): 0.3102; AL/AW (anal plate surface, "Anal form index"): 0.5283019; and AS/SVL (√(AL*AW)*100/SVL, relative anal plate size with respect to the total length, "Anal size index"): 4.7332.

Scalation: Supraciliar Granula (GrS): 9 in right and 8 in left side; Gularia (GUL): 22; Collaria (COLL): 10; Dorsalia (DORS): 51; Ventralia (VENT): 27; Femoralia right (FEMr): 18 and left (FEMl): 19; 4th. Digit Lamellae (LAM): 25; and Circumanalia (CIRCA): 6. Absence of contact between Rostral-Internasal (R-I), Supranasal-first Loréal (Sn-Lor) and in Postocular-Parietal (Po-Pa).

Coloration: (in life, outside the breeding period) (Fig.9 E). Pileus with tiny, irregular and vermiculated spotted. Very few and small spots on the sides of the gular area. Some spots in the fourth submaxillary plate. Dorsal tract grayish-orange (6B5) [5YR 7.5/3.9] in life (brownish-gray (52C) [6YR 6.8/1.1] in alcohol). First third of the dorsal tract with small spots similar to the pileus ones, coalescing in bigger transversal marks that converge together forming an irregular vertebral band and that covers all the width of the dorsal tract. Temporal and infratemporal bands fused and forming a network, leaving small whitish spots, more clearly in the area within these two original bands and on the lower part of the flanks, and also specially marked in the upper scalloped ridge of the temporal (costal) band. This temporal band starts at the eyes, between the two black lines from the temporal area above mentioned, and runs along the sides, where it narrows and appears faintly on the sides of the tail. The tail, regenerated from near its basis has a clearly marked discontinuous line continuation of these temporal bands, and very faint, the correspondent to the dorsal tract. The tail tip is bifurcated in this concrete specimen. Four blue (21A7) [6PB 5.0/12.4] ocelli in each side: three well marked, of them two are closer to the shoulder, and other more backwards. The fourth one is a very small spot in the upper scalloped temporal-band limit). Small blue dots on the
outermost ventral plates. Venter pastel green (29A4) [6GY 8.7/3.1], more greenish white towards the limits of the gular area (29A2) [4GY 9.0/1.0]. The four external ventral plate ranges with black spotting (the two outermost well developed, complex and connecting with the reticulate of the flanks, surrounding the blue spots; the two intermediate rows only with thin marks on the foremost border). One spot on the posterior free border of anal (preanal) plate, and a bit black in the anterior seam. Blue ocelli on the shoulder UV-reflective, as are the blue spots on the outermost ventral ranges.

Variability. Biometric and scalation values of *I. m. astur* ssp. nov. and comparison with the other *Iberolacerta* species from the NW Iberian Peninsula are shown in Tables 2 (males) and 3 (females). Pictures of *I. m. astur* ssp. nov. from different populations are in Fig.9 A–H and 10 A–H.

Concerning certain singular scalation characters, from 63 lizards studied, 8 exhibit an azygos scale between prefrontals (12.7% of total specimens). This proportion varies among localities: in the numerous Salientes sample (loc. 1–8 in Fig. 1A) is present in the 11.1% (5 out of 45), whereas in South Villabandín (loc. 14 in Fig. 1A) reaches a 27.2% (3 out of 11). None of the Sª de las Tiendas-Suspirón or Babia (loc. 9–11 and 15 in Fig. 1A, respectively) specimens studied presented this anomaly (7 studied in total).

Variability in the coloration of breeding males (Fig. 9 A–D): In life, dorsal tract from green (28A6) to yellowish-green (29A7) [8GY 8.0/6.4 to 7.5GY 8.0/8.7] and to greenish grey (28B2) [5GY 8.0/0.8] in the vermiculated morph, all them during the breeding period (in Salientes area) (Fig. 9 C). Outside breeding period (Fig. 9 E–G, Fig. 10 F), as the holotype, with dorsal tract grayish-orange (6BS) [5YR 7.5/3.9] (base color, less green in general, even yellowish grey (3D2) [4.5Y8.1/1.2], Dorsal tract with irregular dots, slightly transverse, aligned towards the dorsum center, leaving clearer ground tone (without black) areas toward sides. Alternatively, there can be two paravertebral rows of dots, aligned and also slightly transverse, nearly contacting with the costal (temporal) bands. Old males are usually more pigmented, with irregular black dots across all the wide of the dorsal tract, even coalescing among them when bigger. Sides (costal or temporal band) in all adult males reticulated in black, united to the costal-inferior line, and inclosing clearer ocelli inside (or blue in the axilar area). Upper temporal band edge scalloped, very irregular. Shoulder ocelli vivid blue (22A8) [5PB 4.5/14.2] in number of 1 to 5 (usually 2 or more frequently three), highly reflective in UV light (Arribas 2012; photo 20). Ventral punctuation well developed in the two outermost ventral ranges (Fig. 9 B, D). Throat and venter light yellow (sulphur yellow) (1A5) [1GY 8.9/6.2], yellow (2A6) [8.5Y 8.8/8.4], or greenish yellow (1A7) [1GY 8.8/10.4] or a gradation from yellowish green (29A8) [7GY/7.7/11.3] to pale green (29A3) [6GY/8.9/2.1] from inside to outside the ventral ranges, or (30A2) greenish white (1GY/9.1/1.1). Rarely yellowish white (1A2) [9Y/9.1/1.3] or yellowish white pale (2A2) [7Y/9.1/1.3] in the vermiculated morph (Fig. 9 D). The blue maculae in the outermost ventral ranges are highly reflective in UV.

Variability in the coloration of Females (Fig. 9 I–K, Fig. 10 C, E–G): Dorsum pale yellow (3A3) [5.5Y 9.0/2.8], cream (4A3) [3Y 8.9/2.8], dull yellow (3B3) [5.5Y 8.0/2.6], yellowish grey (3D2) [4.5Y 8.1/1.2] or yellowish white (2A2) [6Y 8.1/1.2]. Presumable aged (bigger) females with medium-small dots aligned in the middle of the dorsum (without forming two paravertebral bands), usually leaving clear areas at the sides of the dorsal tracts. Alternatively, the dots are greater and coalesce, but without forming a network as in males, are not transversely oriented, more rounded and without contacting with the temporal bands (except in very old females). Young females are perhaps the most variable sex/age category: some with few and small points in the middle of dorsum, more widespread in the neck and first third of the back; some with two centered paravertebral rows; some with irregular vermiculated marks, or even slightly transverse but without covering all the dorsal tract except in the first third of the body. In some mid-grown females, the costal (temporal) band can appear very faintly reticulated. Temporal bands uniform, with a black slightly serrated upper border but fairly less than in males, and with diffuse limits in the lower parts. The rest of the temporal band, below the black upper limit, is brown and uniform (not reticulated), clearer in tone than in males. From 0 to 4 axillary blue ocelli (numbers of one and two equally frequent, 3 and 4 exceptional). Below the temporal bands, the clear lateral-inferior line can be visible and formed by faint small clear dots, and the dark inferior band can be also visible and composed of small dark dots. Ventral parts (Fig.10 D, H) fairly uniform, only with single and small points in the outermost ventral ranges, as well as some scarce blue points, highly reflective under UV light. Mid-grown females usually lack ventral dark punctuation in the outermost ventral ranges. Belly pigmented in light yellow (2A5) [8.5Y 8.9/6.2], yellow (2A6) [8.5Y 8.8/8.4], or yellowish white (1A2) [9Y 9.1/1.3]. In some individuals, the whole outermost ventral ranges are bluish white (23A2) [2.5PB 8.6/1.8] or pale turquoise (24A3) [6.5B 8.2/2.1].
FIGURE 12. Variability (deviations from the most common disposition depicted in Fig. 11) in the temporal area of *I. monticola astur* ssp. nov. Left side (from top to bottom; oriented to the right): Puerto de los Portillinos; Alto de la Poza del Puerto; El Molar de Montrando; Alto de los Grillos (all four in Salientes, Palacios del Sil, León province); Sierra de Villabandín -Southern slopes-; and Mortera de la Vieja -El Suspirón—(the two in Murias de Paredes, León prov.). Right side (from top to bottom; oriented to the left): Pico Valdiglesias (Salientes, Palacios del Sil, León prov.); Los Bayos; El Campón de Posada; Sierra de las Tiendas (the three in Murias de Paredes, León prov.); and Riolago de Babia (two specimens) (San Emiliano de Babia, León prov.).
Hatchlings and young specimens (1st and 2nd calendar years) (Fig. 9 H): There is sexual dimorphism in the hatchlings, as in other species of the I. monticola group. Dark pattern is similar to the adults one, but more reduced. Specimens with small dorsal points and slightly reticulated temporal bands are males, whereas dorsal tracts with few or barely discernible marks and fairly homogeneous sides indicate a female. Both sexes have low-lateral clear line formed by clear ocelli. Axillary ocelli yellow, very contrasting and dark edged. Dorsum with greyish yellow (2B3 and 2B4) [8Y 8.0/2.5 to 9Y 8.0/4.1] to orange grey (5B2) [6YR 8.0/1.6] background. Venter without color pigmentation, with the first (outer) row of ventral scales densely spotted, but the inner second and third pairs of rows less patterned. The outermost row encircles clear ocelli (precursors of the blue dots?). Tail is blue (2B27 and 23B7)[5PB 4.9/11.0 and 1PB 5.1/9.2] with clear points towards the lateral parts of the caudal prolongation of the dorsal tract, a dark continuation of the temporal band in its sides and, occasionally also a few marked dark band in the dorsal part, prolongation of the dorsal tract.

Comparative notes. Iberolacerta monticola astur ssp. nov. differs significantly (p<0.05; if underlined, p<0.01) from its most closely related taxa in the following characters, analyzed for males (m) and females (f) independently (Tables 2 and 3): from I. monticola s. str. (“cantabrica”) in body length (f), relative length of the pileus (f), relative forelimb length (m), relative hidlimb length (m), relative anal size (m, f), anal plate shape (m), postocular-parietal contact (f), Rostral-Internasal contact (f), left side granula supraciliaria (f), the number of ventral plates (m, f), circumanal plates (f), subdigital lamellae (m, f), ventral punctuation (m, f) and blue ocelli (m). From I. galani differs in collar scales (m, f), supranasal-lookal contact (m, f), dorsal scales (f), ventral plates (f) and right side femoral pores (f), subdigital lamellae (f), rostral-internal contact (f) and ventral punctuation (m). The presence of an azigos scale between prefrontals is moderate I. m. astur ssp. nov. (NML-I: Molar de Montrando, León) presents a genetic distance (Cyt s. str. 1.07 (NML-I) to 1.2 (NML-II), being smaller to the former due to the past introgression. Internal Nei’s distance (microsatellites) between NML-I and NML-II is small (D(NML-I), and to I. galani s. str. (7). Also, it has slightly more maxillary (average 17.25 vs. 16.38 in I. monticola s. str. and 16.5 in I. galani) and clearly more dentary teeth (average 21.5 vs. 17.76 in I. monticola s. str. and 18.5 in I. galani). By other side, its Squamosal bone is arched, similar to the ones of I. monticola s. str. and other Iberolacerta spp. and different to the singular and markedly straight of I. galani.

Genetically, I. m. astur ssp. nov. (NML-I: Molar de Montrando, León) presents a genetic distance (Cyt b) of 5% (20 mutations) to I. galani from Sanabria (Zamora) and 3.5% (14 mutations) to I. monticola s. str. from Peña Ubiña (León) (Carranza com.pers.). Distance among I. galani and I. monticola is 4.5 % (18 mutations), and distances of the three abovementioned (galani, monticola and astur) to I. martinezricai reach 5.2–5.3%. Tree topology is equivalent in Remón (2011) and Remón et al. (2013), who find for CR and Cyt b, a mean neat p-distance of 2.32% to I. monticola s. str. (corresponding to 1.8 [1.46–2.29] My), and 4.20 % and 3.81 5 to I. galani and I. martinezricai, respectively. Mitochondrial relationships among the taxa can be expressed as nearly a trichotomy (martinezricai, galani and (monticola s. str. plus monticola astur ssp. nov.)). Iberolacerta m. astur ssp. nov. is an early offshoot of the I. monticola branch, with distances to I. martinezricai and I. galani equivalent to the ones of the own I. monticola s. str. (Remón, 2011; and Remón et al., 2013; Carranza unpublished). Mitochondrial tree topology is expressed in Fig. 6. Nuclear (Microsatellites) distances to I.galani are 0.64 (NML-II) to 0.82 (NML-I), and to I. monticola s. str. 1.07 (NML-I) to 1.2 (NML-II), being smaller to the former due to the past introgression. Internal Nei’s distance (microsatellites) between NML-I and NML-II is small (D = 0.243) (see Fig. 8 for a tridimensional representation of these taxa).

The karyotype of I. m. astur ssp. nov. is largely similar to the I. monticola s. str. and I galani ones, and composed of 2n = 36 acrocentric macrochromosomes (no microchromosomes). NORs and the major ribosomal genes were located in the subtelomeric region of chromosome pair 6. Hybridization signals of the telomeric sequences (TTAGGG)n, were visualized at the telomeres of all chromosomes and interstitially in five chromosome pairs. C-banding showed constitutive heterochromatin at the centromeres of all chromosomes, as well as clear pericentromeric and light telomeric C-bands in several chromosome pairs. C-banding revealed the presence of a heteromorphic ZW sex chromosome pair, where W is smaller than Z and almost completely heterochromatic. Both NML-I and NML-II haplotyped specimens have the karyotype above described (Rojo et al. 2013).

Derivatio nominis. Astur/és, an adjective. Refers to the inhabitants of the Conventus Iuridicus Astur. Previously to the Roman conquest, Astures were the riverains of the Astura flumen (today the Esla river, in León)
with their capital in Asturica Augusta (Astorga, León) and their territory called by Romans Asturia. Pliny distinguished between Astures Augustani (today León, W Ourense, N Zamora and NE Portugal) south of the Cantabrian Mountains (whose occidental part was called Asturum Iuga) and Astures Transmontani (today Asturias) north of the Cantabrian Mountains.

**Distribution.** Northern Montes de León (Sierra de Gistreo sensu latissimo): Gistreo, Catoute, Tambarón, Nevadín, Villabandín (or Macizo del Alto de la Cañada), Arcos del Agua (or Fernán Pérez), Tiendas and Suspirón (Fig. 1A).

**Habitat.** Geology of the zone is varied: Candama Formation, with Cambrian quartzites, sandstones, and dolomites (Salientes Valley, Tambarón, Sierra de Villabandín). Mora Formation: Cambrian schists, slates and sandstones (Nevadín and Collado de Ocidiello). Los Cabos Series: Cambroordovician quartzites, sandstones and slates (Salientes, Valdeiglesia, Robeza, Catoute, Arcos del Agua, Peña Cefera and Suspirón) (Matas 1982). Summital peaks in the area were converted into “horns” by glacier erosion, whereas their connecting spurs still conserve rounded and extensive forms, locally called “lombos”, of some km long and above 1800 m, marking the pale relief of the area. Huge glaciers developed in this area, due to the combination of abundant precipitation coming from the Atlantic Ocean and increasingly colder (more continental) conditions. The most important rock glacier from all the Western Cantabrian Mountains is just in this area (in Arcos del Agua).

In general, glacier apparatuses during the Pleistocene were more developed in valleys facing to the East (facing to the Castilian Meseta, colder and with greater snow persistence), whereas Western-facing ones had almost no glacier modeling, except the Salientes and Salentinos valleys, that had glacier cirques in the highest parts of the northern slopes. Frequently, valley tongues reached dimensions longer than 4 km, descending to 1400 m a.s.l. in several places. Deglaciation in the area (Laguna de Villaseca) has been dated from 34000±1400 years before present (Jalut et al. 2004).

Climate (and consequently the vegetation) of the area is, as in other NW Iberian zones, deeply conditioned by the oceanic influence. This oceanic-influence gradient decreases from NW (high oceanic influence) to the SE (continental, dry and thermally more extreme). Climate is Temperate-Moist in the classification of Koppen, with 1000–1400 mm of average annual rainfall, 15–20 snow fall days yearly and an average temperature of 7.5º–10ºC. Detailed data on the climate of the area can be found in IGN (1992) and Ninyerola et al. (2005). It is noteworthy among the climatic parameters, the steep gradient in the evaporation across the study area, which can have an influence in lizard scalation (see Arribas et al. 2006). In general this area is fairly sunnier than the main Cantabrian Mountains, and is well known among mountaineers that these mountains frequently offer sun when in the main Cantabrian Range is raining or under a cloud-mantle (Walker 2002; Alvarez-Ruiz 2011).

Concerning vegetation, the area inhabited by lizards is situated above the level of oak forests (Quercus petraea and Q. pyrenaica) or in areas where the upper limits of these natural vegetation has been destroyed, giving pass to siliciculous dwarf-juniper formations with bog bilberry (Juniperion nanae-Vaccinietum uliginosi, with Juniperus communis ssp. alpina, Cytisus oromediterraneus and Vaccinium spp.), broom formations (associations Cytiso cantabrici-Genistetum polygaliphyllae and Cytiso cantabrici-Genistetum obtusirraneae), with Cytisus cantabricus, Genista florida ssp. polygalphylla and G. obtusirranea), heathlands with gorse (assoc. Daboecio cantabricae-Ericetum aragonensis and Daboecio cantabricae-Ulicetum gallii, with Daboecia cantabrica, Erica australis ssp. aragonensis, Pterospartum tridentatum and Ulex gallii). In deeper soils appear hazel (Corylus avellana), holly (Ilex aquifolium), rowan (Sorbus aucuparia), whitebeam (Sorbus aria) and birch (Betula celtiberica) (Navarro-Andrés & Valle-Gutierrez 1987).

*Iberolacerta m. astur* ssp. nov. is sympatric with other reptiles as *Podarcis bocagei*, *Lacerta schreiberi*, *Anguis fragilis* and (exceptionally) with *Zootoca vivipara*, and can be not only sympatric but even syntopic with *Podarcis muralis*. Two snakes, *Coronella austriaca* and *Vipera seoanei* are also syntopic and certainly predators of this lizard.

The study area, the Alto Sil, Las Omañas and nearby zones, is one of the wildest and with best conserved landscapes of the Iberian Peninsula –despite that it is not even a natural park– although it is frequently ignored and almost unknown by the great majority of mountaineers and tourists (Alvarez-Ruiz 2011). An idea of this environmental value is represented by the presence of the brown bear, wolf and capercaillie that still survive in the area, but curiously, only one endemic beetle species, *Iberodorcadion vanhoegaerdeni* (Breuning, 1956) has been described from here (Tomé, Berger & Bahillo 2001) probably due to the lack of prospection in these unexplored mountains. There are no important threats to this lizard that lives well within the silvopastoralism developed in the
area. However, in areas where dry and cold persistent wind blows almost without interruption (as witnessed by the proper name Suspirón, “a mountain which sighs”) and where lizards are scarce per se, there is an increasing and worrying proliferation of wind farms.

Discussion

Our study revealed some discordance between different approaches, especially the patterns of variation of nuclear microsatellites and mtDNA, namely a very low nuclear differentiation between relatively highly differentiated mtDNA lineages. This kind of result is not uncommon in reptiles, and may obey to different, not mutually exclusive causes (see, for example Rato et al. 2010 and references therein). Our case is essentially similar to the picture drawn for Lacerta schreiberi in the mountains of the Iberian Central System (Godinho et al. 2008). Here, as there, different groups of populations persisted through Pleistocene climatic cycles in close geographic areas, and probably experienced multiple episodes of admixture as they cyclically expanded and contracted in response to successive ice ages. Figure 13 depicts schematically four major alternative models of evolution for the populations of the Northern Montes de León. Only the first one is fully compatible with the observed patterns of microsatellite and mitochondrial variation. After a separation from I. monticola ca. 1.8 Mya, the populations in this geographic region must have suffered two different waves of immigration (or almost two different hybridization events) from I. galani, the second one of them not much later than 0.5 Mya (see extension of model I in Fig. 13). These results support a gradual view of speciation, whereby some lineages may persist as distinct genetic entities despite some permeability to exchange from neighboring populations, thus frequently giving rise to cryptic species complexes whenever no phenotypic correlates of such differentiation are evident (Pinho et al. 2007, 2008).

**How many species in the North Montes de León?** Our morphological approach was unable to discriminate any of the NML populations as really different from the others from the same area. The same was confirmed by microsatellite analysis. Thus, we consider them homogeneous and belonging to the same taxon that we described above as a new subspecies: I. monticola astur ssp. nov.

Moreover, the numerous sample of Nevadín-Tambarón-Catoute (loc. 1–8 in Fig. 1A) mountain ridge embraces inside its morphological variability almost all the other samples from the area (see Figs. 2 & 3). The CDAs render non-significant results, even in its first axes that accumulate the largest amount of variability. The ANOSIM analyses were also not significant, which means that there is more variation inside the defined groups than among the groups themselves. Also, the PCA analyses were unable to segregate any single specimen or samples from the main cloud of individuals.

In spite of the presence of an haplotype lineage closely related to the I. galani clade (MNL-II in our text) in some specimens of Sierra de Villabandin (loc. 14 and 15 in Fig. 1A) and Suspirón (loc. 11 in Fig. 1A), these populations are morphologically indistinguishable from the others showing the lineage labeled as “Gistredo” (from loc. 2 and 3 in Fig. 1A) in Remon et al. (2013), here denoted as NML-I, which is phylogenetically closer to I. monticola. Moreover, these NML-II haplotypes are not very recent (see Parham et al. 2001), since they are slightly variant from I. galani. Our microsatellite results also indicate that all the NML populations are genetically similar in terms of their nuclear genomes, independently of their mitochondrial differentiation. All these evidences point towards the fact that there is only one morphotaxon in the area: I. m. astur ssp. nov.

Past hybridization among species is a phenomenon that is increasingly known. Examples of these are the presence of Lissotriton vulgaris (Linnaeus, 1758) mitochondrial haplotypes in a substantial number of L. montandoni (Boulenger, 1880) specimens (otherwise morphologically homogeneous, as our NML-taxon). Lissotriton montandoni haplotypes are distributed in six groups, five of them also including L. vulgaris haplotypes (Babik et al. 2005). In lizards, introgression between species has been found among different Podarcis hispanica-complex cryptic taxa, identified as different taxa first by its morphology and nuclear markers, and not by their own mitochondrial haplotypes, one of them unknown and presumably displaced by an alien mtDNA from the other one (Renault et al. 2009); the presence of shared mtDNA haplotypes in the Podarcis hispanica-complex and in morphologically typical Podarcis bocagei (Seoane, 1884) individuals from the Ria de Arousa (Arntzen & Sá-Sousa 2007), or the presence of Darevskia mixta (Méhely, 1909) mtDNA haplotypes in D. alpina (Darevsky, 1967) specimens (Murphy et al. 2000). All these examples are considered to be the result of past hybridization events.
FIGURE 13. Comparison of the expected outcomes of four major geographic models of evolution. M = *I. monticola* s. str.; G = *I. galant*; NML = *Iberolacerta monticola astur* ssp. nov. from the Northern Montes de León. The three main cladogenetic events dated by mitochondrial markers are indicated by the width of the lines separating the populations. Block arrows indicate direction of gene flow after the initial separation of *I. m. astur* ssp. nov. Arrowheads within text boxes under each model mark the expected tendency (upward or downward) of the corresponding indicators of nuclear genetic divergence. Reference values of divergence, obtained from the observed data, are shown at the bottom of the figure.
If true *I. galani* were present in the NML we should have detected its presence in the multivariate analysis. It is important to take into account that the two areas of *I. m. astur* ssp. nov. that show the NML-II haplotypes correspond to the most continental and harsh localities within its distribution range, facing to the Meseta plain. Since *I. galani* is a more continental-climate dwelling species, this may explain why the contact took place in these two areas. *Iberolacerta galani* probably became isolated and survived in an inland refuge during the cold Pleistocene waves (see Arribas et al. 2006), and when its range extended to the NML area (thus entering into contact with the populations living there), it was following the coldest areas. Alternatively, it might have survived in the region until the last moment, just in these colder and more marginal Meseta-facing areas.

Concerning the main nucleus of *I. monticola astur* ssp. nov., the mountains surrounding the Salientes and Valdewiglesias massifs form an arch from the Nevadin, and across the Puerto del Portillín (or Alto de Vivero), Molar de Montrando, Tambarón, Peñón de Brañalibrán, Picós de la Robeza, Peña Carnicera, Cerneya, Catoute and Montrando, Tambarón, Peñón de Brañalibrán, Picós de la Robeza, Peña Carnicera, Cerneya, Catoute and Valdeiglesias massifs (loc. 1–8 in Fig. 1A). Specimens from these areas are mitochondrially homogeneous (NML-I lineage) and appear depicted in Fig. 9 (A–E) and Fig. 10 (A and C–E). Morphologically, this sample (SALIENTES) had been used to compare with other isolated populations, and despite small local differences (probably a “familiar effect” by endogamy in isolated small populations in shepherd huts, dry stonewalls, etc., surrounded by unsuitable habitats as meadows or broom heathlands), it can be concluded that all of them are fairly homogeneous between populations but fairly variable inside each population (as is characteristic of *Iberolacerta* ssp. populations). Their variability (the cloud of points in Figs. 2 & 3) embraces all or nearly all the variability of the other samples from the NML. Towards E and SE from this main area (SALIENTES), these populations connect across moderately high passes (as Collado de Ocidiello) with the Arcos del Agua massif (at 5 km straight-line; loc. 9 in Fig. 1A), also bearing a NML-I haplotype (genotyped by S. Carranza, com. pers.). The area continues across the narrow ridge of Sierra de las Tiendas until the Suspiróin peak (at a distance of 5 km; loc. 10 and 11 in Fig. 1A). This region is very interesting as it is a ridge without barriers that allows the past and present contact between NML-I and NML-II haplotypes. Arcos del Agua specimens (loc. 9 in Fig. 1A) present a NML-I haplotype and morphologically also fall clearly with the SALIENTES sample, as did one male (Fig. 9 F) and two females from the Sierra de las Tiendas (more to the SE of the area; loc. 10 in Fig. 1A). The only morphological exception in this area is one female that falls more towards the *I. galani* side in a partial CDA (not represented), but without true overlap. More to the East (towards the Meseta, with more continental climate), Sierra de las Tiendas (loc. 10 in Fig. 1A) is a smaller crest that connects Arcos del Agua (=Fernanperez; loc. 9 in Fig. 1A) with the Suspirovón peak (loc. 11 in Fig. 1A; where NML-II haplotypes have been found). The single genetically examined female from this latter locality has a NML-II haplotype, but, morphologically, the three specimens studied from this locality (from Suspirovón and neighboring Mortera de la Vieja) (specimens Fig. 10 O–P) appear like beads-on-a-string situated in partial CDA (not represented) among the typical *I. m. astur* ssp. nov. from SALIENTES (NML-I haplotype) and the true *I. galani* scores. This is one of the two areas where traces of the ancient introgression between *I. galani* and *I. monticola astur* ssp. nov. have been found. Lizards are, however, relatively scarce and live in low densities in this area, probably as a result of the extreme environment, as this is the first mountain range in front of the continental Castilian Meseta and the climate is fairly arid and dry. The second past-hybridization footprint lies at the Sierra de Villabandín (specimens Fig. 9 G and 10 F; loc. 14 in Fig. 1A). Specimens from this area all had the same NML-II haplotype. However, morphologically, all these specimens fall totally within the typical *I. m. astur* ssp. nov. (NML-I haplotype) side in all CDA partial analyses (both for males and females) and never in the *I. galani* side, as could be expected if they were true *I. galani* or any hybrid characters were to be present. In the northern slopes of these same Villabandín mountains, specimens from Riolago de Babia (loc. 15 in Fig. 1A; also NML-II) are morphologically perfectly matching with typical NML-I populations (SALIENTES) of *I. m. astur* ssp. nov. and are even the most different from the true *I. galani* in CDA analyses. On the other hand, true *I. monticola* s. str. live at the other side of the Luna river, situated only 6 or 7 km further North than these *I. m. astur* ssp. nov. populations from the Sierra de Villabandín (inverted triangles in Fig. 1 A & B).

Special mention merit the localities of Los Bayos and the Valdeloso valley (loc. 12 and 13 in Fig. 1A), which enter towards the core of the Sierra de Villabandín (our BAYOS sample), situated in the extreme West of the Sierra de Villabandín. In this area, genetically studied specimens belong to the NML-I lineage, contrary to the main populations of Villabandín, which are NML-II. Morphologically, females (depicted in Fig. 10 B) also fall inside the NML-I side of CDA, but the only studied male (from Valdeloso valley) falls towards the *I. galani* side of the CDA (but not strictly among the true *I. galani* cloud of specimens).
Further East of the Sierra de Villabandin, other prospections resulted unfruitful (Abelgas de Luna, Collado del Remansadero -1520 m-, East of our localities in Fig. 1A). A combination of diverse factors, such as changes in the geologic materials, paleogeographic variations in the ancient river watershed, and an increasing aridity, are probably the causes of the eastern limit of Iberolacerta in these mountains.

Relationships of the Northern Montes de León lizards (I. m. astur ssp. nov.) with other NW Iberian Iberolacerta taxa (I. monticola s. str. and I. galani). Once demonstrated that all the populations of NML are morphologically homogeneous and their microsatellites congruent with this, independently of the mitochondrial haplotypes that they bear (NML-I or NML-II), we have assumed that all belong to the same taxon: I. monticola astur ssp. nov., and we will compare the differences between I. m. astur ssp. nov. (the NML-taxon) and Iberolacerta monticola s. str. (from Central Cantabrian Mountains) and I. galani (from Southern Montes de León). The Discriminant Analysis in males (Fig. 4) best separates I. monticola from the two other samples (I. galani and I. m. astur ssp. nov.). It is significant that the worse classification in ANOSIM was between I. m. astur ssp. nov. and I. galani (0.07). Also, I. galani and I. m. astur ssp. nov. centroids appear closer in the CDA (Fig. 4). Iberolacerta m. astur ssp. nov. specimens are well individualized as a group (percentage of correct discrimination higher), but the hypothetic introgression from I. galani could have had some influence in the fact that, still being significant, the least good separation of male samples in ANOSIM appeared between I. galani and I. m. astur ssp. nov.

The outcome of females analysis is clearly better than that of males, both concerning the total percentage of correct classification in CDA (up to a 80.7 %) and the discrimination of the samples source by ANOSIM. Contrary to males, the first axis separates I. galani from I. monticola s. str. and I. m. astur ssp. nov., that result more similar among them (Fig. 5). As corresponds to their reciprocal species status, the best assignation in the ANOSIM analysis of females was between I. galani and I. monticola s. str., and the worse between I. m. astur ssp. nov. and I. monticola s. str., that we consider here as belonging to the same species. These differences coincide with the results of the mtDNA analyses shown in this paper and previous reports (Remón 2011; Remon et al. 2013).

Osteologically, I. m. astur ssp. nov. has more premaxillary teeth (average 8.3) than I. monticola s. str. (7.15) and I. galani (7), being more similar to I. cyreni (8 or 9, rarely 7; Arribas 1998). Also, it has slightly more maxillary teeth (average 17.25 vs. 16.38 in I. monticola s. str. and 16.5 in I. galani) and clearly more dentary teeth (average 21.5 vs. 17.76 in I. monticola s. str. and 18.5 in I. galani), again more similarly to I. cyreni (average 18.07 maxillary and 22.07 dentary ones; Arribas 1998). Vertebral numbers are largely equivalent to the other NW Iberian Iberolacerta, but we have detected unusual low numbers (25 for a male and 27 for a female) in I. m. astur ssp. nov., values not found in I. galani or I. monticola s. str. (27 is however the common value in females of other European small lizards, as in the Dynaric and Pyrenean species of Iberolacerta, whose males possess 26 vertebrae, but not in the monticola-group). Moreover, just in the two studied specimens from localities where possible introgression has been detected, there appear rare B-type processes coexisting in the same specimen among the usual A-type in the preautotomic vertebrae, although this same phenomenon is not unusual in other taxa of Lacertini (i.e. in 30% of the specimens of I. monticola s. str. from Cantabrian Mountains and in I. cyreni; Arribas 1998). The squamosal bone of the NML-taxon is also similar to other Iberolacerta, except I. galani that has it markedly straight (although one specimen of the NML-taxon, a Suspirón female, has in one side of the head a squamosal bone similar to I. galani). It is hypothesized here that this could be the result of hybridization between I. m. astur ssp. nov. and I. galani in this locality. In the raw numbers of teeth and vertebrae, the NML-taxon seems a bit more similar to I. galani than to I. monticola s. str. but these similitudes can be influenced by ecological factors related to feeding habits or climate.

Concluding, whereas morphological differences in females coincide fully with mtDNA results, in males the relationship is just the contrary (oddly “parallels” the direction of the hypothesized past hybridization). Moreover, in both sexes’ ANOVA, I. m. astur ssp. nov. results more similar (less p<0.05 differences) to I. galani than to I. monticola s. str. Genetically, there is greater nuclear (microsatellite) similitude between I. galani and I. m. astur ssp. nov. and mitocondrially the characteristic haplotype of this taxon (NML-I) is more similar to I. monticola s. str, whereas the other one (NML-II) is clearly a galani- introgressed one.

Could be these discrepancies in morphology an effect of a greater bioclimatic similitude influencing the scalation (as suggested in Arribas et al. 2006 for other NW Iberian Iberolacerta and as the second axis in our CDA suggests), or the phylogenetic signal in morphology of a past local introgression?. Males could have a greater vagility than females so as to decouple, to some extent, the evolution of the nuclear and mitochondrial markers. This can explain why nuclear microsatellites of I. m. astur ssp. nov. and I. galani appear closer due to the hybridization, whereas female-inherited mtDNA reflects the true origin of the taxa, due to the smaller vagility of
this sex (in the unique species of the group where this has been studied, *I. cyreni*, females have smaller territories and greater sedentarity than males; see Perez-Mellado *et al.* 1988). Male biased gene flow has been shown in lizards by several authors (Doughty *et al.* 1994; Stenson *et al.* 2002; Podnar *et al.* 2005). Also, the similitude of the karyotypes of *I. monticola* s. str., *I. monticola astur* ssp. nov. and *I. galani* (Rojo *et al.* 2013) could have facilitated the hybridization of the two later in the studied area.

**Geographic framework and biogeography of the Northern Montes de León.** The current *I. m. astur* ssp. nov. distribution, cornered in the NW of the Northern part of the Montes de León, suggests a possible competitive exclusion between this taxon and *I. galani*, as the *galani* haplotypes (NML-II) appear cornered in the most continental areas, and speak of a very limited, even in the past, presence of this species in the area. However, we lack any proof of this, and the current situation recalls more for a scarce number of *I. galani* representatives, today witnessed by the presence of the NML-II haplotypes, that were soon absorbed by the typical astur-haplotype *I. m. astur* ssp. nov. (with NML-I haplotype).

Variation in watershed limits probably played a crucial role in the isolation of the different *Iberolacerta* colonization waves in this zone (Fig. 1B): Current surface of the different watersheds is very unequal and clearly favoring the Sil river, but this situation departs from a clearly inverse situation during the Neogene, when the watershed divisory was fairly more westwards than nowadays. The sinking of the Bierzo-graben during Miocene caused the Sil River to begin a strong retrogressive erosion (as its river base level is close to 0 m), reaching the Laciana area (part of their current upper course) just in the transit to Pleistocene (Fig. 1B), and capturing great part of the Luna river watershed, whose more splendorous past can be deduced from its disproportionate valley, very wide and deeply eroded for the small river that currently crosses Babia County. This capture phenomenon is probably responsible for the beginning of the isolation and differentiation process of *I. m. astur* ssp. nov. All these capture processes must have been rapid from the beginning of the Quaternary, because when glacial erosion began (Upper Pleistocene) the river watershed was nearly equal to the current one (García de Ce
dis 1997; Alfonso-Gómez 2003, and references therein). Changes in the watershed divisory and the sequential shift of the migration corridors between Cantabrian Mountains and the study area, can be followed in figure 1B (left: Neogene and Plio-Pleistocene; right: Holocene).

These changes in the boundaries among watersheds limited the contact between the NML and the main Cantabrian Mountains, restricting to narrow points (different along time) the contact between the two ranges, and thus, the areas for possible contact between *I. m. astur* ssp. nov. and *I. monticola* s. str. (see Fig.1B). The current contact zone, situated in Puente de las Palomas (1220 m) is too low and dry, devoid of *Iberolacerta* nowadays, both in the contact corridor (slightly wider of 3 km) as well as in the close isolated relieves (as El Pando, 1627m) currently occupied by *Podarcis muralis* and *P. guadarramae* (Boscá, 1916) (=*P. hispanica*-type 1 sensu Auctt.), but contact took place in the past across higher mountain crests (divisory pass theoretically first at 1600 m and later at 1450–1500 m; Alfonso-Gómez 2003) from Cueto del Oso (1904 m) and Cuerno (1932 m) from where the ancestors of *I. m. astur* ssp. nov. possibly crossed to our study area. Henceforth, Sil and Luna contact was cut-off by the Sil river erosion, opening the today so-called Cañones del Sil (“Sil Canyons”). From then on, the Sil and Luna rivers acted as a powerful barrier, and watershed limits shifted progressively towards the East, to lower areas, narrowing, fainting and finally totally interrupting the contact between both *I. monticola* ssp. (see all the sequence of phases in Fig.1B). Final divergence is dated from mtDNA about the end Pliocene or Lower Pleistocene (around 1.8 Mya).

More to the South, upper reaches from the Boeza River valley, nowadays separating the main area of *I. m. astur* ssp. nov. from their conspecifics at Arcos del Agua, Sierra de las Tiendas and Suspirón, flowed across Collado de Ocidiello towards the so-called Valle Gordo and the Omaña River (Matas 1982), constituting, in an indeterminate past moment, a barrier between the NML-II haplotype area and the main typical astur-haplotype area (NML-I), although nowadays there are populations of the latter at both sides (Arcos del Agua can be a recent colonization of NML-I to the East, into the NML-II area), as indicated above.

The Omaña River also had a part of its upper reaches captured by the Sil watershed (the so-called capture of the Puerto de la Magdalena). The shift of the watershed limits towards Omaña, probably posterior to the pass of *I. m. astur* ssp. nov. to the East of this river, towards the Sierra de Villabandín, and left NML-I populations (such as Los Bayos) cut-off at the other shore of the advancing Bayo river (belonging to the Sil watershed). The current higher passes among these Sierras (e.g. Alto Pozo la Mora, 1476m), seem covered by dry broom heathlands with few rock outcrops, and currently devoid of *Iberolacerta*. 

*IBEROLACERTA MONTICOLA ASTUR SPP. NOV. FROM NW SPAIN* Zootaxa 3796 (2) © 2014 Magnolia Press · 231
Concluding, it seems that the current main distribution area of *I. m. astur* ssp. nov. (especially the typical NML-I) gravitates around what was the divisory between watersheds in the past, later shifted to the East during the Quaternary.

Eastern known limits of *I. m. astur* ssp. nov. do not pass away from Collado de Campo Lamoso (1500 m), which today is perfectly suitable for the species, but during the Pliocene and the main part of the Pleistocene, constituted a barrier across which the two northern immediate valleys drained to the southern slopes. The West-East continuity of this massif during the end of the Miocene was broken by changes in the drainage across this pass in the Pliocene (geological datation uncertain). Although nowadays the pass to the East (to the Filera Massif, 1879 m) is possible for *Iberolacerta*, the prospections in these drier limestone areas had been unfruitful. In the north of these Sierras, the species can reach up to Cascaros peak (1854 m), but this extreme has to be confirmed.

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