

A new cryptic rock-dwelling species of the genus *Iberus* Montfort, 1810 (Gastropoda, Stylommatophora, Helicidae) from the southern Iberian Peninsula

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Abstract

A new cryptic rock-dwelling species of the genus *Iberus* Montfort, 1810 (Gastropoda, Stylommatophora, Helicidae) from the southern Iberian Peninsula. Since the description in 1957 of *Iberus ortizi*, a land snail endemic to the south-eastern Iberian Peninsula, there has been limited information about its distribution. It has traditionally been assumed that all populations of rock-dwelling *Iberus* snails inhabiting the Subbetic mountain ranges of Córdoba belonged to *I. ortizi*. In this study, intensive sampling throughout the entire potential area of *I. ortizi* allowed the identification of a new species (named *I. rutensis* sp. nov.) with conchological features somewhat intermediate between *I. ortizi* and *I. loxanus*. This new species occupies a limited area, divided into two populations. The first of these is located south of the Sierras Subbéticas Natural Park and the second is located in the central-eastern part of the massif. Here we present a comprehensive description of the species following an integrative taxonomic approach combining geographical distribution, conchological analysis (including a morphometric study) and phylogenetic position and relationships. We also provide new localities for the cryptic species *I. ortizi* and *I. marmoratus marmoratus*, extending their known distributions to the provinces of Jaén and Córdoba, respectively.

Key words: *Iberus rutensis*, *I. ortizi*, *I. marmoratus*, *I. loxanus*, Integrative taxonomy, Andalucía, Córdoba

Resumen

Una nueva especie críptica rupícola del género *Iberus* Montfort, 1810 (Gastropoda, Stylommatophora, Helicidae) del sur de la Península Ibérica. Desde que en 1957 se describió el caracol terrestre *Iberus ortizi*, autóctono del sureste de la península ibérica, la información sobre su distribución ha sido escasa. Tradicionalmente se ha supuesto que todas las poblaciones de caracoles rupícolas del género *Iberus* que habitan las sierras subbéticas de Córdoba pertenecen a *I. ortizi*. En este estudio, un muestreo intensivo en todo el área geográfica potencial de *I. ortizi* permitió identificar una nueva especie (denominada *I. rutensis* sp. nov.) con características conquiológicas intermedias entre *I. ortizi* e *I. loxanus*. Esta nueva especie habita en un área reducida dividida en dos núcleos de población, uno principal situado al sur del Parque Natural de las Sierras Subbéticas y otro secundario en la parte centro-oriental del macizo. En este trabajo se presenta una descripción detallada de la especie, resultado de un enfoque taxonómico integrativo que combina la distribución geográfica, el análisis conquiológico (incluido el estudio morfométrico) y la posición y relaciones filogenéticas. Además, se aportan nuevas localidades para las especies crípticas *I. ortizi* e *I. marmoratus marmoratus*, que amplían su distribución conocida a las provincias de Jaén y Córdoba, respectivamente.

Palabras clave: *Iberus rutensis*, *I. ortizi*, *I. marmoratus*, *I. loxanus*, Taxonomía integrativa, Andalucía, Córdoba

Introduction

Iberus ortizi García San Nicolás, 1957 is a restricted endemism from the southern Iberian Peninsula. Since its description more than 60 years ago, no further studies have contributed to its exact geographical distribution range, and only a few have focused on its phylogenetic relationships (Elejalde et al 2008a, Neiber et al 2021). Recently, Liétor et al (2024) found *I. ortizi* in a clade of small-sized *Iberus*, located in the southeastern Iberian Peninsula, together with *I. angustatus* (Rossmässler, 1854), *I. guiraoanus* (L. Pfeiffer, 1853) and three new species: *I. axarciensis*, *I. antikarianus* and *I. giennensis* Liétor, Tudela, Jódar, Jowers and Moreno-Rueda, 2024.

To date, *I. ortizi* had been considered the only species of its genus inhabiting the calcareous mountains across the Andalusian province of Córdoba (García San Nicolás 1957, Ruiz-Ruiz et al 2006, Bank and Luijten 2014, Cadevall and Orozco 2016). However, the calcareous ranges surrounding the Subbetic Mountains, where *I. ortizi* is found, are a hotspot for the genus, as up to five *Iberus* species converge there, a density that is significantly higher than the average density typically found in areas of similar size in the south-eastern Iberian Peninsula: *I. ortizi* is found along with *I. alonensis*-like O2 (*sensu* Elejalde et al 2008b), *I. loxanus* (A. Schmidt 1853), *I. giennensis* and *I. marmoratus marmoratus* (A. Férussac 1821) (Ruiz-Ruiz et al 2006, Talaván-Serna and Talaván Gómez 2011, Liétor et al 2024, see results). Clarifying the precise distribution of *I. ortizi* would therefore require further systematic study and intensive sampling across its entire range. To this end, we aimed to: i) delimit *I. ortizi* distribution across the southern region of the province of Córdoba and its bordering areas with the provinces of Jaén and Granada; ii) further explore the contact areas with other congeneric taxa; and iii) assess the genetic identification and phylogenetic position of specimens from key locations.

Material and methods

Field sampling

To precisely delineate the distribution of *I. ortizi*, we opted for intensive sampling that systematically covered all the calcareous mountain ranges and most of the hills of southern Córdoba Province (Spain). We established 72 sampling locations (fig. 1). Difficulties in accessing private farms and hunting grounds and the scarcity of specimens prevented us from conducting intensive prospecting in several of the originally planned locations. Therefore, whereas most locations were thoroughly sampled, resulting in large sets of shells and many other field observations, a few others were reviewed solely for the presence of *Iberus* snails.

Planning of the network of sampling points was based on: i) previous citations documented in specialised literature; ii) the presence of calcareous or, at least, sedimentary lithology (the need for lithologies that provide adequate levels of calcium to form the shells of terrestrial pulmonates has been widely demonstrated; e.g. Fournié and Chéteil, 1984); iii) the existence of relatively undisturbed mountain karstic habitats, and iv)

prior knowledge and field experience of the researchers. Although priority was given to locations that were accessible by road, we also included sampling points involving long routes (> 10 km) on foot.

Good quality shells sampled were cleaned later, and some of them were properly photographed for the figures which are included in this work. All the shells, including those that were sun-bleached and a few sub-fossils, were measured for morphometric parameters to complementarily support taxonomic work. Some live specimens were collected from key locations. All samples, both shells and biological tissues, were carefully labelled and managed to guarantee optimal traceability and conservation of the specimens.

Molecular analysis

Eight live specimens were sacrificed by drowning and tissue samples extracted for molecular analyses were stored in absolute ethanol and maintained at -20°C. Four specimens were tentatively identified as belonging to a new lineage (coded as M15, M16, M17 and AW1). The remaining four were classified as *I. ortizi* (samples for *I. ortizi* coded as N1, N4, N7 and W1 in table 1s in supplementary material). Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The total alignment comprises all known *Iberus* species from GenBank, as well as *Iberellus minoricensis*, *Rossmässleria sicanooides* and *Eremina dillwyniana* as outgroup taxa (table 1s in supplementary material).

We amplified fractions of the mitochondrial cytochrome oxidase I (COI), 16S rRNA and the nuclear large ribosomal subunit (LSU) following Jowers et al (2024).

Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and GenBank's BLASTn search (Altschul et al 1990) were used to edit sequences and to check for potential contaminants, respectively. Sequences were aligned in Seaview v.4.2.11 (Gouy et al 2010) with MAFFT V 7.505 with Q-INS-i settings (Katoh et al 2002). Uncorrected *p*-distances with partial deletion were computed in MEGA X (Kumar et al 2018).

Phylogenetic tree reconstructions (on the basis of three concatenated gene fragments with total length 1,984 bp) were performed using maximum likelihood (ML) and Bayesian inference (BI), through RAXML v8.2.12 (Stamatakis 2014) and MrBayes v3.2.673 (Ronquist and Huelsenbeck 2003), respectively. The Akaike Information Criterion (AICc) implemented in PartitionFinder v2.1.1 (Lanfear et al 2016) applied a greedy search (Lanfear et al 2012) to select the best-fit evolutionary model for each partition. The subsequent models and partitions were GTR+I (COI pos1), F81+I+G (COI pos2), GTR+I+G (COI pos3), GTR+I+G (16S rRNA) and HKY+G (LSU).

From the BI, we conducted two independent runs (each with four Markov chains for 10x10⁷ generations). Trees and parameters were sampled every 1,000 generations. The majority-rule consensus tree was estimated by combining results from duplicated analyses after the 25% of the total samples were discarded as burn-in. ML searches were performed under GTRGAMMA and support was evaluated using 1,000 bootstrapped replicates. All phylogenetic analyses

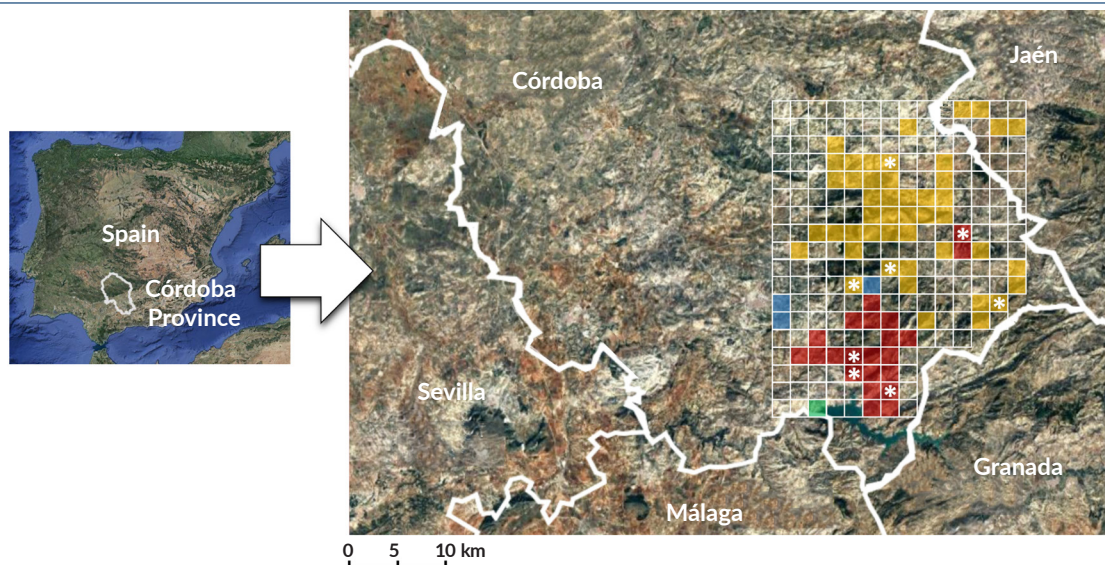


Fig. 1. Distribution of *I. ortizi* (light orange) and assumed samples from the new lineage (see results) (red) in the south-east of Córdoba Province (Spain). The nearest populations of *I. marmoratus marmoratus* (blue) and *I. loxanus* (green) to the core distribution of the new lineage have also been included. Each grid represents an area of 4 km² with the coloured grids showing the presence of *Iberus* land snails. The sample locations of the tissue samples used for the genetic sequencing of *I. ortizi* and the new lineage are indicated by asterisks.

Fig. 1. Distribución de *I. ortizi* (naranja claro) y muestras atribuidas al supuesto nuevo linaje (véanse los resultados) (rojo) en el extremo sureste de la provincia de Córdoba (España). También se han incluido las poblaciones de *I. marmoratus marmoratus* (azul) e *I. loxanus* (verde) más próximas al núcleo poblacional del nuevo linaje. Cada cuadrícula representa una superficie de 4 km² y las cuadrículas coloreadas indican la presencia de caracoles terrestres del género *Iberus*. Las ubicaciones de las muestras de tejido utilizadas para la secuenciación genética de *I. ortizi* y el nuevo linaje se indican con asteriscos.

were performed in the CIPRES platform (Miller et al 2010). The consensus tree was visualised and rooted using FigTree v1.4.4 (Rambaut 2018), and then prepared as a graphic with the software Inkscape v1.0.1 (<http://www.inkscape.org>).

Morphometric analysis

Shell morphometric parameters were obtained following López-Alcántara et al (1985) and Polo (2016). Measurements were taken with a digital calliper (accuracy 0.01 mm): major and minor diameters (Ø) of the shell, shell height, and major and minor external Ø of the peristome. According to these data, we estimated shell and peristome areas by considering that both the shell and the peristome may resemble an ellipse, thus applying the formula

$$\text{Area} = \pi \times [(\text{major } \text{Ø})/2] \times [(\text{minor } \text{Ø})/2]$$

On the basis of these main measurements, we estimated a set of morphological ratios: shell height/major shell Ø, as an indicator of shell globosity (more globose shells having a higher ratio); major shell Ø/minor shell Ø, as an indicator of shell circularity (the closer this rate is to unity, the greater the degree of shell circularity); major external peristome Ø/minor external peristome Ø, as an indicator of peristome circularity; and percentage of the whole shell area occupied by the peristome, calculated as (peristome area × 100)/shell area. All measurements were carried out by the same researcher (JL).

Statistical comparisons of morphometric measurements were conducted using ANOVA tests when the variables were homoscedastic and normally distributed; otherwise, the non-parametric Kruskal-Wallis test was used. In addition, a principal components analysis (PCA) was carried out to determine the overlap between the species under study in the morphospace. Each sampling location within the PCA was characterised by the average morphometric parameters of all the collected shells. This method could be seen as reducing scatter, potentially underestimating the overlap between the population clusters of the compared species. In truth, however, the PCA was not significantly influenced by conchological variation as the coefficients of variation for the five main morphometric parameters were low, averaging around 10% at interpopulation level (fig. 2A) and 7% at intrapopulation level (fig. 2B).

Results

Phylogenetic analyses and genetic distances

Phylogenetic analyses of the genus *Iberus* recovered three well-supported clades (fig. 3). The most basal clade is composed of *I. guiraoanus*, *I. angustatus*, *I. ortizi* and the recently described *I. axarciensis*, *I. antikarianus* and *I. giennensis*. The middle or central clade consists of a basal *loxanus* and an ancestral lineage (hereafter referred to as the 'new lineage') represented by samples M15, M16, M17, and AW1, which holds particular significance for this study. This clade is also com-

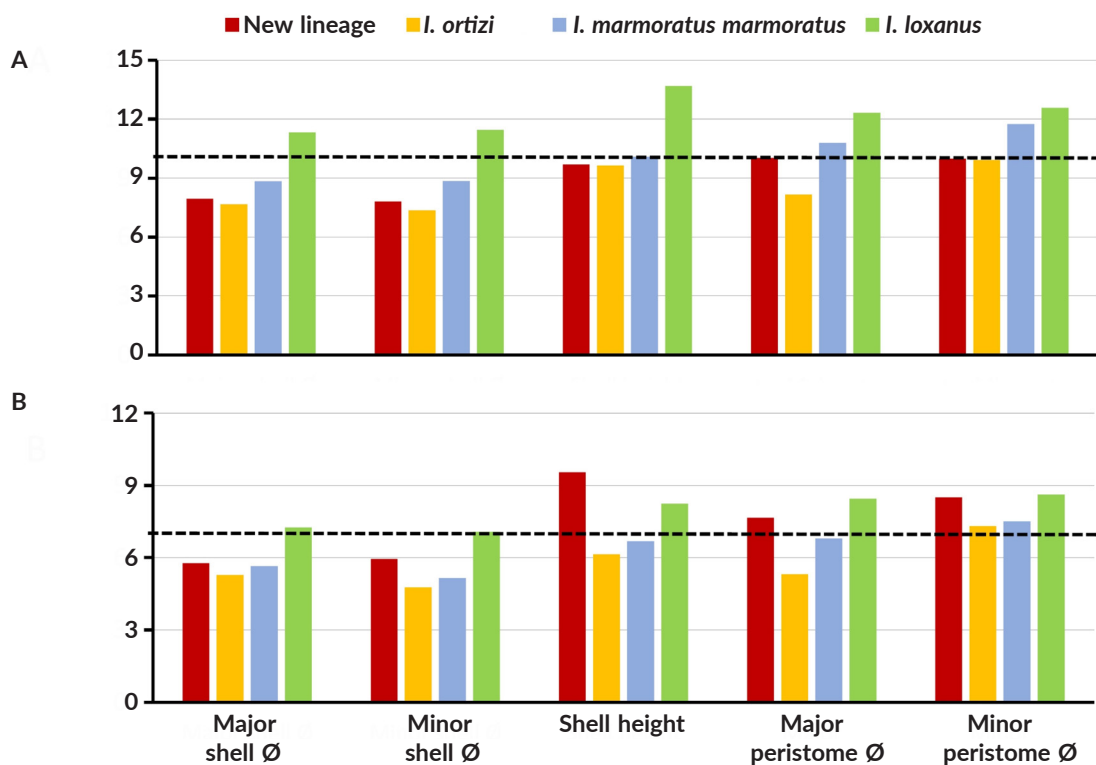


Fig. 2. Coefficients of variation of the main morphometric parameters considered in this work: A, at interpopulation level (2,367 shells); B, at intrapopulation level (27, 58, 31 and 65 shells from single populations of the new lineage, *I. ortizi*, *I. marmoratus marmoratus* and *I. loxanus*, respectively). The average coefficients of variation of the whole sets of morphometric parameters are shown by dashed black lines.

Fig. 2. Coeficientes de variación de los principales parámetros morfométricos considerados en este trabajo: A, a escala interpoblacional (2.367 conchas); B, a escala intrapoblacional (27, 58, 31 y 65 conchas de poblaciones del nuevo linaje, de *I. ortizi*, de *I. marmoratus marmoratus* y de *I. loxanus*, respectivamente). Los promedios de los coeficientes de variación del total de parámetros morfométricos se muestran con líneas negras discontinuas.

posed of *I. cilbanus* (Altaba and Ríos-Jiménez 2021), *I. marmoratus cobosi* Ibáñez and Alonso, 1978, *I. marmoratus rositai* de Fez, 1950, *I. marmoratus marmoratus* and a new subspecies of *I. marmoratus* (see Liétor et al in press a). The third clade is composed of *I. punicus* Tudela, Liétor, Jódar, Galán-Luque and Jowers, 2024, *I. alonensis* (A. Férussac, 1821), *I. xerophilus* Liétor, Jowers, Jódar, Galán-Luque and Tudela, 2024, *I. globulosus* C. R. Boettger, 1913, *I. carthaginiensis* (Rossmässler, 1853), *I. campesinus* (L. Pfeiffer, 1846), *I. rhodopeplus* (Liétor et al in press b), *I. mariae* Cobos, 1979 and *I. gaultieranus* (Linnaeus, 1758).

The new lineage exhibits intraspecific differences of only 0.4% in the COI gene (table 1), the smallest within its clade. Furthermore, it shows divergence from all other *Iberus* species in the sister clades (fig. 3), with distances of approximately 10–13% and 3–6% for the COI and 16S rRNA genes, respectively (table 2).

Morphometric comparison between shells of the new lineage and those of the other *Iberus* species inhabiting the province of Córdoba

A set of 2,367 shells were measured and distributed as follows: 625 for the new lineage (coming from 15 sample points of 12 different locations), 326 for

I. ortizi, 808 for *I. loxanus* and 608 for *I. marmoratus marmoratus* (fig. 1s in supplementary material shows a representative shell of each of these taxa).

Most shell size indicators, such as shell and peristome diameters and areas, were significantly greater in the newly recovered lineage than in *I. ortizi*. Although no significant differences were detected between them in shell height, the globosity indicator was significantly higher in *I. ortizi*. Differences were also observed in the degree of circularity of shell samples, with circularity being greater in *I. ortizi* than in the new lineage, though this did not apply to peristome circularity. Proportionally, the peristome area of shells in the new lineage was larger in relation to the total shell area than that of *I. ortizi* (table 3.1).

The shells of the new lineage had a significantly smaller average size than *I. loxanus* and a more circular shape. There were no significant differences between the two taxa regarding shell globosity or peristome circularity indicators. Likewise, no differences were observed in the proportion of shell area occupied by the peristome relative to the total shell area (table 3.2).

The shells and their peristomes in the new lineage had significantly larger diameters compared to those of *I. marmoratus marmoratus*, resulting in correspondingly

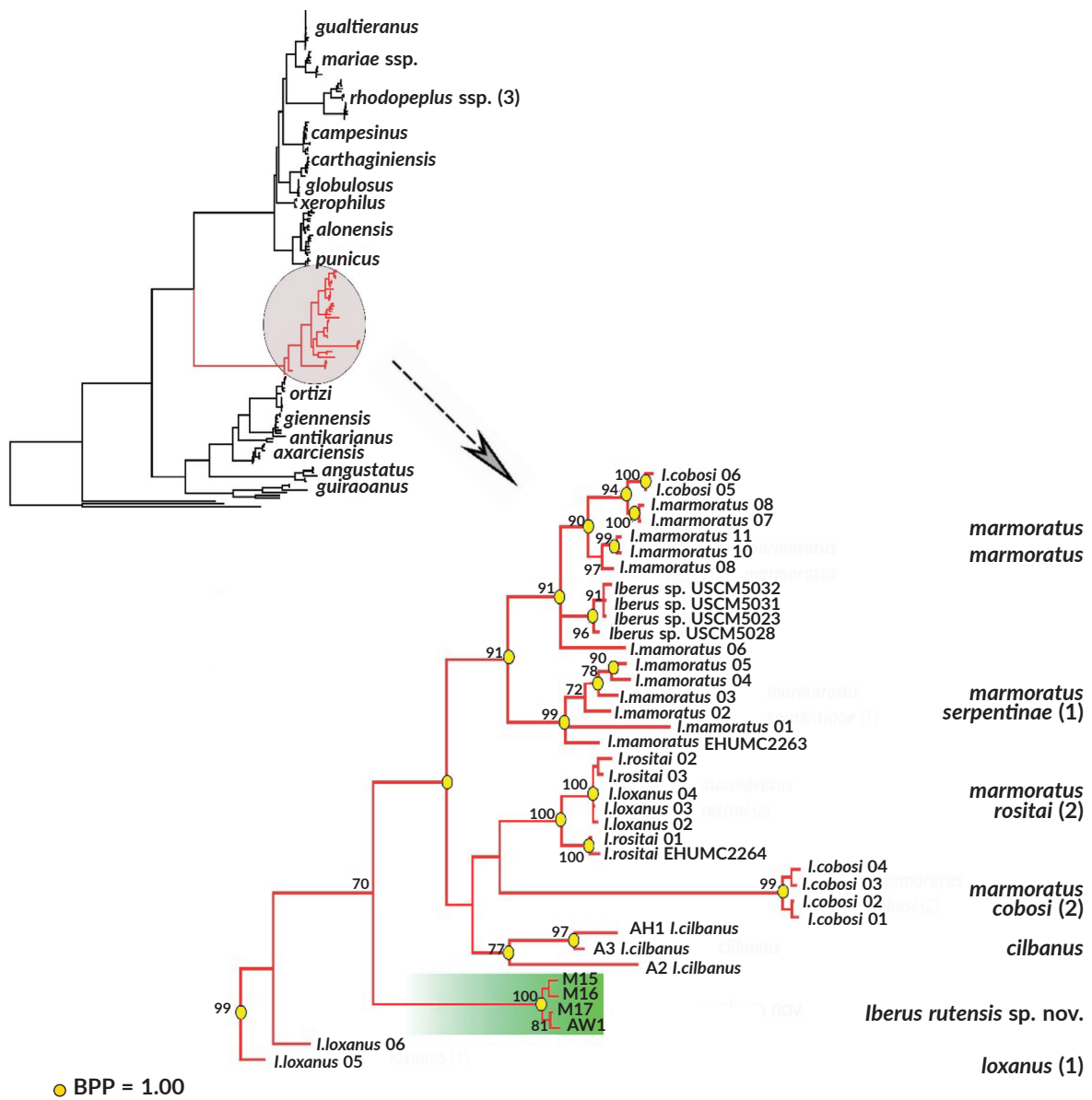


Fig. 3. Maximum likelihood tree of *Iberus*. Values by nodes represent bootstrap values for the ML analyses (> 70%). The yellow filled circles are BPP = 1 for the BI analyses. (1) In Liétor et al (in press a), the taxonomic status of *I. serpentinae* Ahuir (2020) is revised to *I. marmoratus serpentinae*, and the taxonomic name *I. loxanus* is reinstated, thereby invalidating the name *I. marmoratus loxanus* used in recent years. (2) The genetic distances with *I. marmoratus marmoratus* shown in table 2, together with other geographical and morphological evidence (unpublished data), suggest that *I. marmoratus cobosi* and *I. marmoratus rositai* should no longer be regarded as subspecies of *I. marmoratus* but instead as valid species, as suggested by Elejalde et al (2008a). (3) The name *I. rhodopeplus* ssp. replaces the earlier, provisional designation *I. alonensis*-like O2, as stated by Liétor et al (in press b). Some additional taxonomic clarifications: the placement of *I. loxanus* within two different clades is misleading, as the specimens of *I. loxanus* within the clade of *I. marmoratus rositai* were likely misidentified during the GenBank registration process (study currently in progress). The placement of *I. cobosi* within two different clades is also inaccurate, as the *I. cobosi* 05 and 06 specimens, located at the top of the clade under study, correspond to hybrids with *I. marmoratus marmoratus* (unpublished data), again misidentified during the GenBank registration process. Liétor et al (in press a) confirm that the specimens referenced as *Iberus* sp. correspond to *I. marmoratus marmoratus*.

Fig. 3. Árbol de máxima verosimilitud de *Iberus*. Los valores en los nodos representan los valores de bootstrap para los análisis ML (> 70%). Los círculos amarillos tienen un BPP = 1 para los análisis BI. (1) En Liétor et al (en prensa a), el estado taxonómico de *I. serpentinae* Ahuir, 2020 se rectifica a *I. marmoratus serpentinae* y se restablece la denominación taxonómica *I. loxanus*, con lo que se invalida el nombre *I. marmoratus loxanus* utilizado durante los últimos años. (2) Las distancias genéticas con *I. marmoratus marmoratus* que se muestran en la tabla 2, junto con otras evidencias geográficas y morfológicas (datos no publicados), nos llevan a concluir que *I. marmoratus cobosi* e *I. marmoratus rositai* no deberían considerarse subspecies de *I. marmoratus*, sino especies válidas, tal y como sugieren Elejalde et al (2008a). (3) El nombre *I. rhodopeplus* ssp. reemplaza la designación provisional anterior *I. alonensis*-like O2, como establecen Liétor et al (en prensa b). Algunas aclaraciones taxonómicas adicionales: la ubicación de *I. loxanus* en dos cladros diferentes es engañosa, ya que los ejemplares de *I. loxanus* del clado de *I. marmoratus rositai* fueron probablemente identificados de forma errónea durante el proceso de registro en GenBank (estudio en curso). El emplazamiento de *I. cobosi* en dos cladros diferentes es también inexacta, ya que los ejemplares de *I. cobosi* 05 y 06, situados en la parte alta del clado en estudio, corresponden a híbridos con *I. marmoratus marmoratus* (datos no publicados) que también fueron mal identificados durante el proceso de registro en GenBank. Liétor et al (en prensa a) confirman que los ejemplares registrados como *Iberus* sp. corresponden a *I. marmoratus marmoratus*.

Table 1. Intraspecific genetic divergence (p -uncorrected distances) within the new lineage as well as within other clades of interest in reference to figure 3. See the clarifications regarding the taxonomic designations used in this table that are shown in the legend of figure 3.

Tabla 1. Divergencia genética intraespecífica (distancias p no corregidas) dentro del nuevo linaje, así como dentro de otros clados de interés en referencia a la figura 3. Véanse las aclaraciones relativas a las designaciones taxonómicas utilizadas en esta tabla que se muestran en la leyenda de la figura 3.

Lineage	COI	16S rRNA
<i>I. marmoratus marmoratus</i>	3.14%	1.19%
<i>I. marmoratus serpentinae</i>	3.20%	1.41%
<i>I. marmoratus rositai</i>	1.93%	0.09%
<i>I. marmoratus cobosi</i>	0.83%	0.14%
<i>I. cilbanus</i>	5.65%	2.34%
New lineage	0.36%	0.55%
<i>I. loxanus</i>	2.61%	1.35%

larger shell and peristome areas. However, *I. marmoratus marmoratus* displayed the highest degree of globosity due to its significantly greater average shell height. The new lineage exhibited a more circular shell but a less circular peristome than *I. marmoratus marmoratus*. No differences were found between the two taxa in the proportion of shell area occupied by the peristome relative to the total shell area (table 3.3).

In addition to the quantitative measures previously analysed, the ratio of morphotypes (continuous vs. discontinuous bands) proved valuable in distinguishing between shells of the new lineage and those of *I. loxanus*. Continuous bands predominated in the former, while

discontinuous bands were more common in the latter. No other notable differences were observed in the ratio of banding patterns or umbilicus types in any of the remaining interspecies comparisons (table 4).

A PCA provided a first component (PC1) accounting for 60.82% of the total variance in shell morphology, which was determined by major shell diameter and shell height. PC1 can therefore be interpreted as a gradient of shell size. The second component (PC2) was less relevant for species clustering because it explained 39.14% of the total data variance. PC2 was determined by the index of globosity, thus grouping species by shell shape.

The centroids of the four species compared were distributed across the four quadrants of morphospace, indicating conchological differentiation at a metapopulation scale. Confirming our initial field observations, shells from the new lineage appear to occupy a morphological space near that of *I. loxanus* and *I. ortizi*, with a stronger overlap observed with *I. loxanus*. On one hand, the globosity of the new lineage shells closely resembles that of *I. loxanus*, though average shell size is greater in the latter. On the other hand, the new lineage shows shell sizes similar to those of *I. ortizi*, although *I. ortizi* exhibits greater shell globosity (fig. 4).

A new species for the genus *Iberus*

Based on morphological differences, genetic distances that fall primarily within the upper range for the genus (Elejalde et al 2008a, Liétor et al 2024, Tudela et al 2024), and the phylogenetic separation between samples of the new lineage and other *Iberus* species, along with the recovery of an ancestral position and the absence of shared haplotypes with any GenBank samples, we conclude that the clade encompassing our four biological samples represents a new species, proposed here as *Iberus rutensis* sp. nov. A detailed description follows.

Table 2. P -uncorrected distances among taxa for the clades of interest and the new lineage. The lower matrix is the COI data and the upper matrix is the 16S rRNA data. Comparisons in which the new lineage is involved are highlighted in grey. See the clarifications regarding the taxonomic designations used in this table that are shown in the legend of figure 3.

Tabla 2. Distancias p no corregidas entre taxones para los clados de interés y el nuevo linaje. La matriz inferior corresponde a los datos del gen COI y la matriz superior corresponde a los del gen ARNr 16S. Las comparaciones en las que figura el nuevo linaje se han resaltado en gris. Véanse las aclaraciones relativas a las designaciones taxonómicas utilizadas en esta tabla que se muestran en la leyenda de la figura 3.

Lineage	IMM	IMS	IMR	IMC	ICI	NWL	ILO
<i>I. marmoratus marmoratus</i> (IMM)	-	2.41%	3.56%	4.74%	3.54%	3.74%	3.06%
<i>I. marmoratus serpentinae</i> (IMS)	6.57%	-	3.57%	5.05%	2.77%	4.64%	2.97%
<i>I. marmoratus rositai</i> (IMR)	8.71%	8.45%	-	4.73%	2.07%	4.64%	1.89%
<i>I. marmoratus cobosi</i> (IMC)	12.37%	11.54%	10.22%	-	4.37%	5.73%	3.92%
<i>I. cilbanus</i> (ICI)	8.83%	8.14%	7.88%	11.23%	-	4.12%	1.26%
New lineage (NWL)	10.11%	9.90%	11.97%	13.24%	11.60%	-	2.87%
<i>I. loxanus</i> (ILO)	11.81%	11.27%	11.74%	13.86%	10.94%	10.84%	-

Table 3. Morphometric comparisons between the new lineage and the other three taxa of the genus *Iberus* inhabiting Córdoba Province (1, *I. ortizi*; 2, *I. loxanus*; 3, *I. marmoratus marmoratus*). For *I. ortizi*, *I. marmoratus marmoratus* and *I. loxanus*, not only shells from the populations of the Province of Córdoba were measured, but also all those obtained in the other Andalusian provinces during the authors' intensive field sampling. Different letters in the superscripts indicate significant differences (p -value < 0.05) according to one-way ANOVA plus post hoc HSD Tukey test (^A) or Kruskal Wallis plus 2-tailed multiple comparison test (^K); 'ns' means non-significant. Liétor et al (in press a) suggest that the taxonomic name *I. marmoratus loxanus* (Bank and Luijten 2014) is not valid and propose to reinstate the name *I. loxanus*, which is the designation we use throughout this work.

Tabla 3. Comparaciones morfométricas entre el nuevo linaje y los otros tres taxones del género *Iberus* que habitan en la provincia de Córdoba (1, *I. ortizi*; 2, *I. loxanus*; 3, *I. marmoratus marmoratus*). En el caso de *I. ortizi*, *I. marmoratus marmoratus* e *I. loxanus*, no solo se midieron las conchas de las poblaciones de la provincia de Córdoba, sino también todas las obtenidas en el resto de las provincias andaluzas durante el intensivo muestreo de campo de los autores. Letras diferentes en los superíndices indican diferencias significativas ($p < 0,05$) según ANOVA de una vía más prueba post hoc HSD Tukey (^A) o Kruskal Wallis más prueba de comparación múltiple de 2 colas (^K); 'ns' significa no significativo. Liétor et al (en prensa a) sugieren que la denominación taxonómica *I. marmoratus loxanus* (Bank y Luijten 2014) no es válida y proponen recuperar la denominación *I. loxanus*, que es la que estamos usando en este trabajo.

1. Parameters and ratios	p-values	New lineage (n = 625)	<i>I. ortizi</i> (n = 326)
Major Ø of the shell (mm) ^A	0.000009	21.26 ± 1.69 ^a	19.11 ± 1.47 ^b
Minor Ø of the shell (mm) ^A	0.000009	18.00 ± 1.41 ^a	16.49 ± 1.21 ^b
Shell height (mm) ^A	0.3754 ns	11.01 ± 1.07 ^a	10.95 ± 1.05 ^a
Major external Ø of the peristome (mm) ^K	< 0.001	12.73 ± 1.28 ^a	11.08 ± 0.90 ^b
Minor external Ø of the peristome (mm) ^K	< 0.001	10.29 ± 1.03 ^a	8.92 ± 0.89 ^b
Shell height/major Ø of the shell (ratio) ^K	< 0.001	0.52 ± 0.03 ^b	0.57 ± 0.03 ^a
Shell area (mm ²) ^A	0.000009	302.25 ± 46.56 ^a	248.88 ± 37.15 ^b
Peristome area (mm ²) ^K	< 0.001	103.69 ± 19.50 ^a	78.05 ± 13.49 ^b
Major Ø/minor Ø of the shell (ratio) ^A	0.000009	1.18 ± 0.02 ^a	1.16 ± 0.03 ^b
Major Ø/Minor Ø of the peristome (ratio) ^A	0.0928 ns	1.24 ± 0.07 ^a	1.25 ± 0.08 ^a
Peristome area x 100/shell area (%) ^A	0.000009	34.21 ± 2.81 ^a	31.34 ± 2.28 ^b
2. Parameters and ratios	p-values	New lineage (n = 625)	<i>I. loxanus</i> (n = 808)
Major Ø of the shell (mm) ^K	< 0.01	21.26 ± 1.69 ^b	22.42 ± 2.54 ^a
Minor Ø of the shell (mm) ^K	< 0.01	18.00 ± 1.41 ^b	18.85 ± 2.16 ^a
Shell height (mm) ^A	< 0.01	11.01 ± 1.07 ^b	11.59 ± 1.59 ^a
Major external Ø of the peristome (mm) ^A	0.000009	12.73 ± 1.28 ^b	13.39 ± 1.65 ^a
Minor external Ø of the peristome (mm) ^K	< 0.01	10.29 ± 1.03 ^b	10.86 ± 1.37 ^a
Shell height/major Ø of the shell (ratio) ^A	0.5058 ns	0.52 ± 0.03 ^a	0.52 ± 0.03 ^a
Shell area (mm ²) ^K	< 0.01	302.25 ± 46.56 ^b	336.22 ± 77.57 ^a
Peristome area (mm ²) ^K	< 0.01	103.69 ± 19.50 ^b	115.82 ± 28.92 ^a
Major Ø/minor Ø of the shell (ratio) ^A	0.000009	1.18 ± 0.02 ^b	1.19 ± 0.03 ^a
Major Ø/minor Ø of the peristome (ratio) ^A	0.4749 ns	1.24 ± 0.07 ^a	1.24 ± 0.08 ^a
Peristome area x 100/shell area (%) ^A	0.0932 ns	34.21 ± 2.81 ^a	34.47 ± 3.05 ^a
3. Parameters and ratios	p-values	New lineage (n = 625)	<i>I. marmoratus marmoratus</i> (n = 608)
Major Ø of the shell (mm) ^A	0.000009	21.26 ± 1.69 ^a	20.71 ± 1.83 ^b
Minor Ø of the shell (mm) ^A	0.000009	18.00 ± 1.41 ^a	17.43 ± 1.54 ^b
Shell height (mm) ^K	0.000009	11.01 ± 1.07 ^b	11.84 ± 1.19 ^a
Major external Ø of the peristome (mm) ^A	0.010000	12.73 ± 1.28 ^a	12.20 ± 1.32 ^b
Minor external Ø of the peristome (mm) ^A	0.000120	10.29 ± 1.03 ^a	10.07 ± 1.18 ^b
Shell height/major Ø of the shell (ratio) ^K	0.001000	0.52 ± 0.03 ^b	0.57 ± 0.03 ^a
Shell area (mm ²) ^K	0.000009	302.25 ± 46.56 ^a	283.68 ± 53.00 ^b
Peristome area (mm ²) ^K	< 0.000001	103.69 ± 19.50 ^a	98.81 ± 21.95 ^b
Major Ø/minor Ø of the shell (ratio) ^A	0.000030	1.18 ± 0.02 ^b	1.20 ± 0.10 ^a
Major Ø/minor Ø of the peristome (ratio) ^A	< 0.000001	1.24 ± 0.07 ^a	1.23 ± 0.08 ^b
Peristome area x 100/shell area (%) ^A	0.3754 ns	34.21 ± 2.81 ^a	34.85 ± 4.07 ^a

Table 4. Percentages of banding patterns and umbilicus opening measured in small-sized *Iberus* species from Córdoba Province (southern Spain).

Tabla 4. Porcentajes de patrones de bandeado y apertura del ombligo en especies de *Iberus* de pequeño tamaño de la provincia de Córdoba (sur de España).

	New lineage	<i>I. ortizi</i>	<i>I. loxanus</i>	<i>I. marmoratus marmoratus</i>
Lateral band pattern				
Shells with continuous bands	81.7	83.6	37.6	64.0
Shells with discontinuous bands	16.2	14.9	53.2	20.7
Umbilicus				
Shells with closed umbilicus	97.0	89.6	95.8	99.0
Shells with unclosed umbilicus	3.0	10.4	4.2	1.0

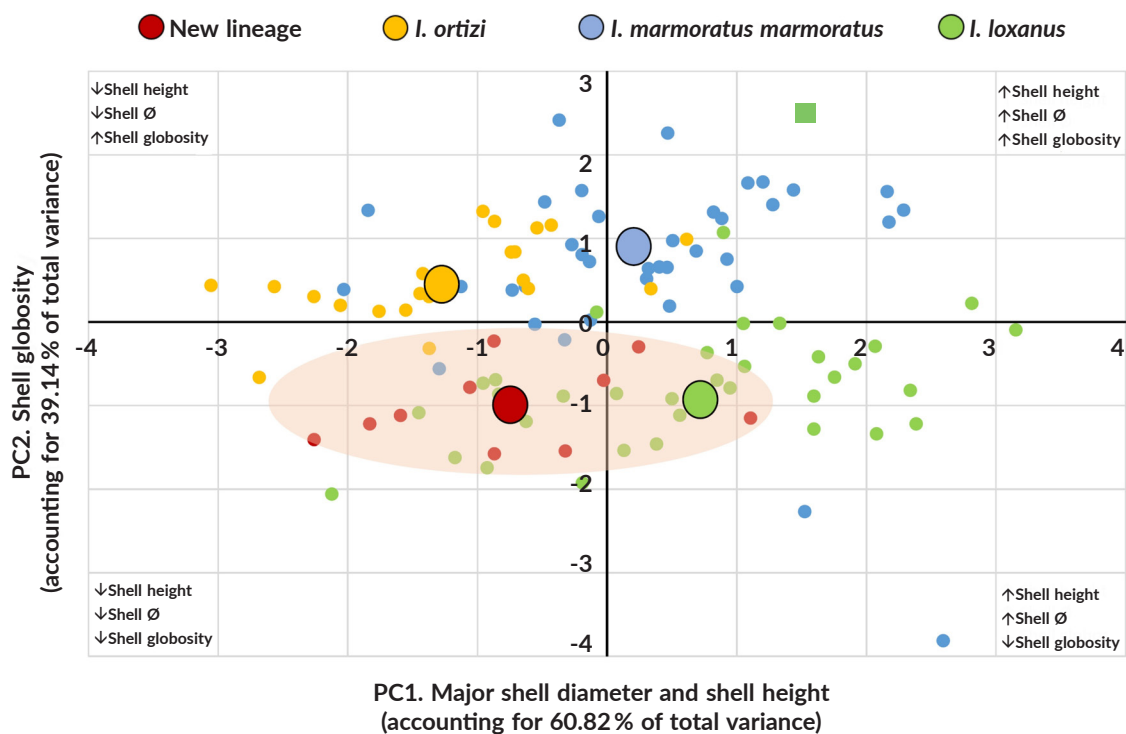


Fig. 4. Distribution of the new lineage (10 locations), *I. ortizi* (21 locations), *I. loxanus* (36 locations) and *I. marmoratus marmoratus* (36 locations) in the bi-dimensional morphospace provided by the two first principal components of a PCA. Each point in the graph represents a single sampling locality. Coordinates of centroids for each species (bigger points) were calculated as the average X and Y coordinates of the points included in the corresponding clouds. The cloud of points for the new lineage has been highlighted in light red.

Fig. 4. Distribución del nuevo linaje (10 localidades), *I. ortizi* (21 localidades), *I. loxanus* (36 localidades) e *I. marmoratus marmoratus* (36 localidades) en el morfoespacio bidimensional generado por los dos componentes principales de un análisis de componentes principales. Cada punto del gráfico representa una única localidad de muestreo. Las coordenadas de los centroides de cada especie (puntos más grandes) se calcularon como el promedio de las coordenadas X e Y de los puntos incluidos en las nubes correspondientes. La nube de puntos del nuevo linaje se ha resaltado en color rojo claro.

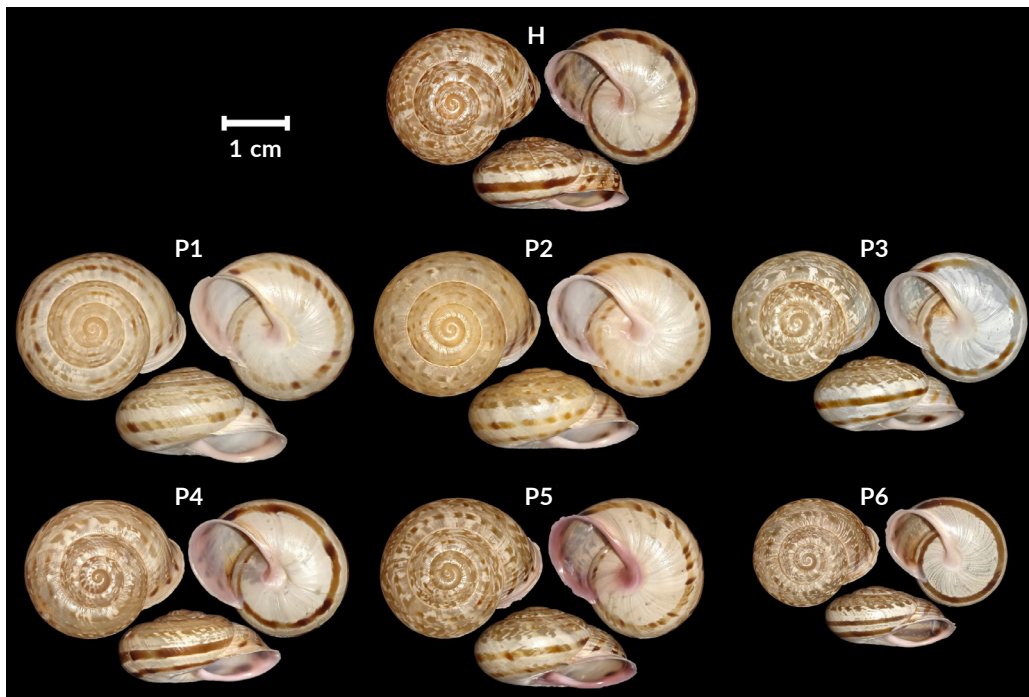


Fig. 5. Photographs of the holotype (H) and the six paratypes (P1 to P6) of *I. rutensis* sp. nov.

Fig. 5. Fotografías del holotipo (H) y los 6 paratipos (P1 a P6) de *I. rutensis* sp. nov.

Systematic

Phylum Mollusca Cuvier, 1795
 Class Gastropoda Cuvier, 1795
 Order Stylommatophora A. Schmidt, 1855
 Family Helicidae Rafinesque, 1815
 Genus *Iberus* Montfort, 1810

Iberus rutensis sp. nov.

Etymology

Given that half of the localities where the new species was found are within the municipality of Rute, with the Lanchar of Rute designated as the type locality, we find it fitting to use the Latin form associated with this municipality to name this new member of the genus *Iberus*.

Holotype and paratypes, and type locality

Holotype and paratype 1 have been deposited in the collections of the Zoology Department of Granada University (Spain). The catalogue codes for holotype and paratype 1 are 22776-CG (CCZ-UGR) and 22777-CG (CCZ-UGR), respectively. Paratypes 2 and 3 have been deposited in the Museo Nacional de Ciencias Naturales- CSIC (Madrid, Spain) with the catalogue numbers 15.05/200552 y 15.05/200553, respectively. Paratypes 4, 5 and 6 correspond to the sequenced samples coded as AW1, M15 and M17, respectively. They remain deposited in the private collection of Dr. José Liétor Gallego (Jaén, Spain), registered as IRP4,

IRP5 and IRP6, respectively. Figure 5 and table 2s in supplementary material provide photographs and additional information on basic morphometrics and the locations of the holotype and paratype shells, respectively.

The type locality of *I. rutensis* sp. nov. (*I. rutensis* hereinafter) consists of karst plains and valleys in the Lanchar of Rute (Córdoba Province, Spain) with coordinates 37° 19' 45" N, -4° 19' 47" W.

Shell variability

The shell variability of *I. rutensis* is shown in fig. 2s in supplementary material. In addition, some live specimens of *I. rutensis* are shown in fig. 3s in supplementary material.

Type shell description

Shell ranging from ~16-25 mm in length, and ~8-15 mm in height. Peristome representing ~27-45% of the total shell area (table 3s in supplementary material).

Shell semi-globose, occasionally depressed, un-keeled and not umbilicated, with 4-5 whorls of regular growth. Last whorl convex and slightly compressed. Suture simple, visible in all whorls. Protoconch of 1-1.5 whorls with smooth and uniform light brown colour. Surface transversely striated, except for protoconch, bearing prominent radial ribs irregularly distributed among a background of less pronounced transverse striations. Spiral striations only appearing on the last whorl, leading to a fine reticulated pattern.

Aperture large, semilunar, wider than high. Peristome slightly reflected, with some populations (especially those from the Rute area) with a remarkable thickening in the columellar fold area, close to the umbilicus. No thickening nor callus on the umbilicus area. Sometimes slightly depressed umbilicus.

Pale to light brown in the first three whorls (excluding the protoconch) and off-white (bone colour) in the rest. Shell body whorl longitudinally crossed by five dark brown bands, with the top three frequently discontinuous, even blurred. Two lower bands wider, continuous or discontinuous, typically with the same thickness, although the upper band sometimes doubles the width of the lower one. Occasionally, area over the two principal bands of the body whorl with a slight spotted pattern of white/light cream tones, dense in some cases, reminiscent of the marmorated pattern of *I. loxanus*. Approximately 1% of shells without bands. Off-white lip, pink or intense fuchsia in some specimens.

Two predominant morphotypes based on their band pattern: (i) Morphotype 1 (fig. 2s in supplementary material): two continuous dark brown lateral bands in the body whorl (82% of sampled shells), (ii) Morphotype 2 (fig. 2s in supplementary material): two intermittent or diffuse lateral bands in the body whorl, sometimes lighter or even pale (16% of sampled shells). Albino shells (pictures 1.8 and 2.19 from fig. 2s in supplementary material) account for 0.8% of the sampled specimens, a proportion notably higher than that found by the authors in other taxa of the genus *Iberus*. Further research could determine which pedoclimatic and ecological factors may influence the relative abundance of each of these morphotypes so as to establish whether they might be considered as ecotypes.

Distribution and habitat

Thirty percent of the sampling localities were finally attributed to *I. rutensis*, most of these placed in the southernmost of the examined geographic area. Fifty percent belonged to the municipality of Rute. The remaining localities were distributed between the municipalities of Priego de Córdoba and Iznájar (33% and 17%, respectively) (see table 4s in supplementary material).

I. rutensis spread over approximately 7,000 hectares, divided into two population nuclei, the main one of about 6,500 hectares to the south of the Sierras Subbéticas Natural Park, and another secondary one of about 500 hectares, in the central-eastern part of the massif. After intensive field sampling, we found no evidence that the two distribution areas were connected.

The ecological niche of *I. rutensis* typically consists of rock formations between 476 and 1,301 m a.s.l. in southern Córdoba Province (Spain). Its habitat is always associated with calcareous-based lithologies. Although it is most common to find this species inside the crevices and cavities of rocky walls, under the stones of limestone pavements, and in rocky ridges or karst formations, it may also inhabit Mediterranean shrubland and, less frequently, areas slightly disturbed due to anthropogenic influence (fig. 4s in supplementary material).

Threat level

The distribution area of *I. rutensis* can be considered small in comparison to other species of the genus *Iberus*. Some populations of *I. rutensis* studied herein can be found on the edges of the Sierras Subbéticas Natural Park, dwelling in the few remnants of Mediterranean shrublands that have not yet been affected by the increasing expansion of olive groves. Furthermore, the area in which *I. rutensis* inhabits is considered one of the Spanish territories where the effects of rising temperatures and increased drought, driven by climate change, will be most intense (Guerrero et al 2014, Jiménez-Donaire et al 2020). Moreover, from a biological perspective, the significantly low intraspecific genetic variability suggests a limited capacity for evolutionary adaptation to changing environmental conditions, which could increase the risk of extinction.

Although further studies will be required to assess the conservation status of the populations of *I. rutensis*, the various threats mentioned should be sufficient to suggest local and regional authorities to provide some degree of environmental protection for this species. Our preliminary recommendation is to classify *I. rutensis* as a Vulnerable species according to the current IUCN categories (IUCN 2024). This decision is based on several subcriteria of criterion B, specifically an area of occupancy of less than 2,000 km² and fragmented populations with a limited number of locations. According to subcriterion D2, these factors could result in plausible future threats of greater magnitude.

Nomenclatural act

This work and the nomenclatural act it contains have been registered at ZooBank. The ZooBank Life Science Identifier (LSID) for this publication is: <https://zoobank.org/urn:lsid:zoobank.org:act:8C19BEC3-C1D8-4693-9C36-5D439A3FFEA1>

Discussion

Since its description, there has been no significant research on the distribution of *I. ortizi* in the southern part of the province of Córdoba, nor has there been any study on the contact zones between this taxon and other congeners. Therefore, this geographical area remains largely unexplored by the scientific community, possibly due to the assumption that all snail populations within it were *I. ortizi*. In fact, only one bibliographic citation was found that mentions one of the localities presented here for *I. rutensis*, specifically the Angosturas of the Salado River in Priego de Córdoba (Talaván Serna and Talaván Gómez 2011). The authors identified the snail at this location as *I. ortizi*, but based on our findings, it is more likely that it was *I. rutensis*.

As a result of the lack of knowledge in the region, our sampling led to the discovery of new populations (subsequently, the first records) for *I. ortizi* in the province of Jaén (Sierra de Orbes and Noguerones in the municipality of Alcaudete, and La Rábita in the municipality of Alcalá la Real) and for *I. marmoratus marmoratus* in the province of Córdoba (Algar village in the municipality of Carcabuey and Sierra de Aras in the town of Lucena).

It is well-known that the genus *Iberus* includes several genetically divergent but morphologically cryptic lineages (Moreno-Rueda 2007, Elejalde et al 2008b, Liétor et al 2024), a common trend in helicids (Chueca et al 2015, Zając et al 2020). *I. rutensis* is part of one of the most conspicuous cryptic complexes within the genus. It has gone unnoticed to date due to its conchological similarity with the surrounding taxa (*I. ortizi*, *I. loxanus*, *I. marmoratus marmoratus* and even the easternmost *I. angustatus*; Liétor 2014). A similar case is reported by Liétor et al (in press b) regarding the *I. alonensis*-like O2 clade from western Andalusia (southern Spain). Until Elejalde et al (2008b) established the existence of this clade, most large globose *Iberus* had been classified as *I. alonensis*. Liétor et al (in press b) not only genetically and morphologically characterised and differentiate this clade from the other taxa involved in the *I. alonensis* morphospecies, but they also found that it contained two well-defined sub-clades. However, the individuals of these subclades are morphologically indistinguishable both in terms of the shell and the soft body parts. The cryptic nature of these taxa is such that distinguishing the shells of both is only possible through molecular analysis or with precise geographical location information. Another paradigmatic case of the cryptic nature of the genus *Iberus*, comparable with *I. rutensis*, is the species pair *I. carthaginiensis*-*I. punicus*. All *Iberus* along the coast of Murcia, from Mazarrón to the Mar Menor (southeastern Spain) had been traditionally classified as *I. carthaginiensis*. Tudela et al (2024) demonstrated that the populations further east, from Cartagena to the Mar Menor, corresponded to a new species, *I. punicus*, while those further west correspond to *I. carthaginiensis*. The latter had been mistakenly referred to as *I. calaensis* over the past decade, and is now considered a synonym of *I. carthaginiensis*. In summary, *I. carthaginiensis*, *I. calaensis* and *I. punicus* had repeatedly been misidentified due to the similarity of their shells.

Initially, we hypothesised that the main population nucleus of *I. rutensis* could consist of intermediate specimens between the southern populations of *I. ortizi* and one of the two *Iberus* subspecies inhabiting the surrounding areas: the *I. loxanus* population from Iznájar to the south or the *I. marmoratus marmoratus* populations from Lucena and Algar to the west and north, respectively (fig. 1). Indeed, the conchological features of *I. rutensis* did not fit the usual patterns for *I. ortizi* or *I. loxanus*. Instead, the shells of *I. rutensis* display characteristics that fall between both species, which likely explains why this taxon has gone unnoticed until now and has been indistinctly attributed to one species or the other. From a molecular perspective, the recovery of a well-supported phylogenetic clade in all analyses and the high genetic distances to the closest taxa as inferred from the phylogenies support the validity that *I. rutensis* is a new species. In addition to the molecular results is the fact that *I. rutensis* has a well-defined geographical distribution and a conchological identity divergent from *I. ortizi* and *I. loxanus*, the geographically closest congeneric species. Future studies should elucidate whether there is a natural corridor connecting the two population nuclei of *I. rutensis*. If

no such corridor exists, the north-eastern population could develop a particularly unique character, making it especially deserving of conservation efforts.

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No conflicts declared

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Supplementary material



Fig. 1s. Comparison of representative shells of the four small *Iberus* species present in the Province of Córdoba (southern Spain): 1, new lineage from Lorite, Rute (22 mm); 2, *I. ortizi* from Doña Mencía (18 mm); 3, *I. loxanus* from Las Chozas, Iznájar (20 mm); 4, *I. marmoratus marmoratus* from Lucena (21 mm). Specimen 4 belongs to one of the first locations recorded for *I. marmoratus marmoratus* in the Province of Córdoba (see blue grids in figure 1).

Fig. 1s. Comparación de conchas representativas de las cuatro especies pequeñas del género *Iberus* presentes en la provincia de Córdoba (sur de España): 1, nuevo linaje de Lorite, Rute (22 mm); 2, *I. ortizi* de Doña Mencía (18 mm); 3, *I. loxanus* de Las Chozas, Iznájar (20 mm); 4, *I. marmoratus marmoratus* de Lucena (21 mm). El ejemplar número 4 pertenece a una de las primeras localidades registradas para *I. marmoratus marmoratus* en la provincia de Córdoba (véanse las cuadrículas azules de la figura 1).



Fig. 2s. Series of conchological variation of *I. rutensis* in the Province of Córdoba (southern Spain): A, serie 1, morfotipo with continuous bands: 1-8, Angosturas of Salado River, Priego de Córdoba (note that number 7 is a teratological specimen with double lip and number 8 is an albino specimen); 9-10, la Celada, Iznájar; 11-18, Northern area of Lanchar of Rute; 19, Las Herreras stream, Lorite, Rute; 20, Los Juncare, Iznájar; 21, Las Labores stream gorge, Las Lagunillas, Priego de Córdoba; 22-23, La Hoz River, Lorite, Rute. B, serie 2, morfotipo with discontinuous bands: 1-2, Rute el Viejo Castle; 3, La Celada, Iznájar; 4-8, Northern of Lanchar of Rute; 9-11, Las Herreras stream, Lorite, Rute; 12-14, Los Juncare, Iznájar; 15-19, Las Labores stream gorge, Las Lagunillas, Priego de Córdoba (note that number 19 is an albino specimen); 20, Anzur River, Rute; 21, La Hoz River, Lorite, Rute.

Fig. 2s. Serie de variación conquiológica de *I. rutensis* en la provincia de Córdoba (sur de España): A, serie 1, morfotipo con bandas continuas: 1-8, Angosturas del río Salado, Priego de Córdoba (nótese que el número 7 es un ejemplar teratológico con doble labio y el número 8 es un ejemplar albino); 9-10, La Celada, Iznájar; 11-18, Área norte del Lanchar de Rute; 19, Arroyo las Herreras, Lorite, Rute; 20, Los Juncare, Iznájar; 21, Arroyo de las Labores, Las Lagunillas, Priego de Córdoba; 22-23, Río la Hoz, Lorite, Rute. B, serie 2, morfotipo con bandas discontinuas: 1-2, Castillo de Rute el Viejo; 3, La Celada, Iznájar; 4-8, Área norte del Lanchar de Rute; 9-11, Arroyo las Herreras, Lorite, Rute; 12-14, Los Juncare, Iznájar; 15-19, Arroyo de las Labores, Las Lagunillas, Priego de Córdoba (nótese que el número 19 es un ejemplar albino); 20, Río Anzur, Rute; 21, Río la Hoz, Lorite, Rute.

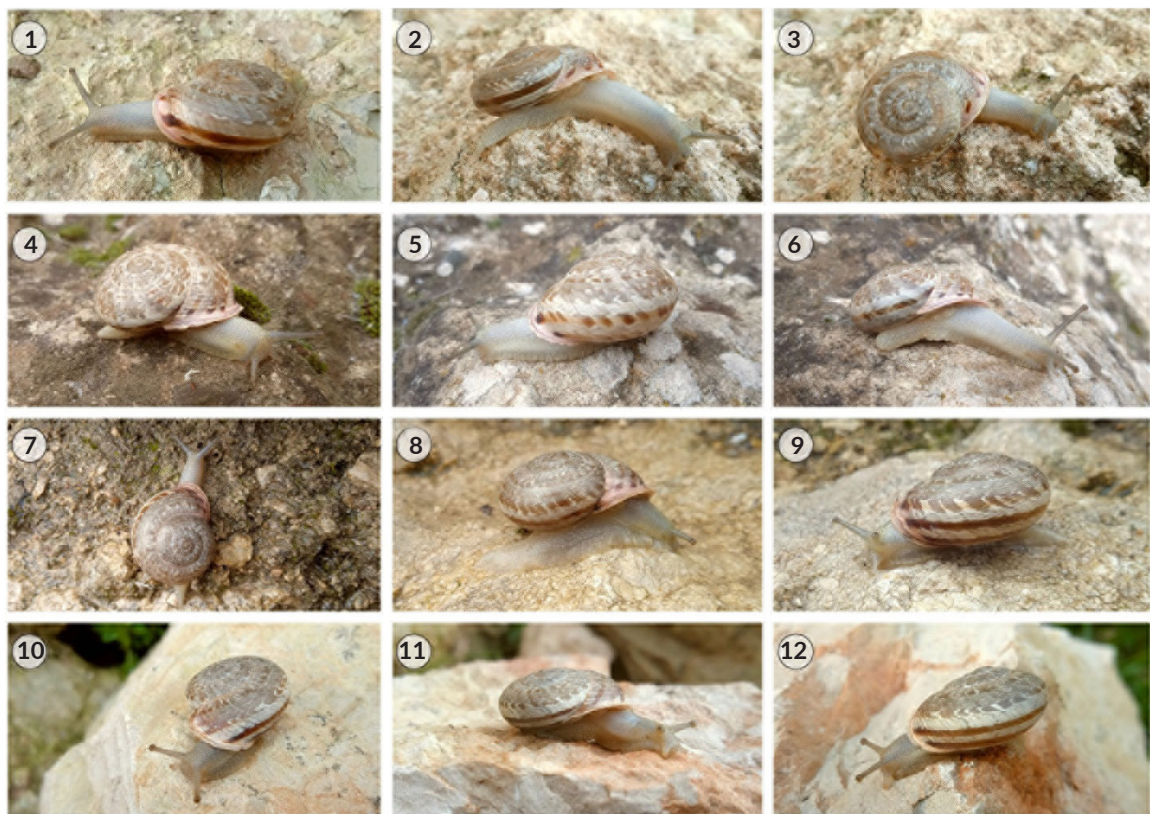


Fig. 3s. Some live specimens of *I. rutensis* from the Province of Córdoba photographed *in situ*: 1-3, Angosturas of Salado River, Priego de Córdoba; 4-6, Northern area of Lanchar of Rute; 7-9, La Hoz River, Lorite, Rute; 10-12, Los Juncares, Iznájar.

Fig. 3s. Algunos ejemplares vivos de *I. rutensis* de la provincia de Córdoba fotografiados *in situ*: 1-3, Angosturas del río Salado, Priego de Córdoba; 4-6, Área norte del Lanchar de Rute; 7-9, Río la Hoz, Lorite, Rute; 10-12, Los Juncares, Iznájar.

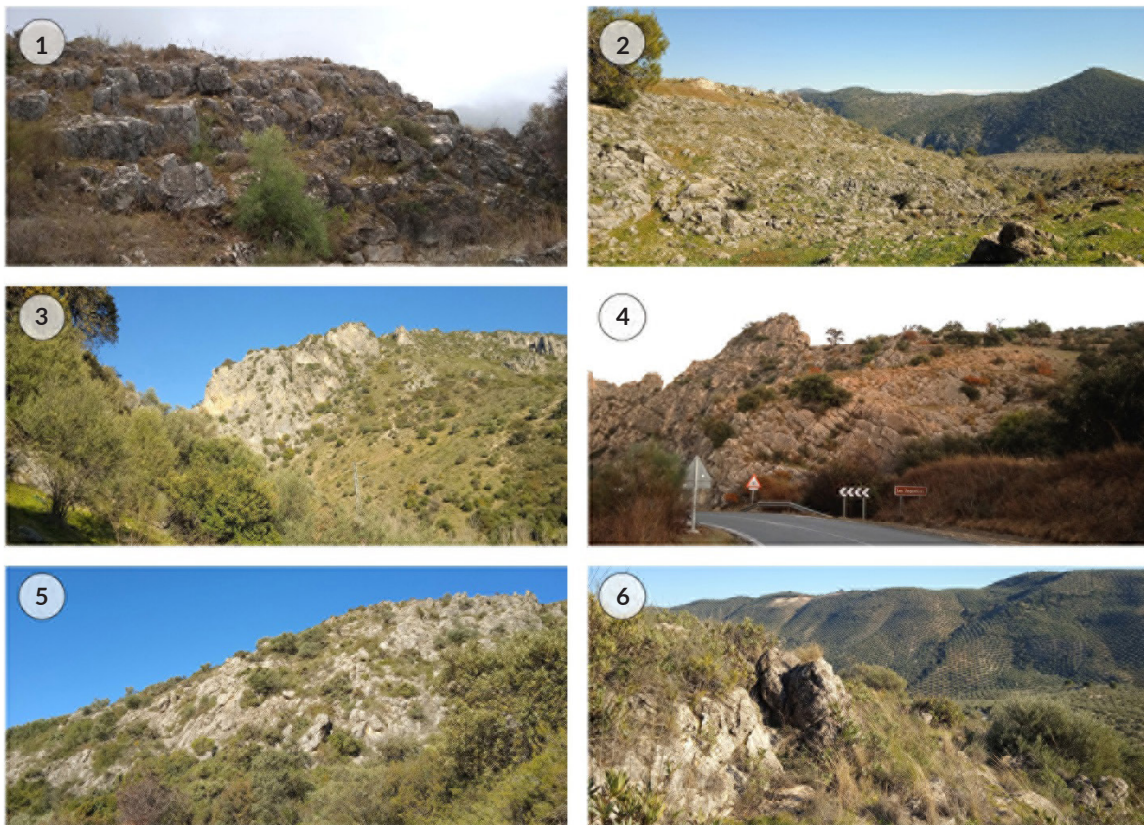


Fig. 4s. Some habitats of *I. rutensis* in the province of Córdoba: 1, Las Herreras stream, Rute; 2, Northern of Lanchar of Rute; 3, La Hoz River, Rute; 4, Angosturas of Salado River, Priego de Córdoba; 5, Los Villares, Priego de Córdoba; 6, Los Juncares, Iznájar.

Fig. 4s. Algunos hábitats de *I. rutensis* en la provincia de Córdoba: 1, Arroyo de las Herreras, Rute; 2, Área norte del Lanchar de Rute; 3, Río La Hoz, Rute; 4, Angosturas del río Salado, Priego de Córdoba; 5, Los Villares, Priego de Córdoba; 6, Los Juncares, Iznájar.

Table 1s. GenBank data: A, samples of the new species; B, GenBank voucher abbreviations, species names, localities, coordinates and GenBank accessions. The samples referenced as N1, N4, N7, and W1 correspond to the four specimens of *I. ortizi* provided by this work.

Tabla 1s. Datos de GenBank: A, muestras de la nueva especie; B, abreviaturas de las referencias de GenBank, nombres de especies, localidades, coordenadas y números de adhesión de GenBank. Las muestras registradas como N1, N4, N7 y W1 se corresponden con los cuatro ejemplares de *I. ortizi* aportados por este trabajo.

A

Voucher	Species	Locality		GenBank Accession Number		
		Lat. (N)	Long. (W)	COI	16S rRNA	LSU
M15	<i>Iberus rutensis</i>	37° 19' 42.1"	4° 19' 44.1"	PQ488232	PQ488687	PQ488696
M16	<i>Iberus rutensis</i>	37° 19' 3.3"	4° 19' 47.4"	PQ488234	PQ488689	PQ488698
M17	<i>Iberus rutensis</i>	37° 17' 32"	4° 17' 41"	PQ488233	PQ488688	PQ488697
AW1	<i>Iberus rutensis</i>	37° 28' 56.7"	4° 10' 52.1"	PQ488235	PQ488690	PQ488699

B

Voucher	Species	Locality (UTM)	GenBank Accession Number		
			COI	16S rRNA	LSU
I.angustatus05	<i>Iberus angustatus</i>	30SVG37	EF440242	EF440192	-
I.angustatus04	<i>Iberus angustatus</i>	30SVG17	EF440241	EF440191	-
I.angustatus03	<i>Iberus angustatus</i>	30SVG52	EF440240	EF440190	MW817736
I.angus_MVHN-091012DS06-1	<i>Iberus angustatus</i>	-	KC693589	-	-
I.angustatus02	<i>Iberus angustatus</i>	30SVG57	EF440239	EF440189	-
I.angustatus01	<i>Iberus angustatus</i>	30SVG67	EF440238	EF440188	-
Iberus sp. MAE-2007-03	<i>Iberus giennensis</i>	30SVG23	EF440274	EF440224	-
Iberus sp. MAE-2007-02	<i>Iberus giennensis</i>	30SVG14	EF440273	EF440223	-
Iberus sp. MAE-2007-01	<i>Iberus giennensis</i>	30SVG14	EF440272	EF440222	MW817751
Iberus sp. MAE-2007-04	<i>Iberus giennensis</i>	30SVG22	EF440275	EF440225	-
I.giennensisB1	<i>Iberus giennensis</i>	37° 20' 18" N 3° 34' 16.7" W	OR800625	-	-
I.giennensisB5	<i>Iberus giennensis</i>	37° 39' 33" N 3° 42' 59" W	OR800624	-	-
I.giennensisB6	<i>Iberus giennensis</i>	37° 39' 49.4" N 3° 55' 6.3" W	OR800623	-	-
I.giennensisB7	<i>Iberus giennensis</i>	37° 26' 14.2" N 3° 26' 16.6" W	OR800626	-	-
I.giennensisB8	<i>Iberus giennensis</i>	37° 20' 21.4" N 3° 54' 51.4" W	OR800627	-	-
I.giennensisU3	<i>Iberus axarciensis</i>	37° 04' 20.5" N 4° 14' 47.2" W	OR800631	-	-
I.axarciensisU4	<i>Iberus axarciensis</i>	37° 04' 20.5" N 4° 14' 47.2" W	OR800630	-	-
I.axarciensisU5	<i>Iberus axarciensis</i>	37° 00' 15.4" N 4° 16' 30.6" W	OR800635	-	-
I.axarciensisU7	<i>Iberus axarciensis</i>	37° 04' 51" N 4° 14' 26" W	OR800632	-	-

Table 1s. (Cont.)

Voucher	Species	Locality (UTM)	GenBank Accession Number		
			COI	16S rRNA	LSU
I.axarciensisAJ1	<i>Iberus axarciensis</i>	37° 01' 56" N 4° 14' 57" W	OR800634	-	-
I.axarciensisAJ2	<i>Iberus axarciensis</i>	37° 01' 56" N 4° 14' 57" W	OR800633	-	-
I.axarciensisAM1	<i>Iberus axarciensis</i>	36° 58' 15" N 4° 13' 06" W	OR800628	-	-
I.axarciensisAM3	<i>Iberus axarciensis</i>	37° 00' 00" N 4° 08' 21" W	OR800629	-	-
I.ortizi09	<i>Iberus ortizi</i>	30SUG74	EF440284	EF440234	-
I.ortizi08	<i>Iberus ortizi</i>	30SUG74	EF440283	EF440233	MW817749
I.ortizi07	<i>Iberus ortizi</i>	30SUG84	EF440282	EF440232	-
I.ortizi06	<i>Iberus ortizi</i>	30SUG74	EF440281	EF440231	-
I.ortizi05	<i>Iberus ortizi</i>	30SUG74	EF440280	EF440230	-
I.ortizi04	<i>Iberus ortizi</i>	30SUG84	EF440279	EF440229	-
I.ortizi03	<i>Iberus ortizi</i>	30SUG84	EF440278	EF440228	-
I.ortizi02	<i>Iberus ortizi</i>	30SUG84	EF440277	EF440227	-
I.ortizi01	<i>Iberus ortizi</i>	30SUG84	EF440276	EF440226	-
I.ortiziN1	<i>Iberus ortizi</i>	37° 26' 40" N 4° 16' 17" W	PQ488237	-	-
I.ortiziN4	<i>Iberus ortizi</i>	37° 33' 34" N 4° 16' 32" W	PQ488239	PQ488693	-
I.ortiziN7	<i>Iberus ortizi</i>	37° 24' 46.6" N 4° 20' 8.7" W	PQ488238	PQ488692	-
I.ortiziW1	<i>Iberus ortizi</i>	37° 23' 47.9" N 4° 08' 05.0" W	PQ488236	PQ488691	-
I.loxanus01	<i>Iberus antikarianus</i>	30SUF79	EF440255	EF440205	MW817741
I.antikarianusU1	<i>Iberus antikarianus</i>	37° 03' 34.7" N 4° 29' 29.3" W	OR800636	-	-
I.guiraoanus06	<i>Iberus guiraoanus</i>	30SWG18	EF440254	EF440204	-
I.guiraoanus05	<i>Iberus guiraoanus</i>	30SWG192	EF440253	EF440203	-
I.guiraoanus04	<i>Iberus guiraoanus</i>	30SWH21	EF440252	EF440202	-
I.guiraoanus03	<i>Iberus guiraoanus</i>	30SWG09	EF440251	EF440201	-
I.guiraoanus02	<i>Iberus guiraoanus</i>	30SWG09	EF440250	EF440200	-
I.guiraoanus01	<i>Iberus guiraoanus</i>	30SWH33	EF440249	EF440199	MW817746
Iberus sp.	<i>Iberus sp.</i>	PN Alcornocales	MF982904	-	-
Iberus sp.	<i>Iberus sp.</i>	PN Alcornocales	MF982905	-	-
Iberus sp.	<i>Iberus sp.</i>	PN Alcornocales	MF982902	-	-
Iberus sp.	<i>Iberus sp.</i>	PN Alcornocales	MF982903	-	-
I. cobosi06	<i>Iberus cobosi</i>	30SUF48	EF440248	EF440198	-
I. cobosi05	<i>Iberus cobosi</i>	30SUF48	EF440247	EF440197	-
I.marmoratus08	<i>Iberus marmoratus</i>	30SUF58	EF440268	EF440218	-
I.marmoratus07	<i>Iberus marmoratus</i>	30SUF58	EF440267	EF440217	-

Table 1s. (Cont.)

Voucher	Species	Locality (UTM)	GenBank Accession Number		
			COI	16S rRNA	LSU
I.marmoratus11	<i>Iberus marmoratus</i>	30SUF48	EF440271	EF440221	-
I.marmoratus10	<i>Iberus marmoratus</i>	30SUF49	EF440270	EF440220	-
I.marmoratus09	<i>Iberus marmoratus</i>	30SUF76	EF440269	EF440219	-
I.marmoratus06	<i>Iberus marmoratus</i>	30SUG09	EF440266	EF440216	-
I.marmoratus05	<i>Iberus marmoratus</i>	30STF96	EF440265	EF440215	-
I.marmoratus04	<i>Iberus marmoratus</i>	30SUF15	EF440264	EF440214	-
I.marmoratus03	<i>Iberus marmoratus</i>	30SUF34	EF440263	EF440213	-
I.marmoratus02	<i>Iberus marmoratus</i>	30SUF24	EF440262	EF440212	-
I.marmEoratus		36° 26' 56" N	MW803160	MW817709	MW817748
HUMC2263	<i>Iberus marmoratus</i>	5° 12' 33" E			
marmoratus-01	<i>Iberus marmoratus</i>	30SUF17	EF440261	EF440211	-
I.rositai02	<i>Iberus rositai</i>	30SUF69	EF440286	EF440236	-
I.rositai03	<i>Iberus rositai</i>	30SUF69	EF440287	EF440237	-
I.loxanus04	<i>Iberus loxanus</i>	30SUF69	EF440258	EF440208	-
I.loxanus03	<i>Iberus loxanus</i>	30SUF69	EF440257	EF440207	-
I.loxanus02	<i>Iberus loxanus</i>	30SUF69	EF440256	EF440206	-
I.rositai01	<i>Iberus rositai</i>	30SUF69	EF440285	EF440235	-
IrositaiEHUMC2264	<i>Iberus rositai</i>	36° 56' 49" N 4° 33' 48" E	MW803161	MW817711	MW817750
I.cobosi04	<i>Iberus cobosi</i>	30SUF48	EF440246	EF440196	-
I.cobosi03	<i>Iberus cobosi</i>	30SUF48	EF440245	EF440195	-
I.cobosi02	<i>Iberus cobosi</i>	30SUF48	EF440244	EF440194	-
I.cobosi01	<i>Iberus cobosi</i>	30SUF48	EF440243	EF440193	MW817742
I.loxanus06	<i>Iberus loxanus</i>	30SUG91	EF440260	EF440210	-
I.loxanus-05	<i>Iberus loxanus</i>	30SVF57	EF440259	EF440209	MW817747
Ialo25	<i>Iberus gualtieranus alonensis</i>	30SUG84	AY928559	AY928587	-
Ialo24	<i>Iberus gualtieranus alonensis</i>	30SXH71	DQ822148	DQ822098	-
Ialo23	<i>Iberus gualtieranus alonensis</i>	30SXH62	DQ822147	DQ822097	-
Ialo22	<i>Iberus gualtieranus alonensis</i>	30SYH26	DQ822146	DQ822096	-
Ialo21	<i>Iberus gualtieranus alonensis</i>	30SYH05	DQ822145	DQ822095	-
Ialo20	<i>Iberus gualtieranus alonensis</i>	30TWM58	DQ822144	DQ822094	-
Ialo19	<i>Iberus gualtieranus alonensis</i>	30SXH51	DQ822143	DQ822093	-
Ialo18	<i>Iberus gualtieranus alonensis</i>	31TCF23	DQ822142	DQ822092	-
Ialonensis_SP493	<i>Iberus alonensis</i>	41° 06' 30" N 0° 52' 33" E	MW803158	MW817696	MW817735
Ialo17	<i>Iberus gualtieranus alonensis</i>	30TYL22	DQ822141	DQ822091	-
Ialo15	<i>Iberus gualtieranus alonensis</i>	30TWL77	DQ822139	DQ822089	-
Ialo13	<i>Iberus gualtieranus alonensis</i>	31TBF60	DQ822137	DQ822087	-
Ialo16	<i>Iberus gualtieranus alonensis</i>	30TYL48	DQ822140	DQ822090	-
Ialo14	<i>Iberus gualtieranus alonensis</i>	30SWH48	DQ822138	DQ822088	-
Ialo12	<i>Iberus gualtieranus alonensis</i>	30TUJ09	DQ822136	DQ822086	MW817740
Ialo11	<i>Iberus gualtieranus alonensis</i>	30SWF59	DQ822135	DQ822085	-

Table 1s. (Cont.)

Voucher	Species	Locality (UTM)	GenBank Accession Number		
			COI	16S rRNA	LSU
lalo10	<i>Iberus gualtieranus alonensis</i>	30SWF58	DQ822134	DQ822084	-
lalo09	<i>Iberus gualtieranus alonensis</i>	30SWF87	DQ822133	DQ822083	-
Iglob01	<i>Iberus gualtieranus globosus</i>	30SWG86	DQ822163	DQ822113	-
lalo07	<i>Iberus gualtieranus alonensis</i>	30SXG17	DQ822131	DQ822081	MW817743
lalo06	<i>Iberus gualtieranus alonensis</i>	30SWG15	DQ822130	DQ822080	-
lalo05	<i>Iberus gualtieranus alonensis</i>	30SWG48	DQ822129	DQ822079	-
lalo04	<i>Iberus gualtieranus alonensis</i>	30SWG15	DQ822128	DQ822078	-
lalo03	<i>Iberus gualtieranus alonensis</i>	30SXG17	DQ822127	DQ822077	-
lalo02	<i>Iberus gualtieranus alonensis</i>	30SWG26	DQ822126	DQ822076	-
Icart04	<i>Iberus gualtieranus carthaginiensis</i>	30SXG76	DQ822162	DQ822112	-
Icart03	<i>Iberus gualtieranus carthaginiensis</i>	30SXG76	DQ822161	DQ822111	-
Icart02	<i>Iberus gualtieranus carthaginiensis</i>	30SXG76	DQ822160	DQ822110	-
Icart01	<i>Iberus gualtieranus carthaginiensis</i>	30SXG76	DQ822159	DQ822109	MW817738
IaAL03	<i>Iberus gualtieranus</i>	30SWG85	AY928554	AY928582	-
Igual06	<i>Iberus gualtieranus gualtieranus</i>	30SWG93	DQ822166	DQ822116	-
Igual05	<i>Iberus gualtieranus gualtieranus</i>	30SWG93	DQ822165	DQ822115	-
Icamp03	<i>Iberus gualtieranus campesinus</i>	30SXG13	DQ822157	DQ822107	-
Icamp01	<i>Iberus gualtieranus campesinus</i>	30SWG62	DQ822155	DQ822105	MW817737
Ilorc02	<i>Iberus gualtieranus lorcanus</i>	30SXG25	DQ822168	DQ822118	-
Ilorc01	<i>Iberus gualtieranus lorcanus</i>	30SXG25	DQ822167	DQ822117	-
IaAL02	<i>Iberus gualtieranus</i>	30SXG02	AY928553	AY928581	-
Icamp02	<i>Iberus gualtieranus campesinus</i>	30SXG02	DQ822156	DQ822106	-
IaAL01	<i>Iberus gualtieranus</i>	30SWG82	AY928552	AY928580	-
Igual04	<i>Iberus gualtieranus gualtieranus</i>	30SWG93	DQ822164	DQ822114	-
Icamp04	<i>Iberus gualtieranus campesinus</i>	30SWG92	DQ822158	DQ822108	-
IaJ04	<i>Iberus gualtieranus</i>	30SVG98	AY928567	AY928595	-
IaJ03	<i>Iberus gualtieranus</i>	30SVG68	AY928566	AY928594	-
lalo36	<i>Iberus gualtieranus alonensis</i>	30SWF07	DQ822152	DQ822102	-
lalo40	<i>Iberus gualtieranus alonensis</i>	30SWF07	DQ822154	DQ822104	-
lalo39	<i>Iberus gualtieranus alonensis</i>	30SWF07	DQ822153	DQ822103	-
lalo35	<i>Iberus gualtieranus alonensis</i>	30SVG72	DQ822152	DQ822102	-
lalo34	<i>Iberus gualtieranus alonensis</i>	30SWF07	DQ822151	DQ822101	-
IaJ02	<i>Iberus gualtieranus</i>	30SVG37	AY928565	AY928593	-
IaJ01	<i>Iberus gualtieranus</i>	30SVG37	AY928564	AY928592	-
IaCO01	<i>Iberus gualtieranus</i>	30SUG85	AY928559	AY928587	-
IgGR04	<i>Iberus gualtieranus</i>	30SVG32	AY928574	AY928602	-
IgGR03	<i>Iberus gualtieranus</i>	30SVG32	AY928573	AY928601	-
IgGR02	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928572	AY928600	MW817739
IaGR01	<i>Iberus gualtieranus</i>	30SVF57	AY928560	AY928588	-
lalo29	<i>Iberus gualtieranus alonensis</i>	30SVF57	AY928560	AY928588	-
IaAL07	<i>Iberus gualtieranus</i>	30SWF27	AY928558	AY928586	-
IaAL06	<i>Iberus gualtieranus</i>	30SWF37	AY928557	AY928585	-

Table 1s. (Cont.)

Voucher	Species	Locality (UTM)	GenBank Accession Number		
			COI	16S rRNA	LSU
lor03	<i>Iberus gualtieranus ornatissimus</i>	30SWF39	DQ822173	DQ822123	-
lor02	<i>Iberus gualtieranus ornatissimus</i>	30SWF39	DQ822172	DQ822122	-
lor01	<i>Iberus gualtieranus ornatissimus</i>	30SWF39	DQ822171	DQ822121	MW817745
laAL05	<i>Iberus gualtieranus</i>	30SWF08	AY928556	AY928584	-
Imari02	<i>Iberus gualtieranus mariae</i>	30SWF26	DQ822170	DQ822120	-
Imari01	<i>Iberus gualtieranus mariae</i>	30SWF26	DQ822169	DQ822119	-
laAL04	<i>Iberus gualtieranus</i>	30SWF07	AY928555	AY928583	-
IguaEHUMC2262	<i>Iberus gualtieranus mariae</i>	36°51'00"N 2°57'00"E	MW803159	MW817705	MW817744
IgJ02	<i>Iberus gualtieranus gualtieranus</i>	30SVG28	AY928578	AY928606	-
IgJ01	<i>Iberus gualtieranus gualtieranus</i>	30SVG28	AY928577	AY928605	-
IgGR06	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928576	AY928604	-
IgAL03	<i>Iberus gualtieranus gualtieranus</i>	30SWF48	AY928570	AY928598	-
IgGR04	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928574	AY928602	-
IgGR03	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928573	AY928601	-
IgGR02	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928572	AY928600	-
IgGR01	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928571	AY928599	-
IgAL02	<i>Iberus gualtieranus gualtieranus</i>	30SWF48	AY928569	AY928597	-
IgAL01	<i>Iberus gualtieranus gualtieranus</i>	30SWF48	AY928568	AY928596	-
lalo01A	<i>Iberus gualtieranus alonensis</i>	30SWF58	DQ822125	DQ822075	-
gua331	<i>Iberus gualtieranus</i>	Sierra Elvira	KM592620	KJ458530	KJ458617
guaESP331	<i>Iberus gualtieranus</i>	37°24'56"N 3°43'30"W	KR705034	KR704995	KR705072
IgJ03	<i>Iberus gualtieranus gualtieranus</i>	30SVG28	AY928579	AY928607	-
IgGR05	<i>Iberus gualtieranus gualtieranus</i>	30SVG32	AY928575	AY928603	-
Ilmin-150 (UPV)	<i>Iberellus minoricensis</i>	-	DQ822174	DQ822124	-
MN 2527-Hel-161	<i>Eremina dillwyniana</i>	-	KR705036	KR704999	KR705076
MN 2555-Hel-153	<i>Rossmassleria sicanoides</i>	-	KR705040	KR705003	KR705080

Table 2s. Location and basic morphometrics of the holotype and the paratypes assigned to *I. rutensis* sp. nov.**Tabla 2s.** Ubicación y morfometría básica del holotipo y los paratipos asignados a *I. rutensis* sp. nov.

Specimen	Length (mm)	Width (mm)	Height (mm)	Latitude (N)	Longitude (W)	Location (Province of Córdoba, southern Spain)
Holotype	22.62	19.37	11.66	37° 19' 45"	-4° 19' 47"	Northern of Lanchar of Rute
Paratype 1	24.41	20.49	13.35	37° 22' 39"	-4° 16' 42"	Las Lagunillas, Priego de Córdoba
Paratype 2	23.75	19.83	11.81	37° 20' 15"	-4° 18' 23"	Las Herrera stream, Lorite, Rute
Paratype 3	20.40	17.23	10.61	37° 17' 31"	-4° 18' 21"	La Celada, Iznájar
Paratype 4	22.55	18.6	11.03	37° 28' 57"	-4° 10' 52"	Angosturas of Salado River, Priego de Córdoba
Paratype 5	23.84	19.86	11.95	37° 19' 42"	-4° 19' 44"	Northern Lanchar of Rute
Paratype 6	17.92	15.07	8.76	37° 17' 32"	-4° 17' 14"	Los Juncares, Iznájar

Table 3s. Shell morphometric parameters and ratios measured for *I. rutensis* (n = 625): CV, coefficient of variation.**Tabla 3s.** Parámetros y relaciones morfométricas de las conchas de *I. rutensis* (n = 625): CV, coeficiente de variación.

Parameters and ratios	Minimum	Maximum	Average	SD	CV (%)
Major Ø of the shell (mm)	16.16	25.45	21.26	1.69	7.95
Minor Ø of the shell (mm)	13.95	21.60	18.00	1.41	7.81
Shell height (mm)	8.11	14.55	11.01	1.07	9.71
Major external Ø of the peristome (mm)	9.03	17.28	12.73	1.28	10.03
Minor external Ø of the peristome (mm)	7.25	13.23	10.29	1.03	9.99
Shell height/major Ø of the shell (ratio)	0.45	0.63	0.52	0.03	4.95
Shell area (mm ²)	177.18	429.88	302.25	46.56	15.41
Peristome area (mm ²)	51.42	162.27	103.69	19.50	18.80
Major Ø/minor Ø of the shell (ratio)	1.10	1.33	1.18	0.02	2.02
Major Ø/minor Ø of the peristome (ratio)	1.03	1.74	1.24	0.07	5.67
Peristome area x 100/shell area (%)	26.70	46.17	34.21	2.81	8.23

Table 4s. Locations thoroughly sampled for *I. rutensis*.**Tabla 4s.** Localidades donde se realizaron muestreos intensivos de *I. rutensis*.

Municipality			
Location	Latitude (N)	Longitude (W)	Altitude (m a.s.l.)
Rute			
Near Las Herreras stream, Lorite	37° 20' 15"	-4° 18' 23"	699
Near Rígüelo stream, Lorite	37° 19' 59"	-4° 17' 24"	731
Northern of Lanchar of Rute	37° 19' 45"	-4° 19' 47"	788
	37° 19' 42"	-4° 19' 44"	759
Near La Hoz River, Lorite	37° 19' 03"	-4° 19' 47"	476
Near Rute el Viejo Castle	37° 20' 41"	-4° 21' 16"	786
Near Anzur River	37° 21' 05"	-4° 23' 24"	518
Priego de Córdoba			
Angosturas of Salado River	37° 28' 24"	-4° 11' 01"	574
	37° 28' 26"	-4° 11' 10"	516
	37° 28' 57"	-4° 10' 52"	554
Gorge in Las Labores stream, Las Lagunillas	37° 22' 39"	-4° 16' 42"	1,301
La Cañatienda stream, Las Lagunillas	37° 22' 05"	-4° 16' 03"	952
Los Villares	37° 23' 25"	-4° 19' 07"	828
Iznájar			
Los Juncares, Iznájar	37° 17' 32"	-4° 17' 14"	666
La Celada	37° 17' 31"	-4° 18' 21"	729