Who is who? An integrative taxonomic revision of *Iberus carthaginiensis* (Rossmässler, 1853) (Gastropoda: Helicidae) in southeastern Spain

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ABSTRACT: The taxonomically poorly known Iberian genus of land snails *Iberus* exhibits high levels of endemicity, often confined to small regions. An example is a small snail restricted to the southeastern coastal area of the Iberian Peninsula which had been known as *Iberus carthaginiensis* until a new species, *I. calaensis*, was assigned to its westernmost populations. Since then, the taxonomic name *I. carthaginiensis* has been solely applicable to the former eastern populations. The findings of a taxonomic integrative revision of *I. carthaginiensis* by combining geographical, morphological and phylogenetic evidence reveal that the populations of the current eastern distribution for *I. carthaginiensis* correspond to a new species, not previously sequenced, which is named *I. punicus* sp.n. Likewise, the molecular results obtained from samples collected within the type locality of *I. calaensis* confirm that this taxon is a junior synonym of *I. carthaginiensis*. Therefore, *I. carthaginiensis* remains restricted to the western populations of the traditional distribution assigned to this species, and *I. calaensis* is invalidated.

KEY WORDS: *Iberus*, *punicus*, *alonensis*, morphometrics, Cartagena, Murcia, Iberian Peninsula.

Кто есть кто? Интегративная таксономическая ревизия *Iberus carthaginiensis* (Rossmässler, 1853) (Gastropoda: Helicidae) из юго-восточной Испании

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РЕЗЮМЕ: Таксономически мало исследованный род легочных моллюсков *Iberus* демонстрирует высокую степень эндемизма часто ограниченную небольшой территорией. Один из примеров — это вид *Iberus carthaginiensis*, распространение которого ограничено побережьем юго-восточной части Иберийского полуострова. Этот вид был признан популяцией вида *Iberus calaensis*, распространенного в самой восточной части полуострова. С тех пор название вида *I. carthaginiensis* используется исключительно для обозначения восточной популяции рода *Iberus*. Ревизия рода с применением географических, морфологических и молекулярно-генетических данных позволила выявить новый вид *I. punicus* sp.n. Кроме того, молекулярно-генетические исследования показали, что вид *I. calaensis* следует рассматривать как младший синоним *I. carthaginiensis*, а *I. calaensis* является невалидным.

КЛЮЧЕВЫЕ СЛОВА: *Iberus*, *punicus*, *alonensis*, морфометрия, Картахена, Мурсия, Иберийский полуостров.

Introduction

The genus of land snails *Iberus* is endemic to the Iberian Peninsula, and most species have a southern distribution, with restricted small areas leading to high levels of local endemicity. One interesting species, is *Iberus alonensis* (A. Férussac, 1821), as it is the only species of its genus whose distribution extends outside the region of Andalusia and Murcia, along the Mediterranean coast and the northeastern half of the Iberian Peninsula (Robles, Martínez-Ortí, 2009; Alba *et al.*, 2011; Chueca *et al.*, 2018; Cadevall *et al.*, 2020; Zaldívar Ezquerro, 2022). There has been no ambitious study covering this vast distribution area so far.

The *alonensis* complex populations have been extensively referenced in the scientific literature and are mostly characterised by globose shells of sizes typically between 30 and 40 mm (Aguilar Amat, 1925, 1930; García San Nicolás, 1957; Arrébola, 1995; Elejalde *et al.,* 2005; Moreno-Rueda, 2006; Liétor, 2014; Neiber *et al.,* 2021). The broad distribution range of *I. alonensis* together with its conchological

variability have resulted in complex taxonomic decisions for the species and in consequence, several *Iberus* species and subspecies are today synonym of *I. alonensis*: *Helix alcarazana* Rossmässler, 1854, *H. alonensis* var. *lorcana* Rossmässler, 1854, *H. alonensis* var. *minima* Schmidt, 1855, *H. bajoi* Servain, 1880, *Iberus carragines* Aguilar Amat, 1921, *I. posthumus* Haas, 1934 and *I. alvaradoi* García San Nicolás, 1957. Therefore, as suggested by Bank & Luijten (2014), further research to clarify this taxonomic challenge (probably posed by cryptic species) is needed. To date, only Elejalde *et al.* (2008a) has partially contributed to the species delimitation within the *I. alonensis* complex when recognizing two subgroups morphologically, genetically and geographically differentiated within their clade A6. Nevertheless, considering the high degree of uncertainty associated with this clade, the authors could just propose a provisional species (*I. alonensis*-like 02) that remains undescribed.

I. carthaginiensis (Rossmässler, 1853) is one of the species that has been phylogenetically associated with *I. alonensis* (Elejalde *et al.*, 2008a). This species was originally described as *Helix*

carthaginiensis and assigned to a somewhat ambiguous type locality in the surroundings of Cartagena in the province of Murcia (southern Spain), and all records since then are from the same region. More recently, Ahuir-Galindo (2013) found that the shells of the westernmost populations of *I. carthaginiensis* showed certain distinctive morphological features, resulting in the description of *I. calaensis* Ahuir, 2013 with type locality in La Azohía (Sierra de la Muela, Cabo Tiñoso y Roldán, Murcia). This description has not received full support, suggesting that samples named after *I. calaensis* needs further research to clarify its exact position within the genus *Iberus* (Bank, Luijten, 2014). The actual identity of *I. carthaginiensis* populations towards the eastern remain unsolved since there are no sampling records for such in the literature.

The aim of this work is to assess the taxonomic status of *I. carthaginiensis* and its phylogenetic relationship with *I. alonensis* and *I. calaensis* through an integrative taxonomic approach which combines a comprehensive geographical mapping, a morphological analysis involving a high number of shells and new molecular evidence from biological samples taken in key locations. We hope that this work will help clarify taxonomic uncertainties within the genus and unravel the possible presence of unknown cryptic species in the region.

Material and methods

FIELD SAMPLINGS AND SAMPLE PROCESSING. To precisely clarify the geographical distribution and morphological diversity of *I. carthaginiensis* we opted for an intensive sampling that systematically covered the whole potential distribution area throughout the south of the province of Murcia (southeastern Spain). Since previous studies (Elejalde *et al.,* 2008a) had considered *I. carthaginiensis* within the group of *alonensis*-like morphotypes, several localities assignable to *I. alonensis* in the north-east of the Murcia Province were included in the sampling. Given the uncertainty of the taxonomic identity of *I. alonensis* along its northernmost distribution, no populations outside of Murcia were added to the sampling of *I. alonensis*. Finally, 52 sampling points were recorded: 11 for *I. alonensis s.str.* (*I. alonensis* henceforth) and 41 within the classical distribution of *I. carthaginiensis*, which were divided into 20 western points assigned to the *I. calaensis* distribution and 21 to the east. The few sampling localities where we found intermediate

shaped shells were excluded from the study since taxonomic identity was unclear.

The planning of the network of sampling points was based on previous citations documented in specialised literature and the prior knowledge and field experience of the research team. A group of shells of the studied taxa were sampled at most sampling points. Good quality specimens were cleaned and some photographed. All the shells, including those sun-bleached and subfossils, were measured to obtain the set of morphometric parameters for morphological characterization. Some live specimens were collected in key locations. Once in the laboratory, tissue samples were processed for further molecular analyses. Special effort was made to establish labelling and conservation protocols for shells and biological tissues to guarantee optimal traceability of the specimens.

MORPHOMETRICS. The procedures and methods used to obtain morphometric measurements of the shells can be found in Jowers *et al.* (2024). Fig. 1 schematically represents how the main morphometric parameters were defined.

According to an integrative taxonomy approach, morphometric measurement cannot be used as an isolated criterion to differentiate species but it can be a useful additional tool for that purpose. Then, three questions arise: i) What minimum amount of shells should be measured to approximate the representative morphometry of a given species?, ii) Are the morphometric parameters steady enough to be considered representative of the size and shape of a given species?, iii) Is ontogenic variation and subsequent shell growth during the adult stage a biasing factor to be taken into account?

Fig. 2A responds to questions i) and ii). It shows that the minimum number of shells required for the morphometric parameters to stabilise (thus, to be representative), ranges from 200 to 300 depending on the taxa. Fig. 2B shows how the coefficients of variation of the morphometric parameters were certainly small, without exceeding 15% in any case. These results allow us to deduce that, when many shells are measured, morphometrics can be valuable as a complementary tool for taxonomic research within *Iberus*.

Regarding question iii), our captive breeding experiences with various *Iberus* species (unpublished data) show that the growth rate of adult snails is small, sometimes imperceptible. Similarly, Polo (2016) reported for a 2-year follow-up over a cohort of adult specimens of *I. gualtieranus*, that there were no significant variations in the shell growth rate once the adult stage was reached. Therefore, we asume that morphometrics is not significantly affected by ontogenic developmet when measurements involve adult specimens (characterised by fully developed thickened lips).

Statistical comparisons between morphometric measurements were carried out with ANOVA tests when the variables were homoscedastic and normally
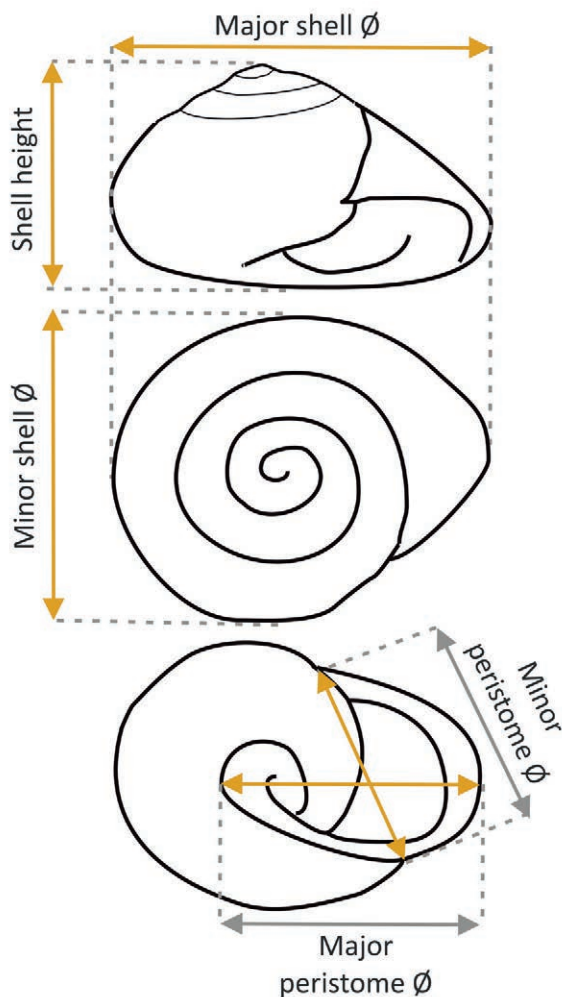
Fig. 1. Shell dimensions considered for morphometric analyses of fully adult specimens. The major diameter of the peristome includes the space occupied by the umbilical callus if present.

Рис. 1. Размеры раковин половозрелых особей, используемые для морфометрического анализа. Наибольший диаметр перистома (устья) включает внутреннюю губу устья и пупочный каллус (если имеется).

distributed, otherwise using the non parametric Kruskal-Wallis test. In addition, a Principal Component Analysis (PCA) was carried out to determine the overlap between the taxa under study in the morphospace.

MOLECULAR ANALYSIS. Among all the specimens collected alive in the field, those from some key locations were selected for genetic analysis. Finally, 11 live specimens were collected and coded as follows: J1, J2, J5 for *I. alonensis*; K3, K5, AR2 and BA1 for *I. calaensis*; K1, AT1, AT3 and AT4 for *I. carthaginiensis*. The specimen K3 was sampled at the entrance of La Azohía (Murcia), the type locality of *I. calaensis*. Therefore, it may be considered as a topotype for this species.

Once in the laboratory, the specimens were sacrificed by drowning and a tissue sample was extracted for molecular analyses. Samples were stored in absolute ethanol and maintained at –20 °C.

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* sequences from Genbank (N = 141) including *Iberellus minoricensis* and two outgroup taxa, *Rossmaessleria sicanoides* and *Eremina dillwyniana* (Table S1). We amplified a fraction of the mitochondrial cytochrome oxidase I (COI) gene with primers from Jowers *et al*. (2024), 16S rRNA (Palumbi, 1996) and a fraction
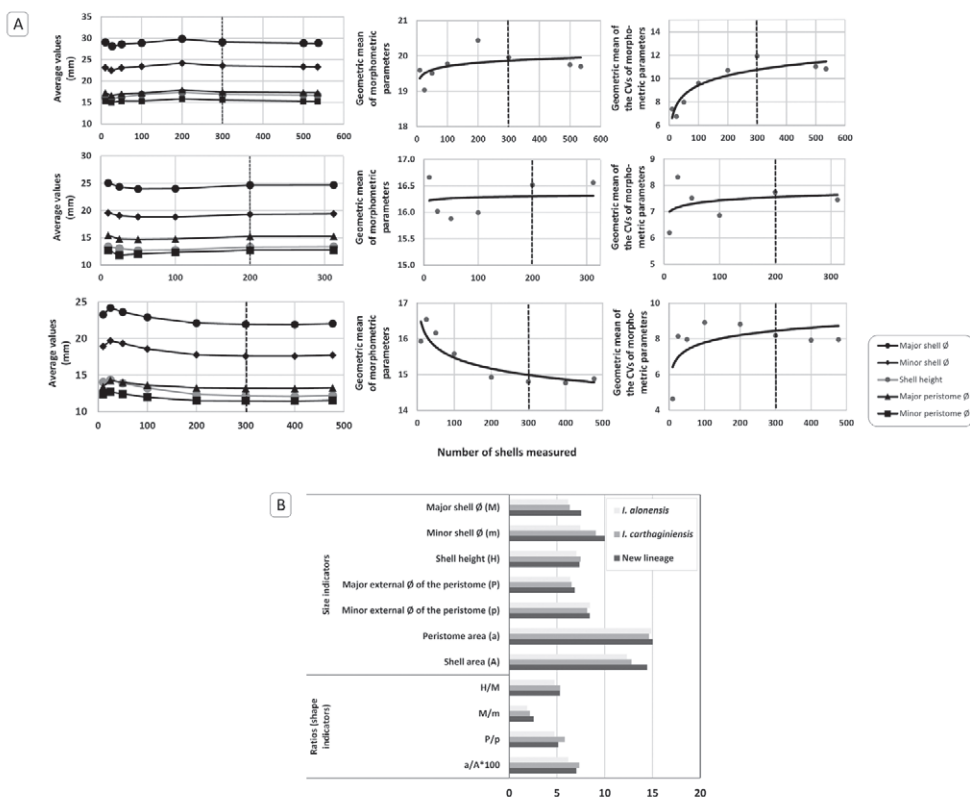
Fig. 2. Indicators on the representativeness and reliability of morphometric analyses. A — quantities of shells of (from top to bottom) *I. alonensis*, *I. carthaginiensis* and a new lineage (see results) from which morphometric parameters stabilise (dashed lines). Left column — 5 main morphometric parameters. Central column — geometric mean of the main morphometric parameters. Right column — geometric mean of the coefficients of variation (CVs) of the main morphometric parameters. Measurements along X-axis were obtained by adding new shells to those already measured in the previous points (i.e. while the abscissa point 100 represents an average value for 100 shells, the abscissa point 200 represents an average value in which another 100 new shells were added to the same 100 previous ones); B — coefficients of variation (%) of the morphometric variables for the whole set of shells considered in this study.

Рис. 2. Индикаторы достоверности и надежности морфометрического анализа. A — число раковин (сверху вниз) *I. alonensis*, *I. carthaginiensis* и нового вида, чьи морфометрические параметры стабильны (отмечены пунктиром). Левая колонка — 5 основных морфометрических параметров. Центральная колонка — геометрическая средняя основных морфометрических параметров. Правая колонка — геометрическая средняя и коэффициент вариации (CVs) основных морфометрических параметров. Измерения по оси X получены для новых дополнительных раковинок и составляют 100 экземпляров, тогда как по оси Y отложены 200 особей (100 новых и 100 уже имевшихся в коллекции); B — коэффициент вариации (%) морфометрических параметров, рассматриваемых в этой статье.

of the nuclear gene large ribosomal subunit (LSU) (Wade *et al.*, 2006).

Sequences were edited with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA), and checked for potential contaminants using GenBank's BLASTn search (Altschul *et al.*, 1990). Sequences were edited in Seaview v.4.2.11 (Gouy *et al.*, 2010) and aligned with MAFFT (Katoh *et al.,* 2002).

Phylogenetic tree reconstructions for the three concatenated gene fragments (total length ~1,980 bp) were performed using maximum likelihood (ML) and Bayesian inference (BI), through RAxML v.7.0.4 (Silvestro, Michalak, 2012) and MrBayes v.3.2, (Ronquist, Huelsenbeck, 2003), respectively. The Akaike Information Criterion (AICc) and partition scheme was implemented in PartitionFinder

v.2.1.1 (Lanfear *et al*., 2016), using a 'greedy' search (Lanfear *et al.*, 2012) to select the best fit evolutionary model for each partition. The chosen models for the BI analyses were GTR+I+G (COI pos1), F81+I (COI pos2), GTR+I+G (COI pos3), GTR+I+G (16S rRNA) and HKY+G (LSU). From the BI analysis, two independent runs (each with four Markov chains for 10×10^7 generations) were performed. Trees and parameters were sampled every 1,000 generations. A majority-rule consensus tree was estimated by combining results from duplicated analyses, after discarding 25% of the total samples as burn-in. ML searches were conducted under GTRGAMMA and support was assessed by using 1,000 bootstrapped replicates. All phylogenetic analyses were performed in the CIPRES platform (Miller *et al.*, 2010). Trees were visualised and edited in Figtree v.1.4.4 (Rambaut, 2018), and later prepared as a graphic with Inkscape v.1.0.1 (http://www.inkscape.org). Uncorrected p-distances with partial deletion were computed in MEGA (Kumar *et al*., 2018).

REASONS FOR THE ABSENCE OF ANATOMICAL STUDY. Given the scenario of plausible widespread hybridization documented for the genus *Iberus* (Moreno-Rueda, 2006; Rodríguez-Perochena, 2006; Elejalde *et al*., 2008b; Martínez-Ortí, Robles, 2012; Liétor, 2014; Jowers *et al.*, 2024), firmly confirmed by the intensive samplings of the authors (unpublished data), the study of the anatomy loses relevance for *Iberus* snails. Indeed, the genus *Tartessiberus* (Altaba, Ríos-Jiménez, 2021) was defined solely based on anatomical criteria and radula morphology and subsequent molecular analyses confirmed its synonymisation with *Iberus* (Jowers *et al.*, 2024).

Results

PHYLOGENETIC POSITION AND GENETIC DIVERGENCE. Phylogenetic relationships of the major *Iberus* clades and species recovered the same tree topologies as Galán-Luque (2023), Liétor *et al*. (2024) and Jowers *et al*. (2024). All clades recovered, as well as the nodes supporting sister clade relationships for this study were highly supported in all phylogenetic analyses (Fig. 3). More specifically, *I. carthaginiensis* is sister clade to *I. globulosus* (*sensu* Elejalde *et al*., 2008a), currently named *I. carthaginiensis globulosus* (*sensu* Bank, Luijten, 2014) and the clade provisionally named *I. alonensis*-like 01 by Elejalde *et al*. is sister clade to the abovementioned clades. The clade into which *I. alonensis* is integrated is sister to a new operational taxonomic unit consisting of the samples preliminary classified as *I.*

carthaginiensis (Fig. 3). This group, that had gone unnoticed until now, will be called 'new lineage' hereafter.

As expected, genetic divergence within the recovered clades were lower than between them (Table 1). The highest within divergence was recovered from *I. carthaginiensis* for COI (3.90%) while the 16S rRNA data set recovered low divergence (0.79%). The genetic differentiation between all 5 clades (Table 2) was on average 10.62% for COI and 5.52% for 16S rRNA. The highest divergence for COI was between the new lineage with *I. alonensis*-like 01 and *I. alonensis*-like 02 (12.60% and 11.73%, respectively). Regarding the 16S rRNA, the highest divergence was between *I. carthaginiensis* and *I. alonensis* (6.99%) and between the new lineage and *I. carthaginiensis* (7.06%). Genetic distance between *I. carthaginiensis* and the new lineage is additionally supported by a divergence of 11.72% for COI.

Samples taken at the type locality of *I. calaensis* fell within the *I. carthaginiensis* clade (Fig. 3). Therefore, the populations preliminarily classified as *I. calaensis* will henceforth be considered *I. carthaginiensis*.

GEOGRAPHICAL DISTRIBUTION. From its western to its eastern distribution, *I. carthaginiensis* occupies about 170 km^2 between Mazarrón and the western end of Sierra de la Muela, Cabo Tiñoso y Roldán. The new lineage is distributed west of this mountain range covering about 225 km^2 from El Portús to Cabo de Palos (Murcia) (Fig. 4). *I. alonensis* is located in the badlands of northern Murcia. The extensive Murcian orchard and the city of Cartagena act as geographical barriers between *I. alonensis* and the coastal taxa (*I. carthaginiensis* and the new lineage). The unique further south population unequivocally identified as *I. alonensis* during sampling inhabited one of the scarce favourable habitats that have remained among the orchards (Fig. 4).

MORPHOLOGICAL COMPARISON. We measured 1,323 shells, distributed as follows: 535 for *I. alonensis,* 311 for *I. carthaginiensis* and 477 for the new lineage. In all cases, the representativeness thresholds reported in Fig. 2A were exceeded.

Due to the geographical and phylogenetic proximity of the new lineage and *I. alonensis*, it was considered as appropriate to explore the morphometric similarities between their type
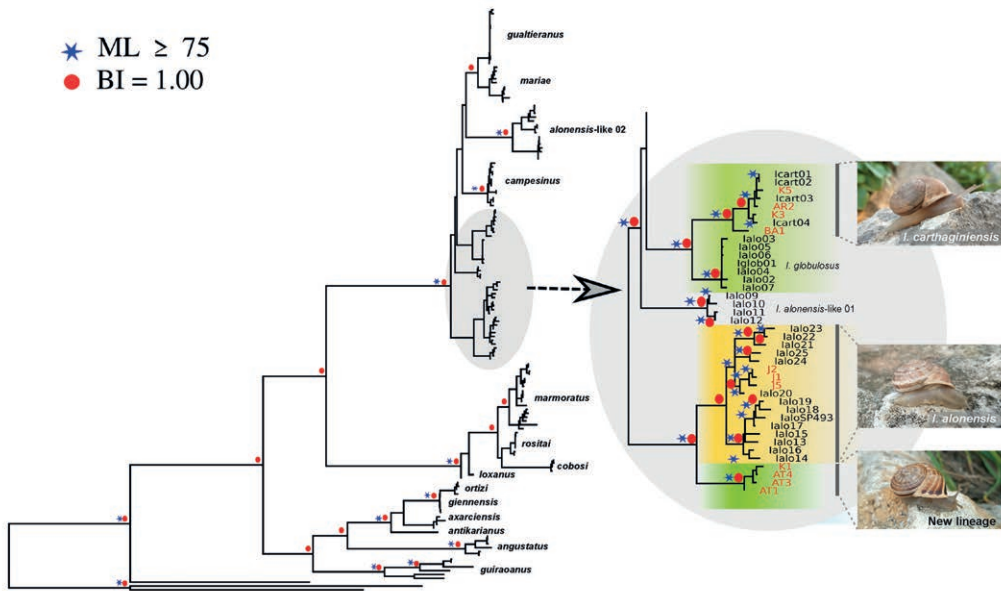
Fig. 3. Maximum Likelihood tree of clade 3 of the genus *Iberus* according to topology of Galán-Luque (2023). Values by nodes represent bootstrap values for the ML analyses (≥ 75%, blue stars). Only the values on major nodes (red filled circles) are represented for the BI analyses (BI =1.00). Samples analysed in this study have been highlighted in red.

Рис. 3. Дерево, построенное методом максимального правдоподобия показывает наличие 3-х клад в роде *Iberus* по топологии Galán-Luque (2023). Уровни бутстреп поддержки больше 75% отмечены синим цветом. Вариации главного узла показаны красным цветом. Пробы, использованные для данной работы, отмечены красным цветом.

Table 1. Within genetic divergence (*p*-uncorrected distances) for the clades of interest for COI and 16S rRNA.

Таблица 1. В пределах генетической дивергенции (р-нескорректированные расстояния) для клад, представляющих интерес для COI и 16S pPHK.

	COI	16S rDNA
I. alonensis-like 02	0.61%	0.29%
I. carthaginiensis	3.90%	0.79%
I. alonensis	0.42%	1.62%
I. alonensis-like 01	0.66%	1.16%
New lineage	1.71%	0.63%

percentage of the shell that is occupied by it. The average shell of the new lineage is significantly smaller than that of *I. carthaginiensis* (e.g. the shell area is 18% smaller). Contrastingly, the shell of *I. carthaginiensis* is more flattened and its peristome is significantly larger (the major diameter and the area of the peristome were 13 and 21% higher, respectively) (Table 3).

The first principal component (PC1) of the PCA explained the population clustering much better than the second principal component (PC2), since it absorbed almost 3 times more variance from the data. PC1 ordered *Iberus* populations according to the size of the shell, while PC2 was related to shell morphology (circularity, globosity and relative area of the peristome) (Fig. 5).

The PCA properly collected the significant differences found for most of the morphometric indicators (Table 3), providing a clustering in which the populations of the compared taxa occupied well-defined positions in the morpho-

shells. Table 3 shows that both species have shells with different average sizes and shapes. The shell of *I. alonensis* is larger, taller, more globose and circular. The only morphometric data that did not show significant differences between both species was the circularity of the peristome and the

Table 2. *p*-uncorrected distances for the taxa of the clades closely associated with *Iberus carthaginiensis* for COI (lower matrix) and 16S rRNA (upper matrix).

Таблица 2. p-нескорректированные расстояния для таксонов клад, тесно связанных с *Iberus carthaginiensis* для COI (нижняя матрица) и 16S рРНК (верхняя матрица).

	I. alonensis-like 02	*I. carthagi-niensis*	*I. alonensis*	*I. alonensis*-like 01	New Lineage
I. alonensis-like 02	–	3.71%	6.25%	5.53%	5.86%
I. carthaginiensis	6.56%	–	6.99%	5.21%	7.06%
I. alonensis	11.29%	11.62%	–	5.82%	2.71%
I. alonensis-like 01	9.85%	11.14%	11.02%	–	6.05%
New Lineage	11.73%	11.72%	8.70%	12.60%	–

Fig. 4. Geographic distribution of the *Iberus* taxa studied in this work throughout the Province of Murcia: *I. carthaginiensis* (triangle), the new lineage (circle) and *I. alonensis* (square). A — view of the general distribution; B — detail of the distribution of *I. carthaginiensis* and the new lineage. The entire territory was intensively sampled. Thus, gaps greater than 20 km^2 with no sampling points represent areas where none of the taxa under study were recorded.

Рис. 4. Географическое распределение представителей рода *Iberus*, обнаруженных в провинции Мурсия: *I. carthaginiensis* (треугольник), новый вид (кружок) и *I. alonensis* (квадрат). A — view of the general distribution; B — детали распространения представителей вида *I. carthaginiensis* и оувого вида. Вся территория была очень тщательно обследована. Таким образом, разрывы более 20 кв.км без точек отбора проб представляют собой области, где не было зарегистрировано ни одного из исследуемых таксонов.

Table 3. Morphometric comparison among *Iberus alonensis, I. carthaginiensis* and the new lineage.
Таблица 3. Морфометрическое сравнение *Iberus alonensis*, *I. carthaginiensis* и новой линии.

Parameters and ratios	*I. alonensis* (n=161)	*I. carthaginiensis* (n=311)	New lineage (n=477)
Major Ø of the shell (mm) [K]	27.71 ± 1.77[a]	24.69 ± 1.61[b]	22.04 ± 1.51[c]
Minor Ø of the shell (mm) [K]	22.40 ± 1.38[a]	19.37 ± 1.23[b]	17.73 ± 1.34[c]
Shell height (mm) [K]	16.17 ± 1.20[a]	13.37 ± 1.21[b]	12.21 ± 1.22[c]
Major external Ø of the peristome (mm) [K]	16.69 ± 1.17[a]	15.28 ± 1.14[b]	13.27 ± 0.98[c]
Minor external Ø of the peristome (mm) [K]	14.48 ± 1.22[a]	12.75 ± 1.04[b]	11.54 ± 0.97[c]
Shell height/Major Ø of the shell (ratio) [K]	0.584 ± 0.028[a]	0.541 ± 0.029[c]	0.553 ± 0.029[b]
Shell area (mm^2) [K]	489.14 ± 60.25[a]	377.07 ± 48.25[b]	308.43 ± 44.55[c]
Peristome area (mm^2) [K]	190.74 ± 28.35[a]	153.65 ± 22.48[b]	120.80 ± 18.15[c]
Major Ø/Minor Ø of the shell (ratio) [A]	1.237 ± 0.023[c]	1.275 ± 0.028[a]	1.244 ± 0.032[b]
Major Ø/Minor Ø of the peristome (ratio) [A]	1.155 ± 0.055[b]	1.201 ± 0.070[a]	1.152 ± 0.059[b]
Peristome area x100/Shell area (%) [A]	38.94 ± 2.40[b]	40.75 ± 2.99[a]	39.23 ± 2.75[b]

Values are given with the mean ± SD. K indicates that the test was a Kruskal-Wallis, while A indicates that an ANOVA test was used. Superscripts with different letters indicate significant differences (p-value < 0.05). Comparisons between pairs of variables were carried out with the Tukey test (HSD) when normally distributed, or with a two-tailed multiple comparison when non-normally distributed. It must be clarified that 161 out of the 535 shells measured for *I. alonensis* were selected for this analysis, just those corresponding to the populations of the Murcia region.

space with no overlapping. *I. carthaginiensis* and the new lineage showed smaller shells than *I. alonensis*. While the new lineage accounted for a range of PC2 scores similar to *I. alonensis* (mostly positive), the populations of *I. carthaginiensis* were distributed in a range of lower scores, always negative, which is indicative of flattened and more elliptical shells with a protruding peristome (Fig. 5).

A NEW SPECIES FOR THE GENUS *IBERUS*. As concluded from the phylogenetic analysis, genetic divergence and complementarily supported by geographical separation and morphological discrimination, we proceed to describe a new species for the genus *Iberus*. This species is named *Iberus punicus* sp.n.

Systematics

Order Stylommatophora Schmidt, 1855
Suborder Helicina Rafinesque, 1815
Superfamily Helicoidea Rafinesque, 1815
Family Helicidae Rafinesque, 1815

Iberus punicus **sp.n.**
Figs 6–9.

ETYMOLOGY. The city of Cartagena was the capital of the Carthaginian dominions in the Iberian Peninsula. When it was conquered by the Roman general Publius Cornelius Scipio it adopted the name of Cartago Nova, from which the current name of Cartagena is derived (in honour of the city of Carthage, capital of the Punic State, in modern-day Tunisia). The new species is found in the surroundings of what was the symbol of Carthage's power in the Iberian Peninsula. The term "punic" (in Latin, *punicus*), literally means Carthaginian.

HOLOTYPE AND PARATYPES. See Fig. 6 to check the holotype and paratype shells assigned to *I. punicus* sp.n. (*I. punicus* hereafter). Holotype and paratypes 1 to 3 remain deposited in the Museo Nacional de Ciencias Naturales – CSIC (Madrid, Spain) with deposit references 15.05/200530 for holotype and 15.05/200531, 15.05/200532 and 15.05/200533 for paratypes, respectively. Paratypes 4 to 8 remain deposited in the private collection of Dr. José Liétor Gallego (Jaén, Spain). Table 4 shows some extra information about sizes and locations of the shells.

Type locality of *I. punicus* consists of Mediterranean thermophilic scrublands with palmettos from
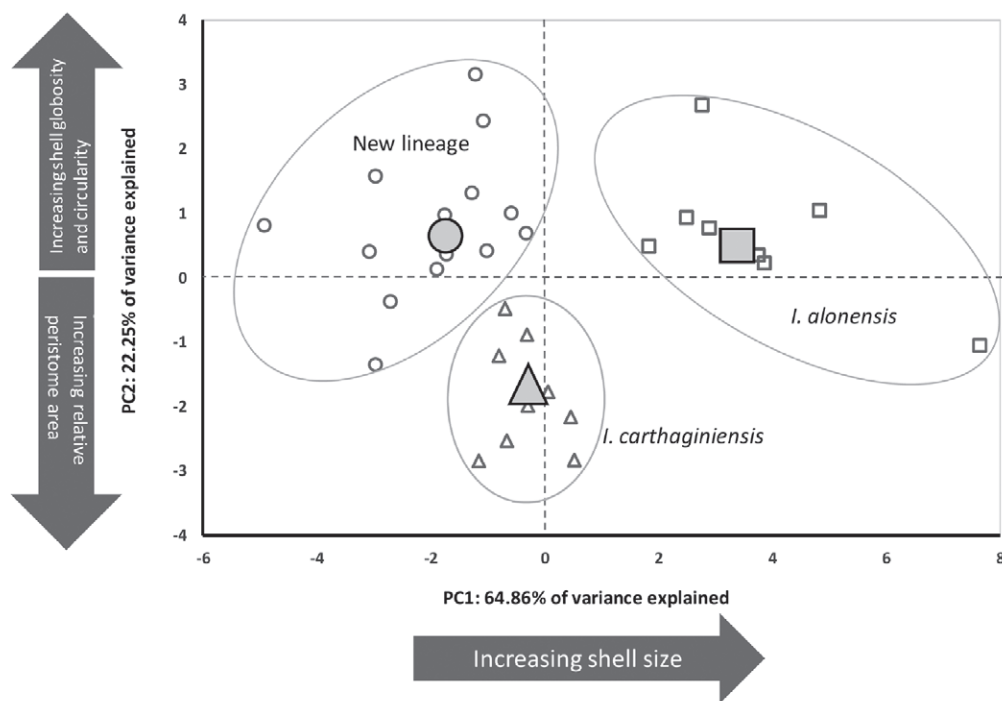
Fig. 5. Clustering of *Iberus alonensis* (8 locations), *I. carthaginiensis* (9 locations), and the new lineage (14 locations) in the two-dimensional morphospace provided by the first two PCs of a PCA. All morphometric parameters and ratios were combined to run the PCA. Each point on the graph represents a sampling location. The coordinates of the centroids for each taxon (large symbols) were calculated as the averages of the X and Y coordinates of the points included in the corresponding clouds.

Рис. 5. Кластеризация *Iberus alonensis* (8 местоположений), *I*. carthaginiensis (9 местоположений) и новой линии (14 местоположений) в двумерном морфопространстве, предоставленном первыми двумя ПК PCA. Все морфометрические параметры и соотношения объединяли для проведения PCA. Каждая точка на графике представляет место отбора проб. Координаты центроидов для каждого таксона (больших символов) рассчитывались как средние значения координат X и Y точек, входящих в соответствующие облака.

Los Belones, Cartagena (Murcia), with the following coordinates: 37°36′51″ N, 0°47′50″ W.

TYPE SHELL DESCRIPTION. A series of conchological variability for *I. punicus* is shown in Fig. 7. Some representative shells of *I. alonensis* and *I. carthaginiensis* are shown in Fig. 8 along with that of *I. punicus* for comparative purposes.

Type shell of *I. punicus* is semi-globose, with acute apex, unkeeled and not umbilicated, with 4–4.5 whorls of regular growth. Major diameter of the shell ranges from 18 to 29 mm. The suture is simple and visible in all whorls. The smooth protoconch occupies the last whorl and shows uniform light to medium brown colour. The surface of the shell (except in the smooth protoconch) shows a fine radial (transver-sal) and spiral (longitudinal) striation, resulting in a reticulated background. The radial striation has a greater density than the spiral one, sometimes turning into ribs or cords, separated from each other by non-periodic distances. Shell aperture is large, from oval to predominantly semilunar, wider than high (equivalent to approximately half the total width of the shell). The peristome is off-white, more or less solid and ranges from cutting to reflected, although the most common is the slightly reflected one. Rarely, the umbilicus area exhibits an off-white to white dilated columellar expansion that becomes a small callus. Subadult shells never show this callus.

The colour of the shell in the main whorls (excluding the protoconch) may vary from off-white to
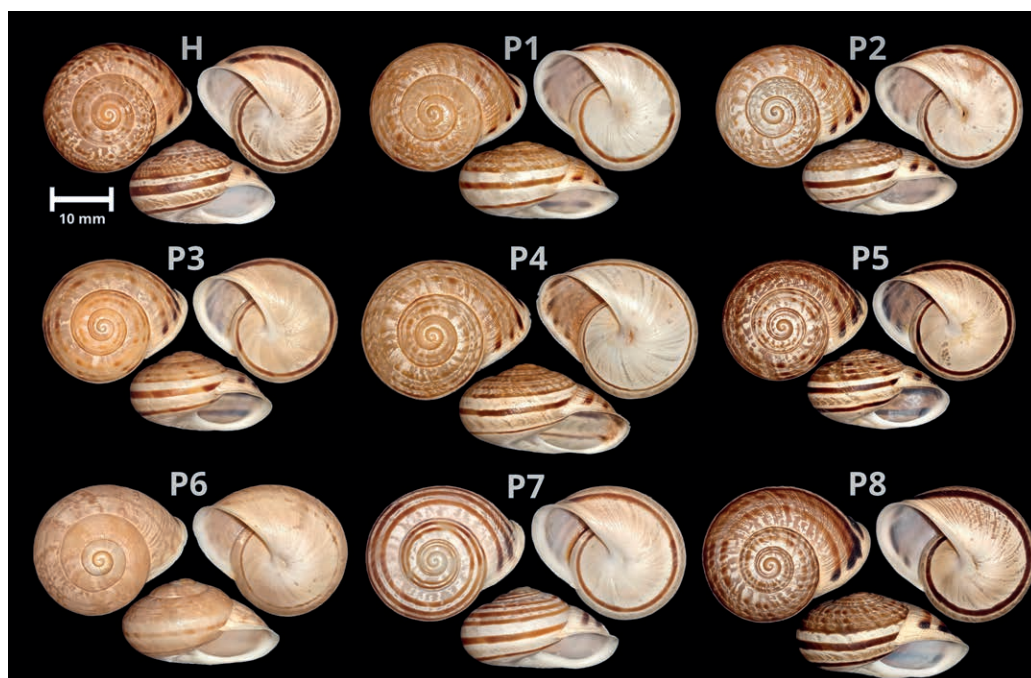
Fig. 6. Photographs of the holotype (H) and the 8 paratypes (P1 to P8) of *Iberus punicus* sp.n.
Рис. 6. Фотографии голотипа (H1) и паратипов (P1–P8) *Iberus punicus* sp.n.

Table 4. Location and basic morphometrics of the holotype and paratypes assigned to *Iberus punicus*.
Таблица 4. Местоположение и основные морфометрические характеристики голотипа и паратипов, приписанных к *Iberus punicus*.

Specimen	Length (mm)	Width (mm)	Height (mm)	Latitude (N)	Longitude (W)	Sampling date
Holotype	22.42	17.89	12.60	37°36′51″	0°47′50″	01/01/2017
Paratype 1	23.60	18.43	11.53	37°35′58″	0°57′32.2″	07/01/2023
Paratype 2	22.99	18.01	11.99	37°35′25.5″	0°47′23.7″	07/01/2023
Paratype 3	22.23	18.13	12.46	37°41′22″	0°50′33″	04/03/2017
Paratype 4	25.26	19.85	12.90	37°35′58″	0°57′32.2″	07/01/2023
Paratype 5	22.07	17.75	12.72	37°35′25.5″	0°47′23.7″	07/01/2023
Paratype 6	23.63	19.04	13.40	37°36′08″	−1°02′50″	01/01/2017
Paratype 7	24.18	19.29	13.09	37°36′08″	−1°02′50″	01/01/2017
Paratype 8	25.04	19.65	13.14	37°37′ 21″	0°43′10.5″	07/01/2023

dark brown. In the most frequent morphotype of *I. punicus*, the last whorl shows five dark brown bands; the lower two are more intensely marked (the top band is usually wider than the bottom one) and located at a greater distance from each other than the upper three. The bands never reach the edge of the lip. The other three bands, somewhat narrower, are placed in the penultimate and antepenultimate whorls of the spiral and sometimes can be merged into a single one. The distance that separates them from the two lower bands usually involves twice the space between them. Sometimes, a band of marmorations accompanies the
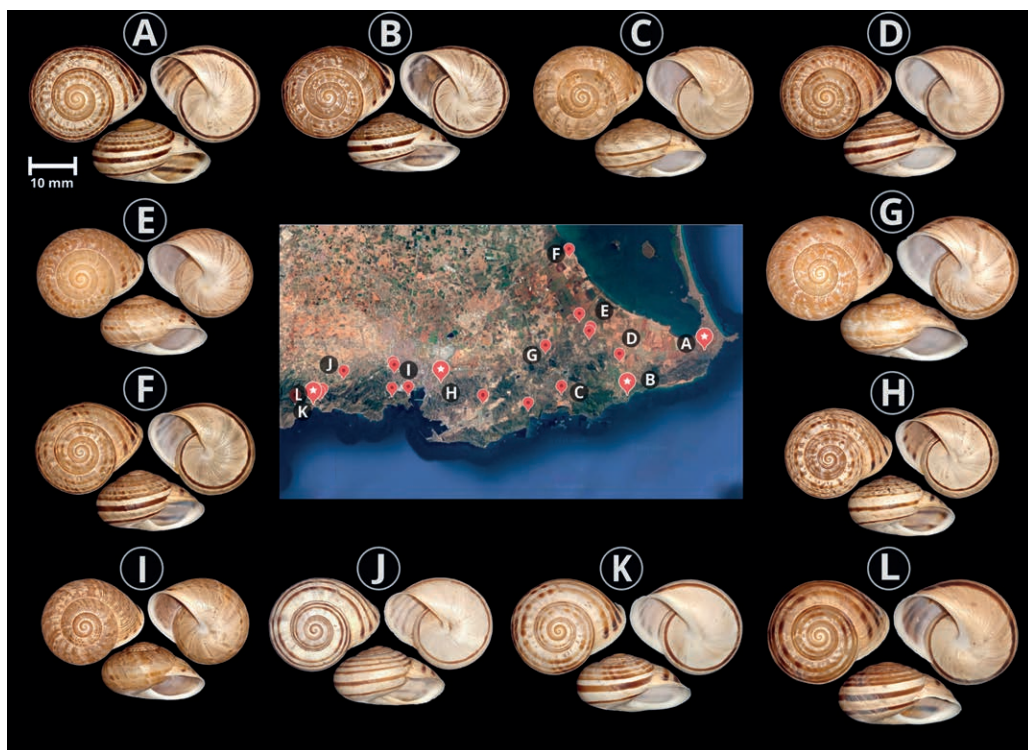
Fig. 7. Series of conchological variation of *Iberus punicus* throughout the region of Murcia. A — Cabo de Palos, Cartagena; B — Atamaría, Cartagena; C — Portman, Cartagena; D — Los Belones, Cartagena; E — Victoria Cave, Estrecho de San Ginés, Cartagena; F — El Carmolí, Cartagena; G — La Unión; H — Santa Lucía Hospital, Cartagena; I — Cartagena Town; J — Sierra de la Muela, Cartagena; K and L — El Portús, Cartagena.
Рис. 7. Серия конхологических вариаций *Iberus punicus* по всему региону Мурсии. A — Кабо-де-Палос, Картахена; B — Атамария, Картахена; C — Портман, Картахена; D — Лос-Белонес, Картахена; Пещера E — Victoria, Estrecho de San Ginés, Картахена; F — El Carmolí, Картахена; G — La Unión; H — Больница Санта-Люсия, Картахена; I — город Картахена; J — Сьерра-де-ла-Муэла, Картахена; K и L — El Portús, Картахена.

suture up to even the very beginning of the protoconch, generating an ornamental pattern that may remind the white marmorations of *I. marmoratus* although, in *I. punicus*, it does not stand out as much because of its off-white or light brown colour. Contrastingly, there is a less common morphotype (13% of the shells sampled) in which the bands tend to reduce their width, becoming even intermittent or blurred. In this case, marmorations are softer when present (see specimens E, G and I in Fig. 7). The two lower lateral bands are bordered by thin whitish bands, which are much more evident in the most common morphotype. A third morphotype accounting for 3% of the shells sampled may be defined (see specimen J in Fig. 7). The upper half of the main whorl shows a continuous band pattern free of marmorations that reaches up to the whorl immediately preceding the protoconch. In this case, the five bands are very thin and no whitish

bands appear. In some specimens, a cloud of small spots of variable density ranging from light grey to dark brown can be found. They are placed between the two lower lateral bands and on the ventral part of the shell.

It is not surprising that traditionally, *I. punicus* has been misidentified as *I. carthaginiensis*. The most apparent morphological character of their shells are somewhat similar (see Fig. 8). Indeed, large specimens of *I. punicus* and small specimens of *I. carthaginiensis* show strong resemblance. At a metapopulation scale, the two first morphotypes just mentioned are equally distributed between both species (around 85% for the morphotype consisting of continuous bands and 10% for the discontinuous one), while the percentage of shells with a partially open umbilicus is very low (1% and 3% for *I. punicus* and *I. carthaginiensis*, respectively). To find morphological differences beyond

Fig. 8. Some representative shells of the two morphotypes (discontinuously and continuously banded) of the species compared in this work. From left to right: *Iberus punicus*, *I. carthaginiensis*, and *I. alonensis.*
Рис. 8. Некоторые раковины двух морфотипов (прерывистые и непрерывные полосы) видов сравнивались в этой работе. Слева направо: *Iberus punicus*, *I. carthaginiensis* и *I. alonensis*.

those detectable with the naked eye, a comparative morphometric study is needed (Table 3, Fig. 5).

HABITAT. *I. punicus* inhabits a narrow altitudinal strip under coastal influence between 20 and 420 m above sea level. The habitat of *I. punicus* typically consists of shrublands and grasslands with fragmented limestone formations and frequent palmettos under which it usually takes refuge (Fig. 9).

NOMENCLATURAL ACT: This work and the nomenclatural act it contains have been registered in ZooBank. The ZooBank Life Science Identifier (LSID) for this publication is: https://zoobank.org/urn:lsid:zoobank.org:act:D4DF39F5-5C6C-4BE0-971F-1F61E1D9E57F

Discussion

IBERUS PUNICUS, A CONTRIBUTION TO UNRAVELLING THE *ALONENSIS* COMPLEX. The analyses of four of our samples, obtained from specimens tentatively identified as *I. carthaginiensis*, revealed a lineage not previously sequenced. From a molecular perspective, the recovery of this well-supported lineage in all analyses and the high genetic distances to the closest taxa (see results section) support the validity of *I. punicus* as a new species. Added to the molecular results is that *I. punicus* has a well-defined geographical distribution (placed on the eastern part of the former distribution assigned to *I. carthaginiensis*) and a conchological identity morphologically divergent from other nearby species. The genetic distance for COI between *I. carthaginiensis* and *I. alonensis* (11.62%) exceed those typically considered between 4 to 10% to define species in land snails (Hebert *et al.*, 2003; Davison *et al.*, 2009; Köhler, Johnson, 2012). For instance, Criscione & Köhler (2014) considered a minimum distance of 5% for COI to discriminate among species of the genus *Kimberleytrachia*; Boonmachai *et al.* (2023) fixed a minimum distance of 5.5% to describe new species of the genus *Diplommatina*, whereas Köhler *et al.* (2024) differentiated two species of the genus *Xanthomelon* with an average COI distance of 8.8%. Within Helicidae family, Greve *et al.* (2010) found that most of *Theba* species from the Canary Islands and Morocco showed divergences in COI between 3.5 and 8.6%; Colomba *et al.* (2015) set between 4 and 7.5% for COI to differentiate among species of the genus *Erctella*; Holyoak *et al.* (2018) established a range from 8.2 to 12.1% to delineate species within the genus *Eremina*.

Despite their morphological similarity, *I. punicus* shows greater genetic divergence with *I. carthaginiensis* than with *I. alonensis* (from which differs morphologically much more). It is well-known that the genus *Iberus* includes several genetically divergent but morphologically cryptic lineages (Elejalde *et al.*, 2008a; Moreno-Rueda, 2014; Liétor *et al.*, 2024) which is a common trend in helicids (Chueca *et al.*, 2015;
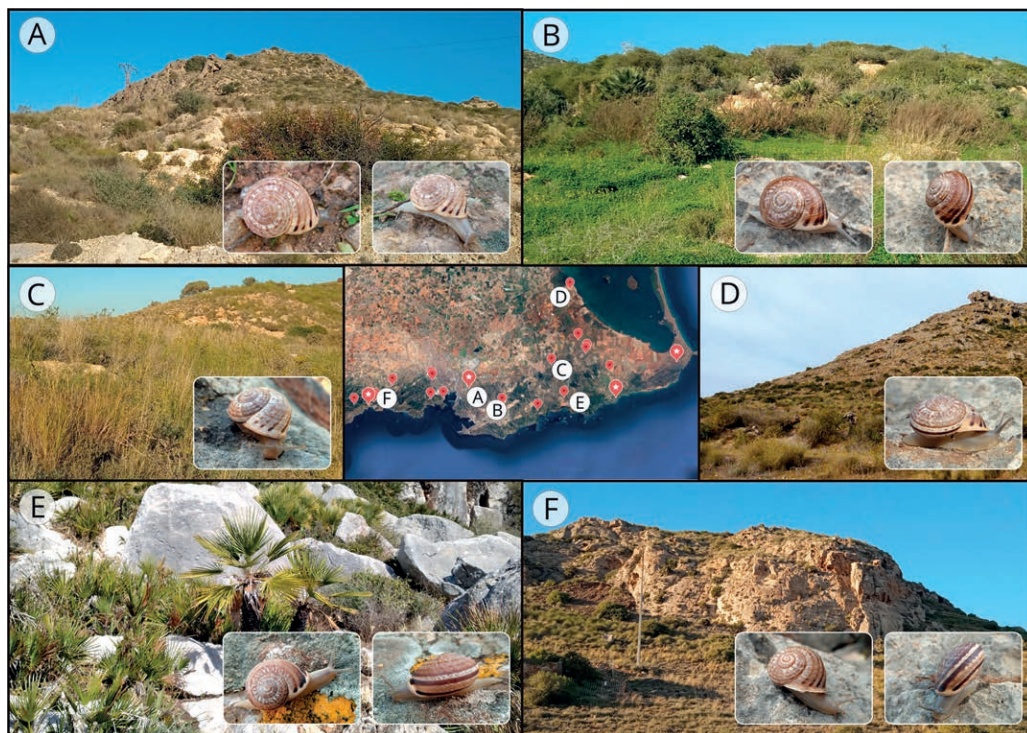
Fig. 9. Some representative habitats and alive specimens of *Iberus punicus* throughout the region of Murcia. A — Santa Lucía Hospital, Cartagena; B — Escombreras, Cartagena; C — La Unión; D — El Carmolí, Cartagena; E — El Portús, Cartagena; F — Victoria Cave, Estrecho de San Ginés, Cartagena.
Рис. 9. Некоторые репрезентативные местообитания и живые экземпляры *Iberus punicus* по всему региону Мурсии. A — больница Санта-Люсия, Картахена; B — Эскомбрерас, Картахена; C — La Unión; D — Эль Кармоли, Картахена; E — El Portús, Картахена; F — Пещера Виктория, Эстречо де Сан-Гинес, Картахена.

Zając *et al.*, 2020). The fact that conchological similarities with *I. carthaginiensis* have caused *I. punicus* to go unnoticed makes the binomial *I. carthaginiensis* – *I. punicus* another example of a cryptic complex within the genus *Iberus*.

The description of *I. punicus* represents a key contribution to cornering, characterising and thus, differentiating the *I. alonensis s. str.* from other cryptic species with which it has been traditionally confused.

SOLVING A TAXONOMIC PUZZLE. Until its description, *I. carthaginiensis* had been traditionally considered a small form of *I. alonensis*. More recently, a phylogenetic study conducted by Elejalde *et al.* (2008a) suggested that it could be considered a valid taxon. In the present work, Elejalde's *et al*. conclusion is reaffirmed with new biological samples collected by us. In addition, we provide a morphometric analysis involving a relevant number of shells that reveal a significant conchological difference between both species, being effectively *I. carthaginiensis*, of smaller size, more flattened and with a peristome proportionally greater than *I. alonensis*. Whereas *I. carthaginiensis* remains isolated over the coastal strip in the southeast tip of Murcia, *I. alonensis* inhabits the badlands of northeastern Murcia, being both species separated by the extensive Murcian orchard.

The catalogue of localities where the presence of *I. carthaginiensis* has been recorded is short and sometimes confusing, with the city of Cartagena being the most repeated reference since it was originally described. According to García San Nicolás, 1957, *I. carthaginiensis* would be distributed throughout the Sierras de Almenara and Carrascoy. Nevertheless, this reference does not seem very reliable since all subsequent citations place *I. carthaginiensis* in localities near the coast, between Cartagena and

Cabo de Palos (Murcia), at least 10 km east of Sierra de Almenara and 20 km south of Sierra de Carrascoy. The few references available in recent literature mention *I. carthaginiensis* mostly in the west of its classic distribution. Indeed, most of the recent citations provided by García-Meseguer *et al.* (2017) are located west of Cartagena. Talaván & Talaván (2006) found *I. carthaginiensis* in El Carmolí and Portman, while Murillo (2012) cited it on Barón Island, in the centre of the Mar Menor (identified as *I. gualtieranus* morpho *alcarazanus*, which was later corrected by García-Meseguer *et al.,* 2017 as *I. carthaginiensis*).

Another species, *I. calaensis* was described from shells sampled close to the geographic area where Elejalde *et al.* (2008a) referenced the samples that defined their A2b clade, assigned to *I. carthaginiensis* (also consistent with the Rossmässler type locality). The description was based solely on morphological criteria despite the high degree of conchological variability ascribable to many species of the genus *Iberus* (Liétor, 2014). In the present work, the molecular results obtained from samples collected within the type locality provided in the description of *I. calaensis* confirmed that this taxon is actually the true *I. carthaginiensis*, so that it must be considered as a junior synonym. Therefore, the name *I. carthaginiensis* has to be assigned from now on solely to the western populations of the traditional distribution for the species (from Mazarrón to the west of Sierra de la Muela, Cabo Tiñoso y Roldán), wrongly called *I. calaensis* during the last decade.

We have documented a contact area between *I. carthaginiensis* and *I. punicus* in the central part of the Sierra de la Muela, with the most significant population being that of El Portús (Cartagena, Murcia). This population is characterised by dwarf specimens (30% smaller than the average size). Significantly lower sizes have been found by the authors in other populations of the genus *Iberus* subjected to plausible interspecific hybridization (Jowers *et al.*, 2024). Introgression has been recognized as a powerful and frequent source of adaptive variation in snails (Shimizu, Ueshima, 2000; Morii *et al.*, 2015). Further studies will be required to assess the implications of such eventual hybridization events on the phylogeographic history of the species involved.

There has been a deep-rooted gastronomic tradition in eastern Spain associated with species of the genus *Iberus* and other helicids (Fajardo *et al*., 2009). Although the access to food and the scarcity of snails influenced by the decline in rainfall has caused the collection rate to have decreased, snails of the genus *Iberus* are still essential in rural areas as ingredients in some recipes. Although our field sampling suggests that *I. carthaginiensis* and *I. punicus* may become locally abundant in specific areas, they are scarce in many others. New studies will have to be conducted to know the density and conservation status of the populations of these species, in order to determine if specific conservation recommendations are convenient.

Compliance with ethical standards
CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

Supplementary data. The following materials are available online.
Table S1. A. Samples used in phylogenetic analyses. B. Genbank voucher abbreviations, species names, localities, coordinates and Genbank accessions.

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