

Searching for the elusive imposter land snails. Description and geographical delimitation of two cryptic *Iberus* subspecies (Gastropoda, Stylommatophora, Helicidae) in southern Spain

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ABSTRACT. The taxonomic identity of *Iberus alonensis* has traditionally been surrounded by great controversy because under this name a large number of similarly-shaped populations distributed over a vast geographical area and probably belonging to different species have been subsumed. The publication of the first phylogenetic study on the genus *Iberus* began to unravel the *alonensis* complex, defining other likely new lineages. One of them is *I. alonensis*-like 02, a provisional name assigned to the traditional populations of *I. alonensis* from Andalusia (southern Spain) but lacks sufficient biogeographic support to be formally defined as a new species. In this study, the potential distribution area of *I. alonensis*-like 02 is thoroughly explored through systematic sampling. The resulting geographical mapping together with a morphometric study and new molecular evidence allow us to confirm the historical name *I. alonensis rhodopeplus* as a valid taxonomic denomination which we divide into two cryptic subspecies named *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* ssp. nov. In addition, the subspecies *I. alonensis labiatus* has to be renamed. Our findings contribute to revealing the species complex involved under the classic name *I. alonensis* and to improving our knowledge on the cryptic nature of numerous taxa within the genus *Iberus*.

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Поиск неуловимых сухопутных улиток. Описание и географическое разграничение двух криптических подвидов *Iberus* (Gastropoda, Stylommatophora, Helicidae) на юге Испании

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РЕЗЮМЕ. Таксономическая идентичность *Iberus alonensis* традиционно была предметом полемики, так как под этим названием объединялись многочисленные популяции с похожей формой раковины, распространенные на обширной географической территории и, вероятно, принадлежащие к разным видам. Публикация первого филогенетического исследования рода *Iberus* начала распутывать комплекс *alonensis*, определяя другие вероятные новые линии. Одной из них является *I. alonensis*-like 02, условное название, присвоенное традиционным популяциям *I. alonensis* из Андалусии (южная Испания), но не имеющее достаточного биogeографического обоснования для формального описания

нового вида. В настоящей работе тщательно исследована потенциальная область распространения *I. alonensis*-like 02 путем систематического отбора проб. Полученное географическое картирование вместе с морфометрическим исследованием и новыми молекулярными доказательствами позволяет нам подтвердить валидность названия *I. alonensis rhodopeplus* как самостоятельного вида, которого мы разделяем на два криптических подвида, *I. rhodopeplus rhodopeplus* и *I. rhodopeplus bastetanus* ssp. nov. Кроме того, подвид *I. alonensis labiatus* необходимо переименовать. Наши выводы способствуют уточнению систематики комплекса видов, объединенных под названием *I. alonensis*, и углублению наших знаний о многочисленных криптических таксонах в роде *Iberus*.

Introduction

Iberus alonensis was originally described as *Helix alonensis* in 1821 by Férussac [1821–1822], to describe a common land snail from southern Spain. It is likely that Férussac did not find sufficient similarities to assign *H. alonensis* into the genus *Iberus* Montfort, 1810 because this genus had been introduced with *I. gualtieranus* (Linnaeus, 1758), a species quite different conchologically from *I. alonensis*. Likewise, other later authors did not take the genus *Iberus* into consideration [Rossmässler, 1853; Roshenhauer, 1856; Bourguignat, 1870; Hidalgo, 1875–1884; Calderón, 1897]. Contrastingly, other authors did differentiate between *I. gualtieranus* and *H. alonensis* [e.g. Kobelt, 1910]. Boettger [1913] considered *I. alonensis* as a subspecies of *I. gualtieranus* so this can be considered the first assignment of *I. alonensis* to the genus *Iberus*. This taxonomic status was supported by some later authors [e.g. Elejalde *et al.*, 2005; Moreno-Rueda, 2006] whilst some others considered *I. alonensis* as a globose morphotype of *I. gualtieranus* [e.g. López Alcántara *et al.*, 1985; Ruiz Ruiz *et al.*, 2006]. Currently, *I. alonensis* is accepted as a valid species [Elejalde *et al.*, 2008a; Bank, Luijten, 2014]. Martínez Orti *et al.* [2005] reviewed the type series of *H. alonensis* deposited in the Muséum National d’Histoire Naturelle, Paris, selecting a specimen from Alicante in eastern Spain as the lectotype for the species [Martínez Orti, Robles, 2012].

During the last decades, the denomination *I. alonensis* has been used interchangeably to refer to a wide spectrum of populations of the genus *Iberus* characterised by medium to large globose shells distributed throughout a vast geographical area across eastern Andalusia and Murcia, in the southeast of the Iberian Peninsula [Arrébola, 1995; Elejalde *et al.*, 2005; Ruiz Ruiz *et al.*, 2006; Liétor, 2014; García-Meseguer *et al.*, 2017]. It has been reported that morphological differences among populations

of *I. alonensis* may be found involving both the soft parts and the shell. For instance, Aguilar Amat [1925] suggested that the bright pink colour of the mantle let to differentiate a new population of *I. alonensis* sampled in Jaén (southern Spain) from others in the easternmost area of its distribution, and concluded that further data and an anatomical study could lead to the description of a local race which should be named *I. alonensis rhodopeplus*. More recently, Ahuir Galindo [2016] described *I. alonensis labiatus* from Nerja (Sierras de Tejada, Almirajara y Alhama, Málaga, Spain) easily distinguishable by a large shell size and a lip more developed than average. Nevertheless, a large body of scientific evidence has proved shell morphology to be often unsuitable as an isolated tool for delimiting and recognising species of pulmonate gastropods [Giusti, Manganelli, 1992; Schilthuizen, Gittenberger, 1996; Young *et al.*, 2001; Korte, Armbruster, 2003; Uit de Weerd *et al.*, 2004; Geenen *et al.*, 2006; Pfenninger *et al.*, 2006; Triantis *et al.*, 2016; Collado *et al.*, 2019; Vinarski *et al.*, 2020; Liétor *et al.*, 2024a].

Altogether, the controversial taxonomic history, the extensive geographic distribution and the conchological heterogeneity of *I. alonensis* have led to a serious problem of synonymy. Currently the taxa *H. alcarazana* Rossmässler, 1854, *H. alonensis* var. *lorcana* Rossmässler, 1854, *H. alonensis* var. *minima* A. Schmidt, 1855, *H. bajoi* Servain, 1880, *I. posthumus* Haas, 1934 and *I. alvaradoi* García San Nicolás, 1957, are considered junior synonyms of *I. alonensis*. Therefore, further research to clarify this taxonomic challenge, probably boosted by cryptic species, is needed.

The taxonomic integrative approach using molecular and morphological information has been shown to be reliable to delineate species of land snails and also to reveal if differences in morphology are merely attributable to polymorphism [Pfenninger, Posada, 2002; Jordaens *et al.*, 2003; Parmakelis *et al.*, 2003; Hurt, 2004; Walther *et al.*, 2016; Pholyotha *et al.*, 2020; Sawada *et al.*, 2021]. Among helicids, there are good examples of how the application of molecular techniques usually ends up changing the taxonomy based on morphology. After carrying out phylogenetic analyses, Neiber, Hausdorf [2015] moved two species, traditionally included on the basis of shell morphology in the genus *Cepaea*, to two different genera, i.e., *Caucasotachea* and *Macularia*, and similarly, molecular evidence led Korábek *et al.* [2016] to define a new divergent lineage for *Helix pomatia* populations with shells fitting the typical morphology for the species. Genetic analyses conducted by Korábek *et al.* [2022] placed *H. godetiana* back in the genus *Helix*, invalidating the position within the genus *Maltzanella* as proposed by Neubert [2014] on the basis of morphological and anatomical data. A paradigmatic case of a morphological approach lead-

ing to a wrong taxonomic conclusion is found within the genus *Iberus*: the genus *Tartessiberus* Altaba et Ríos Jiménez, 2021 was defined based on anatomical criteria and radula morphology and subsequent molecular analyses confirmed its synonymization with *Iberus* [Jowers *et al.*, 2024]. As a consequence, classical classifications of the genus *Iberus* based on shell shape [García San Nicolás, 1957] have become often unreliable on themselves alone, giving way to those relying on molecular analyses [Elejalde *et al.*, 2008a,b].

According to Elejalde *et al.* [2008a], the *I. alonensis* specimens from outside Andalusia are grouped in a different clade compared to those sampled within Andalusia, which are largest in size and show a distinctive shell colour and sculpture. Elejalde *et al.*'s clade for the Andalusian *alonensis* was structured into three subgroups, two of them distributed through the Subbetic and Penibetic mountains to the west, and the last placed to the east, whose geographical distribution remained to be clarified. Given the high degree of uncertainty associated with this clade, the authors proposed a provisional name (*I. alonensis*-like 02) that has been maintained until now.

The aim of this work is to carry out a systematic sampling and a subsequent comprehensive mapping of the populations of the genus *Iberus* attributable to the Elejalde *et al.*'s *I. alonensis*-like 02 clade, through an integrative taxonomical approach combining geographical data, morphometric analyses of a large quantity of shells and phylogenetic analyses.

Material and methods

Molecular analysis

After reviewing geographical coordinates of samples assigned to *I. alonensis*-like 02 clade from Elejalde *et al.* [2008a], we chose two new samples from key locations (coded as I11 and I16) for sequencing. No samples for *I. alonensis labiatus* were sequenced. The specimens were killed in the laboratory 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 by using QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines and instructions. The total alignment comprises all available *Iberus* sequences from Genbank (n=141) along with three outgroup taxa: *Rossmassleria sicanoides* (Kobelt, 1881), *Eremina dillwyniana* (L. Pfeiffer, 1853), and *Iberellus minoricensis* (Mittre, 1842) (see Supplementary Table 1).

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

Reconstructions of the phylogenetic tree for the three concatenated gene fragments (total length 1,984 bp) were carried out using maximum likelihood (ML) and Bayesian inference (BI), through RAXML v7.0.4 [Silvestro, Michalak, 2012] and MrBayes v3.2 [Ronquist, Huelsenbeck, 2003], respectively. The Akaike Information Criterion (AICc) and partition scheme were implemented in PartitionFinder v2.1.1 [Lanfear *et al.*, 2016], applying a 'greedy' search [Lanfear *et al.*, 2012] to select the best fit evolutionary model for each partition. The resulting partitions (for the BI and ML analyses) and models for the BI analyses were the following: GTR+I+G (COI pos1), F81+I (COI pos2), GTR+I+G (COI pos3), GTR+I+G (16S rDNA) and HKY+G (LSU). From the BI, two independent runs (each with four Markov chains for 10×10^7 generations) were implemented. Trees and parameters were sampled every 1,000 generations. The estimation of the majority-rule consensus tree was made by combining results from duplicated analyses, after discarding 25% of the whole set of samples as burn-in. ML searches were conducted under GTRGAMMA and support was evaluated by using 1,000 bootstrapped replicates. The ML partitions are the same as in the BI analysis; the difference is the selection of models as inferred from PartitionFinder 2. All phylogenetic analyses were carried out in the CIPRES platform [Miller *et al.*, 2010]. The consensus tree was visualised and rooted with FigTree v1.4.4 [Rambaut, 2018], and later graphically represented with the software Inkscape v1.0.1 (<http://www.inkscape.org>). Uncorrected p-distances with partial deletion were computed in MEGA X [Kumar *et al.*, 2018].

Field samplings and sample processing

Since *I. alonensis*-like 02 is considered to be distributed throughout the entire region of Andalusia, we opted for an intensive sampling that systematically covered all the potential distribution area within the Provinces of Seville, Málaga, Granada, Córdoba, Jaén and Almería. In addition, a particularly intense sampling throughout the potential distribution area of *I. alonensis labiatus* was carried out. Finally, 225 sampling points and many other field observations were recorded.

The sampling program was planned after a comprehensive review of available citations in the specialised literature and based on the field experience of the research team. Shells of the taxa studied were collected at most of the sampling points, with those of better quality being cleaned and photographed. Freshly collected and sun-bleached shells as well as subfossils were measured for the morphometric study. Some live specimens were collected at key locations. Once in the laboratory, tissue samples were taken from them for molecular analysis. Special effort was made to establish labelling and conserva-

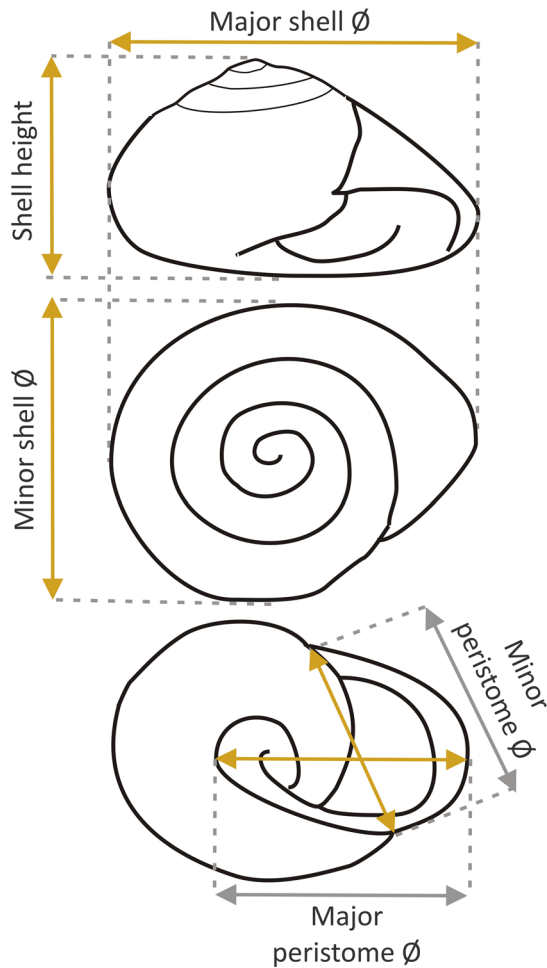


FIG. 1. Shell dimensions considered for morphometric analyses. The major diameter of the peristome includes the space occupied by the umbilical callus if present.

tion protocols for shells and biological tissues to guarantee the traceability of the specimens.

Morphometrics

The same researcher (JL) was in charge of measuring all shells sampled for morphometric study by using a digital calliper (accuracy 0.01 mm). The following were the five main morphometric parameters recorded: major and minor diameters (\emptyset) of the shell, shell height, and major and minor external \emptyset of the peristome. According to these measurements, shell and peristome areas were estimated by considering that both the shell and the peristome may resemble an ellipse, thus applying the formula $\text{Area} = \pi \times [(\text{major } \emptyset)/2] \times [(\text{minor } \emptyset)/2]$. On the basis of these measurements, a subsequent set of morphological ratios were obtained: i) Shell height/major \emptyset of the shell, as an indicator of shell globosity, so that more globose shells show a higher ratio; ii) Major \emptyset /minor \emptyset of the shell, as an indicator of shell circularity, so that circularity is higher as this ratio becomes closer to unity; iii) Major external \emptyset /minor external \emptyset of the peristome, as an indicator of peristome circu-

larity; iv) Percentage of the total shell area that is occupied by the peristome, calculated as $(\text{peristome area} \times 100)/\text{shell area}$.

Fig. 1 schematically represents how the main morphometric parameters were defined.

A morphological study based on morphometric measurements cannot be used as a sole criterion to differentiate species, but it can be a useful complementary tool. Two questions then arise: i) From what minimum number of shells can we approach the standard morphometry of a land snail species? and ii) When can morphometric parameters be considered truly representative of the size and shape of a land snail species? Fig. 2A shows that a minimum quantity of shells varying from 200 to 400, depending on the taxon, is required to be measured for the morphometric parameters to reach a stabilisation threshold and therefore, to be representative. The coefficients of variation of the morphometric parameters were small (Fig. 2B), with only the shell and peristome areas exceeding 15%. Therefore, when measuring a remarkably high number of shells, morphometry can be considered a reliable complementary tool for taxonomic research within the genus *Iberus*.

ANOVA tests were used for statistical comparisons between morphometric measurements when the variables were homoscedastic and normally distributed. Otherwise, the non-parametric Kruskal-Wallis test was used. Additionally, a Principal Component Analysis (PCA) was carried out to determine the clustering and degree of overlap of the taxa under study in the morphospace. Statistica 7 was the software used to conduct the analyses.

Results

Phylogenetic position and genetic divergence

Phylogenetic relationships of the major *Iberus* clades and species recovered the same tree topologies as Liétor *et al.* [2024a] and Jowers *et al.* [2024]. Most clades recovered, as well as the nodes supporting sister clade relationships for this study were highly supported in all phylogenetic analyses (Fig. 3).

We recover a sister clade relationship between Elejalde *et al.*'s *I. alonensis*-like 02 clade, now including our sample I16, geographically positioned to the west of the geographic distribution of Elejalde *et al.*'s *I. alonensis*-like 02, and another with our sample I11, to the east. Henceforth, we name these clades 'new lineage 1' and 'new lineage 2', respectively. As expected, genetic divergence within the recovered new clades (0.83–3.60% and 0.20–2.71% for COI and 16S rRNA, respectively) is consistent with that of other close taxa (Table 1) and lower than between them (Table 2). The two new lineages are sister to the *I. alonensis* clade but weakly supported. Genetic distances among clades are high, and comparative

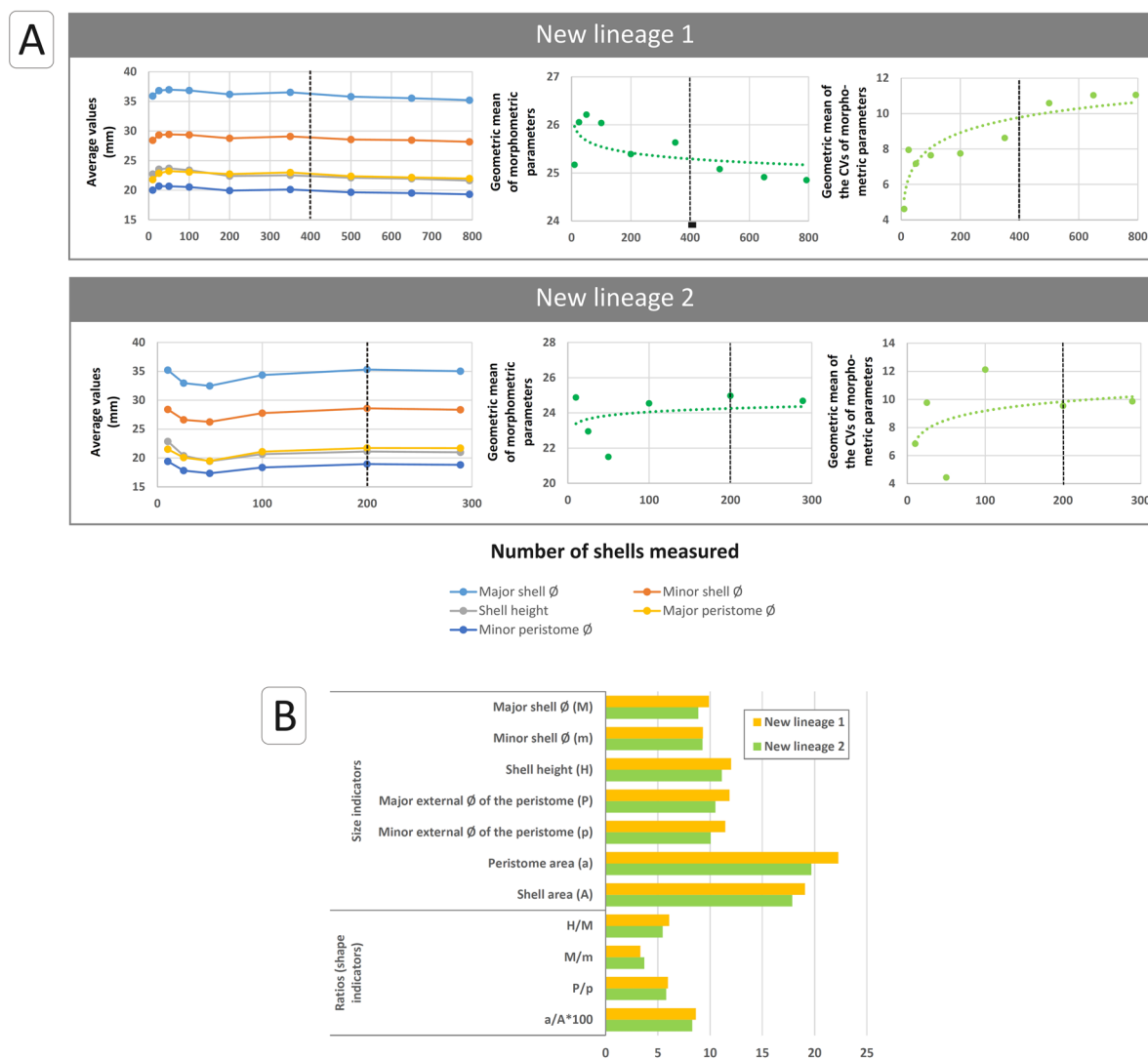


FIG. 2. Indicators on the representativeness and reliability of morphometric analyses. **A.** Quantities of shells of the new lineages from which morphometric parameters stabilise (dotted lines). Left column: 5 main morphometric parameters. Central column: geometric mean of the main morphometric parameters. 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 (%) for the morphometric variables of shells considered in this study.

divergences among all other clades and the new lineages are similarly high ($\sim 11\%$ to $\sim 15\%$ for COI, and ~ 7 to $\sim 10\%$ for 16S rRNA), as well as between the two new lineages (9.54% for COI and 4.82% for 16S rRNA) (Table 2).

Geographical distribution

We estimate that the new lineages occupy an area of 1.84 million hectares (Fig. 4). Such a vast distribution makes these taxa some of the most widely spread within the genus *Iberus* along with *I. alonensis* and *I. globulosus* (Liétor *et al.*, 2024b).

The new lineages are geographically positioned according to a longitudinal gradient with the new

Table 1. Intraspecific genetic divergence (p -uncorrected distances) within the new lineages as well as within other clades of interest in reference to Fig. 3.

	COI	16S rRNA
<i>I. gualtieranus</i>	0.46%	0.14%
<i>I. mariae</i>	3.48%	2.10%
New lineage 1	3.60%	2.71%
New lineage 2	0.83%	0.20%
<i>I. campesinus</i>	2.03%	1.29%
<i>I. globulosus</i>	0.45%	0.29%
<i>I. carthaginiensis</i>	1.33%	0.13%
<i>I. alonensis</i> -like 01	0.69%	1.28%
<i>I. alonensis</i>	3.89%	1.70%

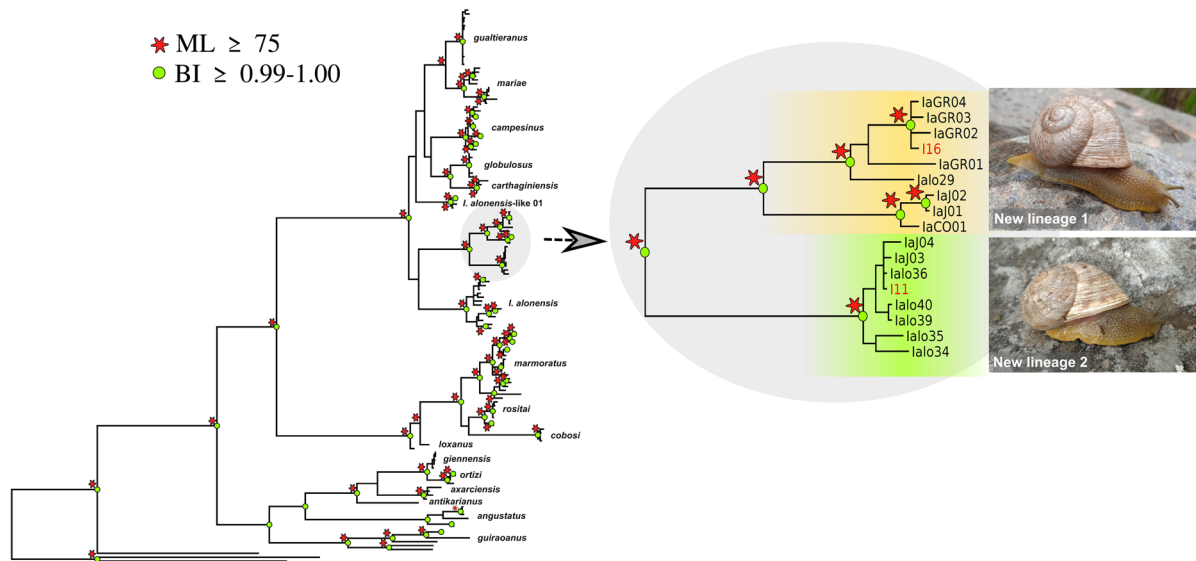


FIG. 3. Maximum Likelihood tree of *Iberus* for the concatenated COI, 16S rRNA and LSU gene fragments. Values by nodes represent bootstrap values for the ML analyses ($\geq 75\%$, red stars). Green filled circles are represented for the BI analyses ($BI \geq 0.99-1.00$). Samples analysed in this study have been highlighted in red.

lineage 1 to the west and the new lineage 2 to the east. Both taxa show a metapopulation structure consisting of main population centres connected by corridors of sparse populations, except for the new lineage 1 centre in the south of Jaén Province, which remains isolated (Fig. 4). The geographical transition between the distributions of both lineages is irregular, albeit three zones may be differentiated: i) To the north (south of the province of Jaén), the new lineages 1 and 2 are distributed in disjunctive areas, across the western and eastern ends of Sierra Mágina Natural Park, respectively. This separation cannot be explained by a geographical or anthropogenic barrier, rather a competitive exclusion one, since the empty area is inhabited by *I. angustatus* (Rossmässler, 1854). ii) The central contact zone (in the middle of Granada Province) shows a corridor between the two new lineages. During the sampling,

we found a single population within this area composed of dwarf-sized specimens that were attributed to likely intermediate shells between both lineages (Fig. 5). iii) To the south, the new lineages become separated by an area of 250 km² between the towns of La Mamola and Adra, on the border between the provinces of Granada and Almería, respectively. In this case, the barrier seems to be imposed by an unfavourable habitat, since six sampling points were recorded there with no presence of *Iberus*. Further research will be needed to clarify if, as it seems, there is no contact between the southernmost populations of the two new lineages.

The populations assignable to *I. alonensis labiatus* are concentrated in an area of approximately 6,500 hectares just on the southern limit of the provinces of Granada and Málaga (Fig. 4), surrounded on all sides by populations of the new lineage 1.

Table 2. *P*-uncorrected distances for the clades of interest and new lineages 1 and 2. The lower matrix is the COI data and the upper matrix is the 16S rRNA. Comparisons in which the new lineages are involved have been highlighted in grey.

	IGU	IMA	NL2	NL1	ICA	IGL	ICT	IL1	IAL
<i>I. gualtieranus</i> (IGU)	-	2.05%	9.25%	8.65%	5.03%	4.47%	5.98%	5.97%	6.85%
<i>I. mariae</i> (IMA)	7.84%	-	9.80%	9.22%	5.57%	5.10%	6.52%	6.45%	7.45%
New lineage 2 (NL2)	12.04%	13.51%	-	4.82%	9.67%	9.16%	9.47%	7.42%	8.42%
New lineage 1 (NL1)	12.20%	13.65%	9.54%	-	8.93%	9.15%	8.66%	7.23%	8.19%
<i>I. campesinus</i> (ICA)	10.80%	11.65%	12.73%	14.18%	-	4.74%	6.41%	5.05%	6.02%
<i>I. globulosus</i> (IGL)	9.41%	10.54%	11.01%	12.85%	8.96%	-	3.53%	5.52%	6.18%
<i>I. carthaginiensis</i> (ICT)	9.68%	10.37%	12.44%	13.69%	9.49%	6.55%	-	5.17%	6.92%
<i>I. alonensis</i> -like 01 (IL1)	11.03%	11.42%	11.60%	14.53%	10.52%	9.80%	10.99%	-	5.75%
<i>I. alonensis</i> (IAL)	11.63%	12.17%	13.14%	12.88%	11.35%	11.14%	11.25%	10.83%	-



FIG. 4. Distribution of the two new lineages and *I. alonensis labiatus* throughout the Andalusia region (southern Spain). For the genetic samples obtained from GenBank, the central points of the 10 x 10 km² UTM grids available have been marked in the map. Populations that showed intermediate conchological features, presumably involving hybridization with other congeneric species, were excluded from the map. The entire territory was intensively sampled. Thus, gaps greater than 10 km² with no sampling points represent areas where none of the taxa under study were recorded. Scale in kilometers.



FIG. 5. Some dwarf *Iberus* shells tentatively classified as intermediate between the two new lineages sampled in Cenes de la Vega (Granada, Spain). The shells of this population show an average major diameter 35% smaller than those of the two new lineages.

Morphological comparison between the two new lineages

We measured 1,428 shells, distributed as follows: 858 for the new lineage 1, 504 for the new lineage 2 and 66 for *I. alonensis labiatus*. The representativeness thresholds reported in Fig. 2A were exceeded by the new lineages.

The minor diameter of the shell was the only morphometric parameter which did not show significant differences when the two new lineages were morphologically compared (Table 3). The average shell of the new lineage 1 is larger and taller, which results in a greater area and a higher globosity than that of the new lineage 2. The peristome is slightly more circular and larger in the new lineage 1, so that its contribution to the entire shell area is also greater.

Fig. 6 shows that the population clouds of the two new lineages are completely overlapping throughout the morphospace resulting from a PCA that brings together the 11 parameters and ratios measured for the morphometric analysis. No differences were found between the new lineages both in size-based or shape-based clusterings determined by Principal Components (PCs) 1 and 2, respectively.

Morphological analysis of *Iberus alonensis labiatus* and comparison with the new lineage 1

Though the shells of *I. alonensis labiatus* occupy the morphometric range of greatest size and relative area of the peristome, a significant number of populations of the two new lineages show similar values for both variables (Fig. 6). The overlap in shell traits between *I. alonensis labiatus* and the new lineage 1 may be verified in Fig. 7. The average major shell diameter for the populations of *I. alonensis labiatus* here measured was 40.76 mm, rather similar to the averaged 40.38 mm obtained for the new lineage in the surroundings (Table 4). Other indicators of shell size (minor shell diameter and shell area) or shape (shell globosity as well as shell and peristome circularity) were not significantly different either. Besides, the morphometric differences found for height and peristome projection might be consistent with an interpopulation variability scenario.

Fig. 8A shows that the minor shell diameter of *I. alonensis labiatus* populations ranges from 15th to 80th percentile when combined with those of the new lineage 1, whilst the last quintile of the size distribution is assignable to populations of the new lineage 1. Furthermore, although the two populations

Table 3. Morphometric comparison between the two new lineages within Elejalde *et al.*'s *I. alonensis*-like 02 clade. Values are given with the mean \pm SD. K indicates that the test was a Kruskal-Wallis, while A indicates that an ANOVA test was used. Superscripts with different letters show significant differences (p-value < 0.05). Comparisons between pairs of variables were carried out with the Tukey test (HSD) when normally distributed, or with two-tailed multiple comparisons when non-normally distributed.

Parameters and ratios	New lineage 1 (n=858)	New lineage 2 (n=504)
Major \emptyset of the shell (mm) ^A	35.37 \pm 3.50 ^a	34.84 \pm 3.09 ^b
Minor \emptyset of the shell (mm) ^K	28.22 \pm 2.62 ^a	28.06 \pm 2.60 ^a
Shell height (mm) ^K	21.57 \pm 2.59 ^a	20.68 \pm 2.30 ^b
Major external \emptyset of the peristome (mm) ^A	22.12 \pm 2.62 ^a	21.62 \pm 2.27 ^b
Minor external \emptyset of the peristome (mm) ^A	19.39 \pm 2.22 ^a	18.80 \pm 1.89 ^b
Shell height/Major \emptyset of the shell (ratio) ^A	0.61 \pm 0.04 ^a	0.59 \pm 0.03 ^b
Shell area (mm ²) ^A	790.96 \pm 150.80 ^a	773.90 \pm 138.25 ^b
Peristome area (mm ²) ^A	340.98 \pm 75.93 ^a	322.07 \pm 63.39 ^b
Major \emptyset /Minor \emptyset of the shell (ratio) ^K	1.25 \pm 0.04 ^a	1.24 \pm 0.05 ^b
Major \emptyset /Minor \emptyset of the peristome (ratio) ^A	1.14 \pm 0.07 ^b	1.15 \pm 0.07 ^a
Peristome area x100/Shell area (%) ^A	42.95 \pm 3.71 ^a	41.60 \pm 3.45 ^b

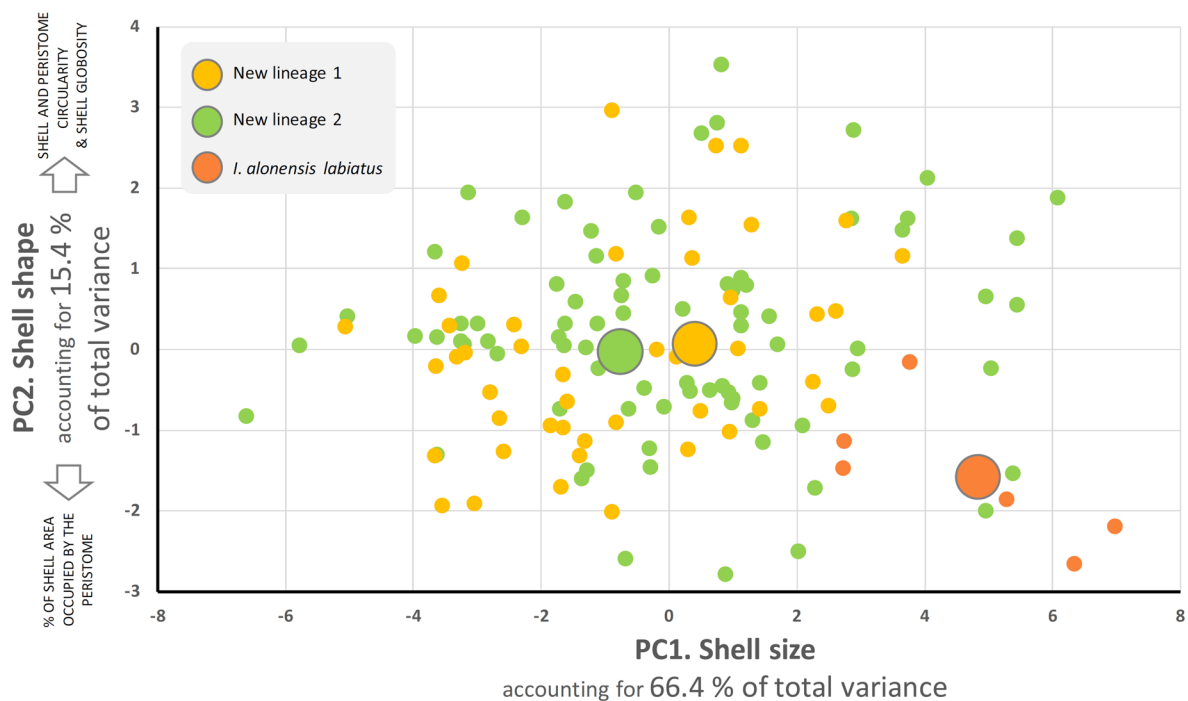


FIG. 6. Clustering of new lineage 1 (81 populations), new lineage 2 (46 populations) and *I. alonensis labiatus* (6 populations) in the two-dimensional morphospace provided by the first two PCs of a PCA. All morphometric parameters and ratios were combined to run the PCA. Each point on the graph represents a sampling location. The coordinates of the centroids for each taxon (large points) were calculated as the averages of the X and Y coordinates of the points included in the corresponding clouds. To facilitate the understanding of clustering, the original negative sign of the PC1 scores was changed.

with the largest shell peristome area corresponded to *I. alonensis labiatus*, half of the populations of this taxon were placed between the 25th and the 50th percentile (Fig. 8B).

A new taxonomic structure for *Iberus alonensis*-like 02 consisting of two subspecies

The results of this work let us to adequately char-

acterise the species provisionally named *I. alonensis*-like 02 [Elejalde *et al.*, 2008a] which is proposed to be divided into two subspecies named *Iberus rhodopeplus rhodopeplus* and *Iberus rhodopeplus bastetanus* ssp. nov. Both subspecies are detached from the nominal species *I. alonensis* proposed by Aguilar Amat [1925] since we currently know that it constitutes a differentiated lineage distributed from

Table 4. Morphometric comparison between the new lineage 1 and *I. alonensis labiatus*. An ANOVA test followed by a Tukey test (HSD) for paired comparisons was applied for all morphometrics. Superscripts with different letters show significant differences (p -value < 0.05). For the new lineage 1, only shells from populations surrounding the *I. alonensis labiatus* distribution area were selected.

Parameters and ratios	New lineage 1 (n=61)	<i>I. alonensis labiatus</i> (n=66)
Major \emptyset of the shell (mm)	40.38 \pm 2.35 ^a	40.76 \pm 2.41 ^a
Minor \emptyset of the shell (mm)	31.94 \pm 1.89 ^a	31.94 \pm 1.72 ^a
Shell height (mm)	25.48 \pm 1.92 ^a	24.30 \pm 1.29 ^b
Major external \emptyset of the peristome (mm)	25.20 \pm 1.88 ^b	26.56 \pm 2.07 ^a
Minor external \emptyset of the peristome (mm)	21.94 \pm 1.77 ^b	23.05 \pm 1.54 ^a
Shell height/Major \emptyset of the shell (ratio)	0.63 \pm 0.03 ^a	0.62 \pm 0.02 ^a
Shell area (mm ²)	1016.15 \pm 114.67 ^a	1025.48 \pm 113.71 ^a
Peristome area (mm ²)	435.95 \pm 61.35 ^b	482.95 \pm 67.68 ^a
Major \emptyset /Minor \emptyset of the shell (ratio)	1.26 \pm 0.03 ^a	1.27 \pm 0.03 ^a
Major \emptyset /Minor \emptyset of the peristome (ratio)	1.15 \pm 0.07 ^a	1.15 \pm 0.05 ^a
Peristome area x100/Shell area (%)	42.85 \pm 3.01 ^b	47.05 \pm 3.35 ^a

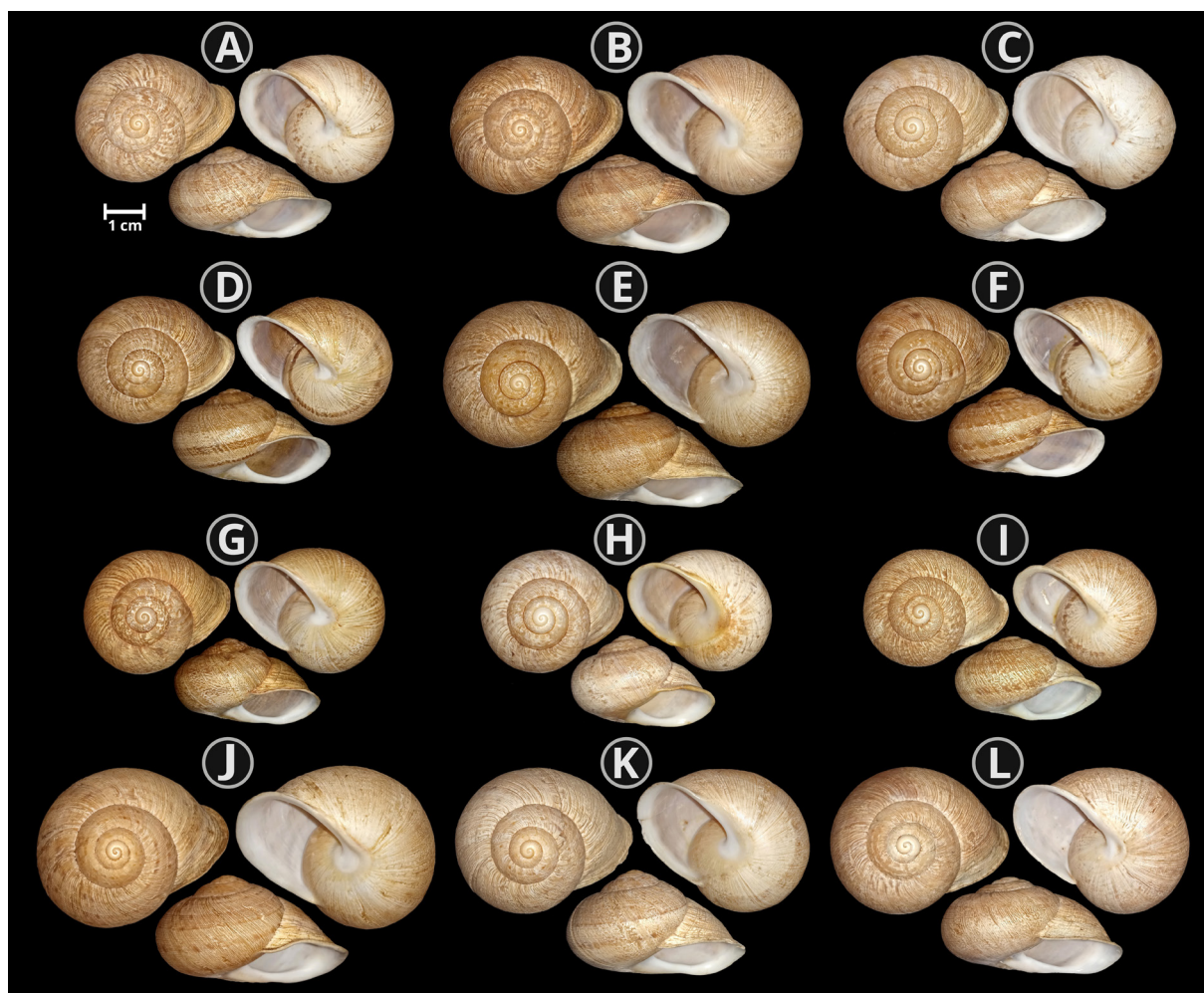


FIG. 7. Comparative iconography of shells of *I. alonensis labiatus* (A–F) and those of the new lineage 1 with developed lip (G–I) and large size (J–L) from different Andalusian locations. A, B, C, Nerja (Málaga); D, E, Almuñécar (Granada); F, Frigiliana (Málaga); G, Almodóvar Mount (Jaén); H, I, Santa Catalina Mountain (Jaén); J, Humilladero (Málaga); K, Órgiva (Granada); L, Lentegí (Granada). Forty-two millimetres was the size threshold from which specimens were considered to have a large size for the species (photographs J–L). This figure was obtained by averaging the diameters reported for Ahuir Galindo (2016) for holotype and 11 paratype shells.

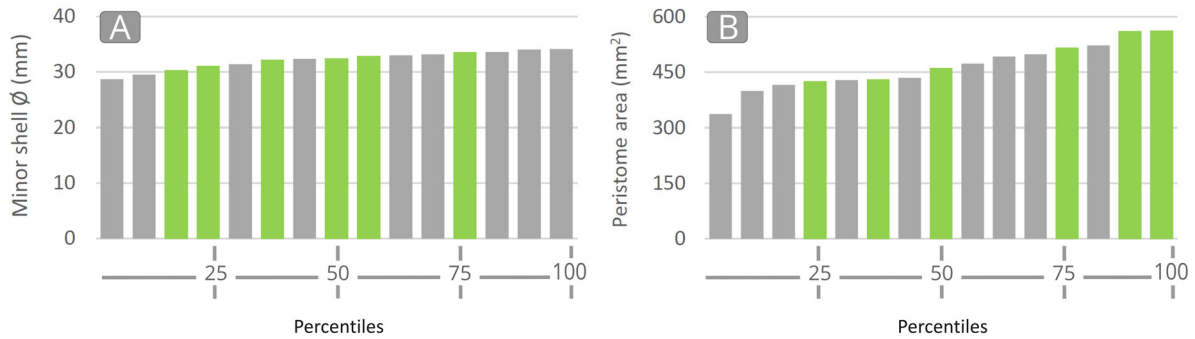


FIG. 8. Populations of the new lineage 1 (grey bars) and *I. alonensis labiatus* (green bars) along an increasing gradient of average minor shell diameter (A) and average peristome area (B). Only populations of the new lineage 1 geographically close to those of *I. alonensis labiatus* were considered. Minor shell diameter was selected as a better representative indicator of size because major shell diameter is affected by the overdeveloped peristome.

northern Murcia in the south of Spain to Catalonia and Navarra in the north [Elejalde *et al.*, 2008a; unpublished data].

The morphological traits of this species, especially those related to the soft parts, are in line with the ones established for *I. alonensis rhodopeplus* by Aguilar Amat [1925] to whom, therefore, the updated name of the species must be attributed. Some years before, Aguilar Amat [1921] had introduced the name ‘*carragines*’ to designate the *Iberus* snails from the Serra de Cardó i de Bràfim in Catalonia (Spain). However, there is hardly any information about this species that allows it to be distinguished from others, which suggests that this taxonomic denomination should be considered invalid, thus having priority Aguilar Amat’s taxonomic proposal from 1925.

Taxonomy

Class GASTROPODA Cuvier, 1795

Subclass HETEROBRANCHIA

Burmeister, 1837

Order STYLOMMATOPHORA

A. Schmidt, 1855

Family HELICIDAE Rafinesque, 1815

Genus *Iberus* Montfort, 1810

Iberus rhodopeplus Aguilar Amat, 1925,
stat. nov.

Iberus rhodopeplus rhodopeplus

Aguilar Amat, 1925

(Figs 9–11)

Iberus alonensis rhodopeplus d’Aguilar Amat, 1925: 266.

Type material. Fig. 9 shows the syntype of *I. rhodopeplus rhodopeplus* which represents the only reference shell available from the Aguilar Amat’s collection, deposited in the Museu de Ciències Naturals de Barcelona (Spain) with register number MZB 89-2580.

Type locality. Since Aguilar Amat [1925] did not designate a holotype, the type locality must be assigned to the only known syntype assigned by the author to ‘El Muruche’, Jaén (Spain). It should be noted that most likely, the type locality stated by the author is actually Mount Morocho, located in the town of Pegalajar, Jaén (Spain). For comparative purposes, a specimen recently sampled by the authors at Aguilar Amat’s historic locality is shown in Fig. 9.

Etymology. Aguilar Amat [1925] suggested the term ‘*rhodopeplus*’ as subspecific epithet to describe a local race characterised by “*showing a mantle of a beautiful salmon colour*”.

Description. Live specimens of *I. rhodopeplus rhodopeplus* typically show a bright yellow body and a pink or salmon-coloured mantle. This is not the only case of pink mantle within the genus *Iberus* [Lietor *et al.*, 2024c]. By contrast, the yellow body can be considered as distinctive for this taxon. Major diameter of *I. rhodopeplus rhodopeplus* shell ranges from 22 to 48 mm.

The typical shell of *I. rhodopeplus rhodopeplus* is globose, occasionally compressed, unkeeled and not umbilicated. It has an acute apex and 4.5–5 whorls of regular growth. The suture is simple and visible in all whorls. Protoconch has 1–1.5 whorls and is smooth, exhibiting a uniform light to dark brown colour. The shell surface is radially (transversally) striated, except in the smooth protoconch. The radial striae show an irregular spatial pattern. Additionally, there is a spiral (longitudinal) striation of variable intensity depending on populations, resulting in a marked reticulation. Shell aperture is large, from oval to semilunar, wider than high (equivalent to approximately half the total width of the shell). The peristome is white, solid and reflected, with the exception of some populations in which it may be slightly cutting. The umbilicus area exhibits a bright white dilated columellar expansion that becomes a callus with variable intensity and extension depending on the populations. The colour of the shell in the first three whorls (excluding the protoconch) may

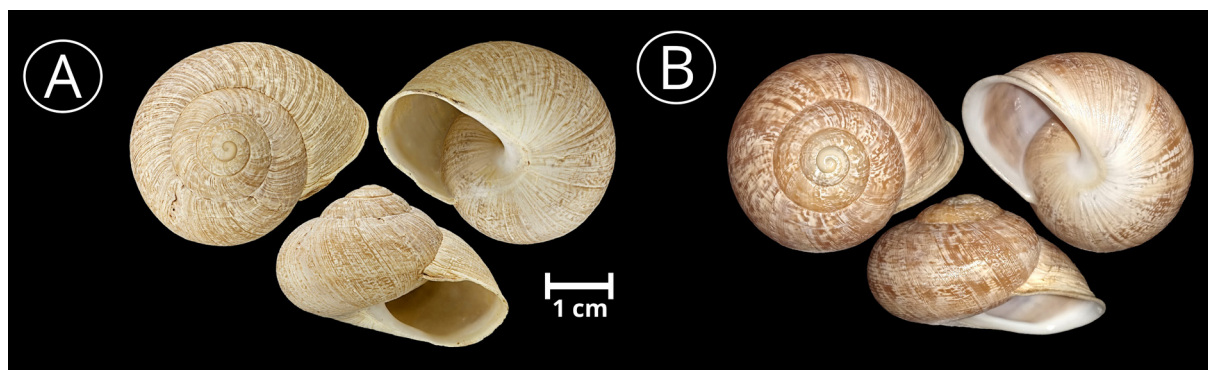


FIG. 9. **A.** Photograph of the syntype of *I. rhodopeplus rhodopeplus* Aguilar Amat, 1925 deposited in the Museu de Ciències Naturals de Barcelona (Spain) and labelled as *I. gualtieranus rhodopeplus*. More information here: <https://www.bioexplora.cat/es/colecciones-abiertas/N880087>. **B.** Specimen sampled in the same historical locality referenced by Aguilar Amat, shown for comparative purposes.

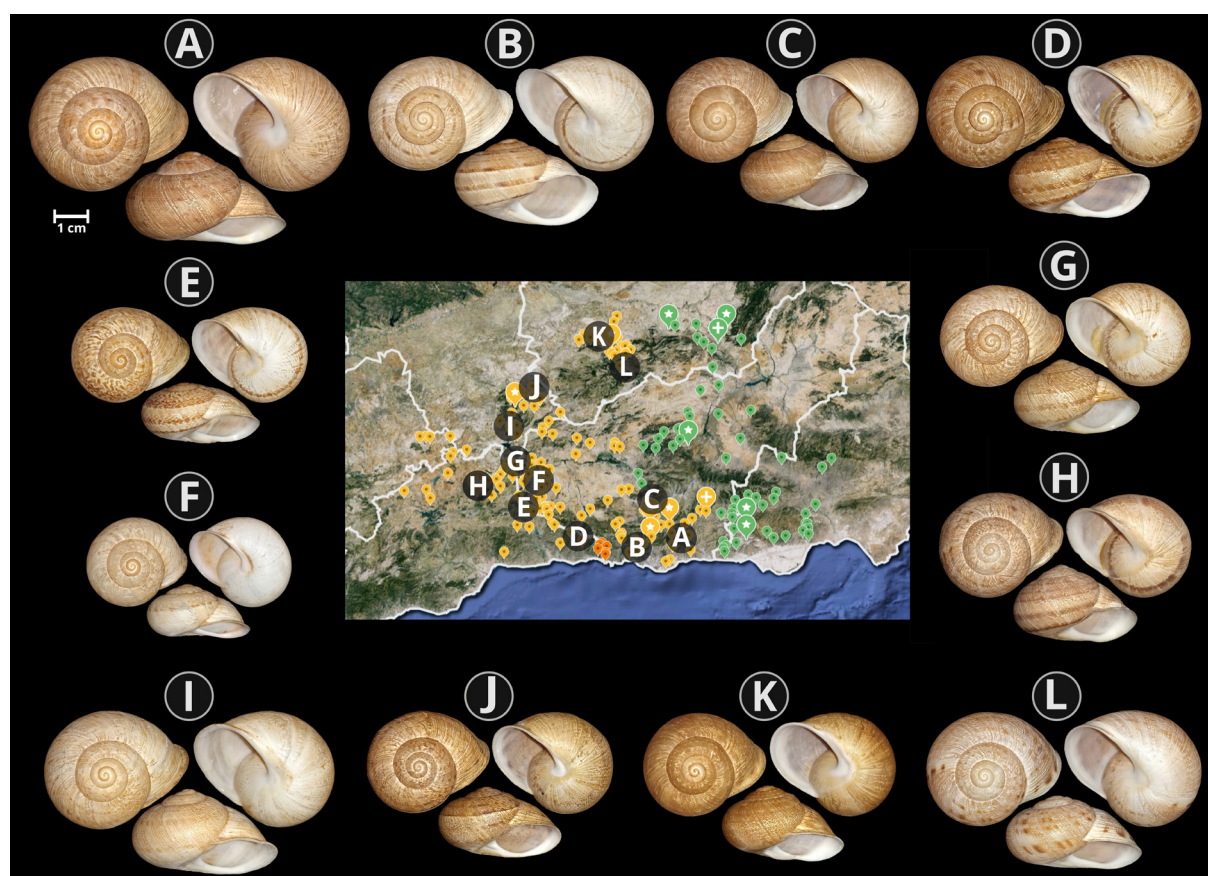


FIG. 10. Series of conchological variation of *I. rhodopeplus rhodopeplus*. **A.** Fregenite, Órgiva (Granada); **B.** Lobres, Salobreña (Granada); **C.** Nigüelas Reservoir (Granada); **D.** Frigiliana (Málaga); **E.** Periana (Málaga); **F.** Boquete de Zafarraya, Ventas de Zafarraya (Granada); **G.** Salar, Sierra Gorda, Loja, (Granada); **H.** Antequera (Málaga); **I.** El Lanchar, Rute (Córdoba); **J.** Sierra Leones, Priego de Córdoba (Córdoba); **K.** Santa Catalina Mount (Jaén); **L.** La Cerradura, Pegalajar (Jaén).

vary from off-white to dark brown. Last whorl of the spire shows five dark brown bands (except for populations with no bands); the lower two are wider, more intensely marked and located at a greater distance from each other than the upper three. They can be continuous, but sometimes they are interrupted by

marmorations that provide an intermittent band pattern. 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, being less marked and interrupted by weaker marmorations in some populations. The distance that

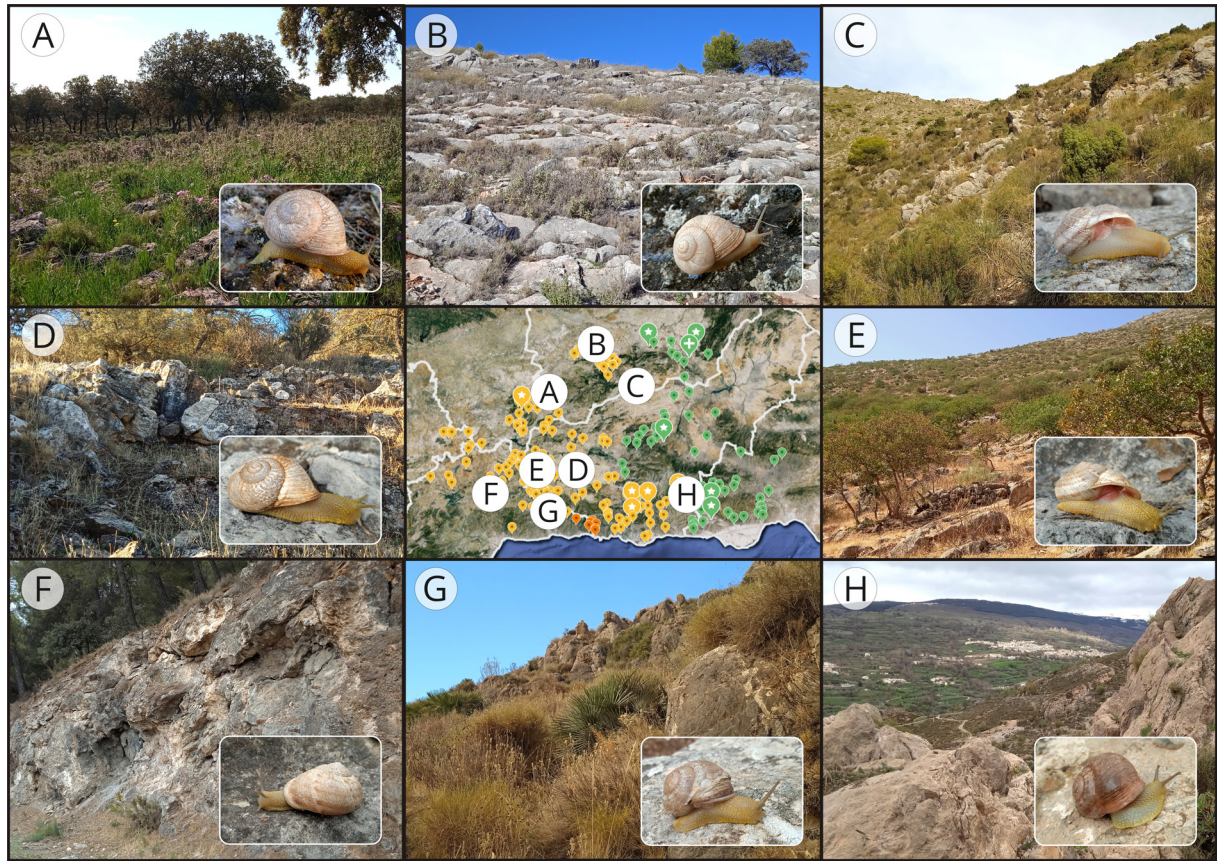


FIG. 11. Some representative habitats and alive specimens of *I. rhodopeplus rhodopeplus*. **A**, Santuario de la Virgen de la Cabeza, Priego de Córdoba (Córdoba); **B**, Santa Catalina Mountain (Jaén); **C**, Barranco de la Cueva del Judío, Cárcel (Jaén); **D**, Northeastern end of Sierra Gorda, Salar (Granada); **E**, Fuente Camacho, Loja (Granada); **F**, Antequera (Málaga); **G**, El Toril, Alcaucín (Málaga); **H**, Cadiar viewpoint, Narila (Granada).

separates them from the two lower bands may be 2–4 times the space among them.

Series of conchological variability of *I. rhodopeplus rhodopeplus* can be checked in Fig. 10.

Habitat. *I. rhodopeplus rhodopeplus* occurs at an altitude range between 9 m and 1,345 m above sea level (a.s.l.). It is one of the most ubiquitous snails within the genus *Iberus*. Although its typical habitat consists of limestone walls and calcareous rock formations inside Mediterranean shrublands or occasionally pine and holm oak forests, it may also occur on siliceous lithology (quartzite and slate) (Fig. 11). When limestone cracks and cavities are not available, snails take refuge deep under bushes and on the lower side of medium to large sized rocks.

Remarks. *I. rhodopeplus rhodopeplus* is one of the taxa that molecular analysis has demonstrated to be part of the traditional *I. alonensis* morphospecies, being attributed to the *I. alonensis* populations throughout the western part of Andalusia (southern Spain). Despite the conchological similarity between *I. rhodopeplus rhodopeplus* and *I. alonensis* s. str., both taxa constitute well-differentiated sister lineages, with a genetic distance of ~13% for COI and ~8% for 16S rRNA (Table 2).

Some morphological features of *I. rhodopeplus*

rhodopeplus are sufficiently distinctive to allow it to be differentiated from the rest of the large-sized *Iberus* from southern Spain. In addition to the above-mentioned striking colours of the mantle and the body, it can be added a significantly larger size than *I. carthaginensis* (Rossmässler, 1853), *I. globulosus* C.R. Boettger, 1913. *I. alonensis*-like 01 (*sensu* Elejalde *et al.*, 2008a) and *I. mariae* Cobos, 1979 (unpublished data). Albeit *I. campesinus* (L. Pfeiffer, 1846) and *I. gualtieranus*, the two remaining taxa of the *I. rhodopeplus rhodopeplus* clade (Fig. 3) can match or even exceed it in size, the highly contrasted morphologies of all of them prevent confusion.

Iberus rhodopeplus bastetanus ssp. nov.
(Figs 12–14)

Zoobank registration urn:lsid:zoobank.org:act:2935FF39-0EF7-467C-890E-0CBED5C42A6C

Type material. Fig. 12 shows the holotype and paratypes of *I. rhodopeplus bastetanus* ssp. nov. (*I. rhodopeplus bastetanus* hereinafter). Holotype and paratypes 1 to 3 are deposited in the Museo Nacional de Ciencias Naturales – CSIC (Madrid, Spain) whereas paratypes 4 to 8 are deposited in the private collection of Dr. José Liétor Gallego (Jaén,

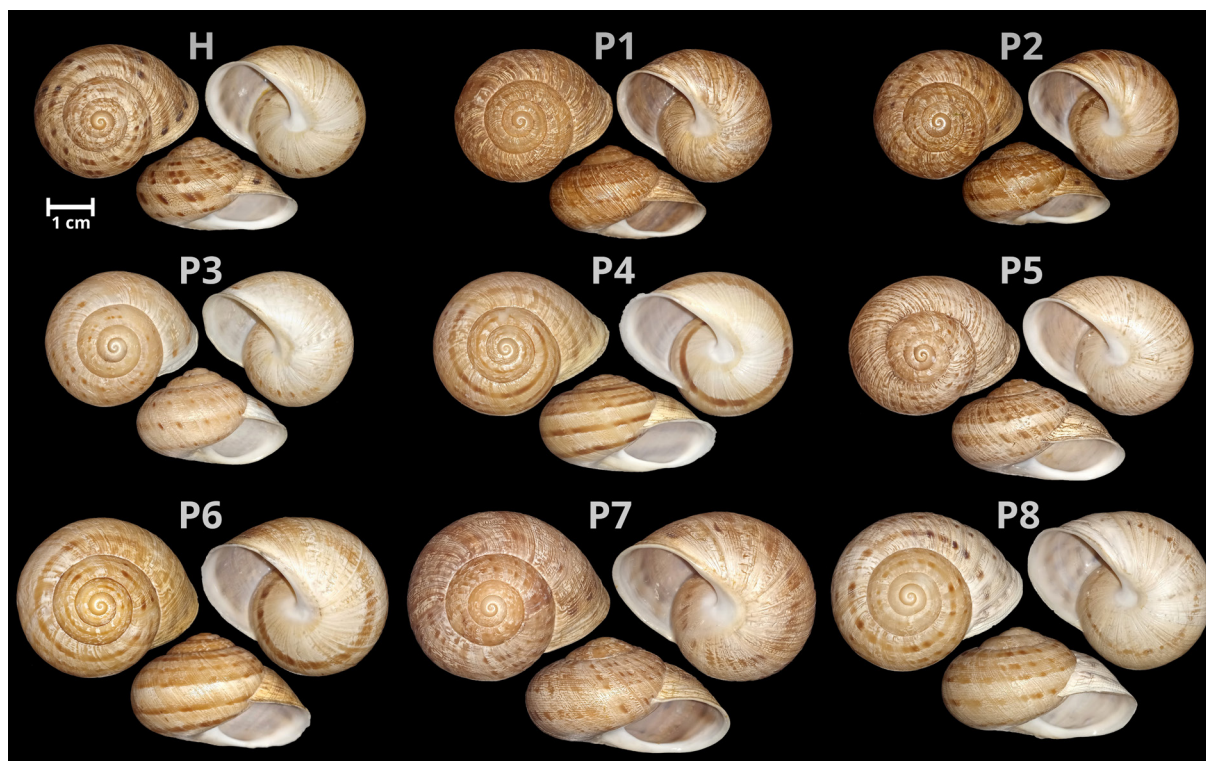


FIG. 12. Photographs of the holotype (H) and the 8 paratypes (P1 to P8) of *I. rhodopeplus bastetanus*. The holotype corresponds with the sequenced sample III1.

Spain). The deposit reference for the holotype of *I. rhodopeplus bastetanus* is 15.05/200543. Those for the paratypes are 15.05/200544 and 15.05/200545. Table 5 shows some extra information about sizes and locations of *I. rhodopeplus bastetanus* types.

Type locality. Type locality for *I. rhodopeplus bastetanus* is located on sloping limestone walls within a Mediterranean scrubland at Picos del Guadiana, Huesa (Jaén, southern Spain), with the following coordinates: 37°44'51"N, 3°06'20"W.

Etymology. The Bastetans people were ancient inhabitants of Bastitania (part of the Iberian civi-

zation), a region covering a significant area of the southeast of the Iberian Peninsula which matches the current distribution of *I. rhodopeplus bastetanus*.

Description. Basically, the same description may be applied to *I. rhodopeplus bastetanus* as for *I. rhodopeplus rhodopeplus*, with major shell diameter of *I. rhodopeplus bastetanus* ranging from 26 to 44 mm.

Series of conchological variability of *I. rhodopeplus bastetanus* can be checked in Fig. 13.

Habitat. *I. rhodopeplus bastetanus* selects similar habitats to those of *I. rhodopeplus rhodopeplus* ranging from 51 m to 1,293 m a.s.l., being also one

Table 5. Location and basic morphometrics of holotype and paratypes assigned to *I. rhodopeplus bastetanus*.

Specimen	Length (mm)	Width (mm)	Height (mm)	Latitude (N)	Longitude (W)	Sampling date
Holotype	34.49	27.06	20.18	37°44'51"	-3°06'20"	13/02/2022
Paratype 1	32.81	26.57	19.15	37°11'2.9"	-3°03'47.5"	24/09/2023
Paratype 2	30.04	23.92	16.53	37°11'2.9"	-3°03'47.5"	24/09/2023
Paratype 3	32.09	26.73	19.80	37°16'34"	-3°17'15"	19/09/2021
Paratype 4	37.21	29.40	20.37	36°47'44"	-3°04'46"	01/10/2023
Paratype 5	35.72	27.63	21.76	37°30'1.8"	-3°12'45.4"	05/02/2022
Paratype 6	38.53	32.09	25.68	36°54'27"	-3°01'34"	25/09/2022
Paratype 7	42.54	33.91	25.19	36°56'55.8"	-3°01'12.1"	25/09/2022
Paratype 8	39.26	31.51	23.51	37°41'38.1"	-3°08'39.5"	31/10/2021

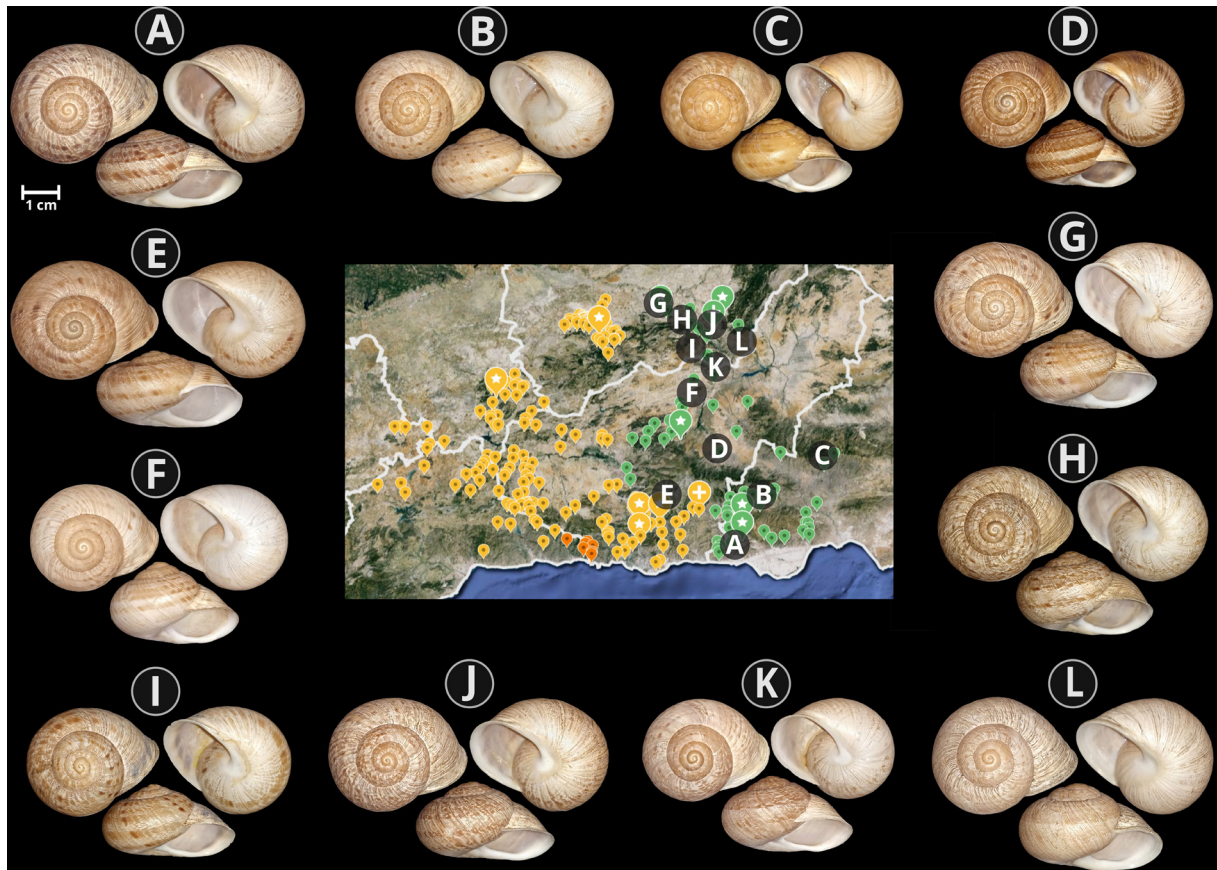


FIG. 13. Series of conchological variation of *I. rhodopeplus bastetanus*. A, El Corral, Adra (Almería); B, Fondón (Almería); C, Castro de Filabres (Almería); D, La Calahorra Castle (Granada); E, Barranco de la Cueva, Soportújar (Granada); F, El Mencil de Pedro Martínez (Granada); G, Serrezuela de Jódar (Jaén); H, Rambla del Fontarrón, Larva to Cabra de Santo Cristo Rd (Jaén); I, Picos del Guadiana, Huesa (Jaén); J, Los Rosales, Quesada (Jaén); K, Alicún de Ortega (Granada); L, Cuenca village, Hinojares (Jaén).

of the *Iberus* taxa with a wider ecological range (Fig. 14). Due to the proximity of the semi-arid steppe of Baza and the Almería desert, *I. rhodopeplus bastetanus* is also highly adapted to xeric habitats devoid of arboreal vegetation in which refuges are provided by esparto grasslands and stony grounds.

Remarks. The ratio of banding patterns in the shells of *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* was found to be different at a metapopulation scale. Although the discontinuous band pattern is the most common in both species, while in *I. rhodopeplus rhodopeplus* 23% and 8% of shells show unbanded and continuous banded patterns, respectively, the same patterns involve 2% and 45% of the shells in *I. rhodopeplus bastetanus*.

Discussion

On the taxonomic status of *Iberus alonensis labiatus*

Ahuir Galindo [2016] considered that two conchological features not matching the standards of the nominal species, a larger average size and a more developed outer lip, were sufficient to define *I. alo-*

nensis labiatus. Now we know that all *alonensis*-like populations throughout Andalusia are *I. rhodopeplus* ssp. The question that arises is: Could this taxon be referred to as *I. rhodopeplus labiatus*? Some evidence resulting from our systematic sampling suggest that the morphological differences argued by Ahuir Galindo are not suitable to support the definition of a new subspecies. On the one hand, the expansion and thickness of the lip are not exclusive traits of the snails of the *alonensis* complex populations inhabiting coastal mountains of eastern Málaga (as exemplified by specimens G to I of Fig. 7; see also Fig. 8B). Furthermore, not all specimens from this area show a typically overdeveloped lip. On the other hand, large specimens of *I. rhodopeplus rhodopeplus* can be found in a number of localities in the provinces of Jaén and Granada (see specimens J to L of Fig. 7 and Fig. 8A).

We consider as appropriate to maintain the taxonomic denomination '*labiatus*' to designate any Andalusian population of *I. rhodopeplus rhodopeplus* with a morph characterised by large shells and overdeveloped lips, calling them *I. rhodopeplus rhodopeplus* f. *labiatus*.

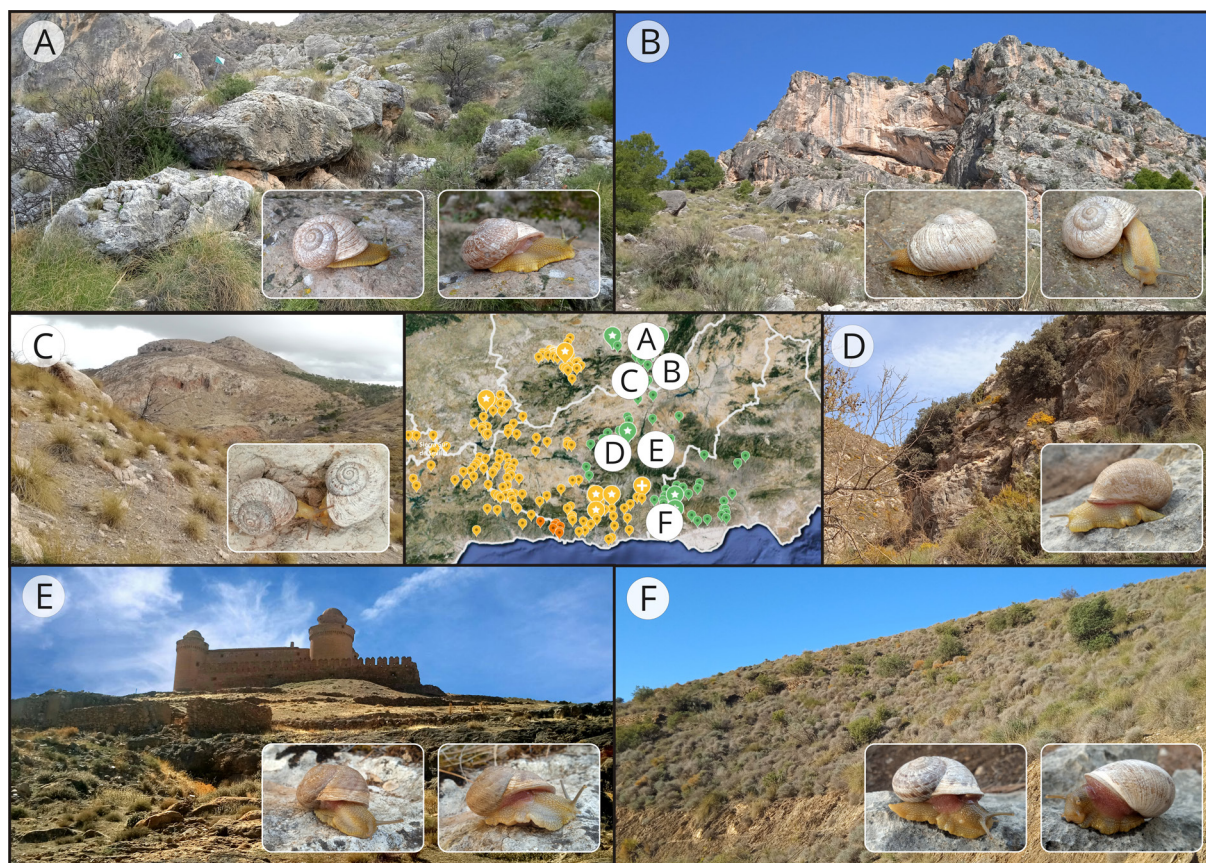


FIG. 14. Some representative habitats and alive specimens of *I. rhodopeplus bastetanus*. **A**, Los Rosales, Quesada (Jaén); **B**, Cuenca village, Hinojares (Jaén); **C**, Rambla del Fontarrón, Larva to Cabra de Santo Cristo Rd (Jaén); **D**, Source of the River Beas, Beas de Granada (Granada); **E**, La Calahorra Castle (Granada); **F**, San Isidro Labrador Ermitage, El Corral, Adra (Almería).

A great step to clarify the *alonensis* puzzle and to understand the prevalent cryptic nature among *Iberus* species

For a long time, the name *I. alonensis* was indiscriminately applied to a large number of similarly-shaped snail populations distributed across the eastern half of the Iberian Peninsula. Elejalde *et al.* [2008a], who addressed for the first time the taxonomic composition of the genus *Iberus* from a molecular perspective, defined two subgroups within their A6 clade, attributable to the Andalusian populations traditionally named *I. alonensis*. The first subgroup was confined to the western part of the distribution range for this clade, whereas the second inhabited the eastern part, with their precise geographical distributions remaining to be clarified. For reasons that are not specified by the authors, they decided to include both subgroups under a single provisional name (*I. alonensis*-like 02) instead of two. Although the notable genetic divergence found in the present work between the two subgroups reveals that two different lineages may be differentiated within Elejalde *et al.*'s A6 clade, their morphological overlap at a metapopulation scale and their geographical

continuity suggest that they are still going through an early phase of the speciation process, therefore being convenient to consider them as two subspecies: *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus*, attributable to the western and eastern Andalusian populations of the former *I. alonensis*-like 02, respectively. Further studies with a greater number of samples in the presumed contact zones and new genetic analyses involving other specific molecular markers will allow us to clarify more precisely this taxonomic status.

Study of shells, although still useful as a complementary taxonomic tool [Korábek *et al.*, 2015], may be misleading depending on the methodological approach employed. It is well known that the power of statistical tests are more reliable by increasing sample size [Columb, Atkinson, 2016; Serdar *et al.*, 2021]. By analysing a large number of shells in this work, we are providing the statistical tests with high sensitivity, capable of detecting small differences in morphometric parameters at a metapopulation scale. As a consequence, despite the differences between the parameters and ratios measured for *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* appear

to be small, high levels of statistical significance are reached by running univariate analysis. In such cases, multivariate analyses have been proved to improve selectivity [Olivieri, 2008] and reliability [Nguyen *et al.*, 2021]. Indeed, contrastingly to the univariate analysis results, PCA shows how clouds of *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* are thoroughly mixed on the morphospace. This explains why identifying both species with the naked eye through shell morphology becomes a hard task, even for experienced malacologists studying the genus *Iberus*.

In conclusion, despite morphological similarities between the shells of *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus*, they are phylogenetically distant. There are a number of taxonomic studies on terrestrial gastropods in which the molecular data do not agree, totally or partially, with the conchological and/or anatomical ones [e.g. Fiorentino *et al.*, 2008]. Recently, Liétor *et al.* [2024a] described several cryptic species of the genus *Iberus* (*I. giennensis*, *I. antikarianus* and *I. axarciensis*) with often indistinguishable shells. In the specific case of *I. axarciensis*, it had gone unnoticed despite having a wide distribution within an easily accessible geographical area. Chance, or as in the case of Liétor *et al.*'s, systematic and intensive sampling are the only ways to find new species when a cryptic complex is involved [Köhler, Burghardt, 2016; Modica *et al.*, 2016].

This work reaffirms that the taxonomic chaos of the *alonensis* complex has been boosted by the prevalence of cryptic species [Elejalde *et al.*, 2008a; Liétor *et al.*, 2024a,c]. Species richness and level of endemism often constitute the basis for managing protected areas and planning conservation strategies [Brooks *et al.*, 2006; Bickford *et al.*, 2007]. Under such a perspective, correct species identification is critical and thus, the presence of undetected cryptic species may become highly problematic. If cryptic species are not identified, disruptive effects like overestimation of species distribution ranges, underestimation of species richness or endemic species overlook may arise. Ultimately, a poor knowledge about cryptic species and their distribution can lead to wrong planning decisions and to inappropriate conservation guidelines [von Oheimb *et al.*, 2019].

Any further taxonomic study on the genus *Iberus* will require an integrative approach supported by robust geographical coverage and custom designed to detect the presence of cryptic species. Scattered sampling over reduced areas within an unknown geographical distribution and a morphology-based methodology will not allow to resolve the challenges that this genus still poses.

The finding of a presumably dwarf hybrid population between *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* is not a surprise since other examples of hybridization between taxa of

the genus *Iberus* entailing dwarf phenotypes have been documented [Jowers *et al.*, 2024]. It will be necessary to intensify research in the contact areas between *I. rhodopeplus rhodopeplus* and *I. rhodopeplus bastetanus* to delineate more precisely their cartography and find new hybrid populations to be genetically characterised. This will contribute to understanding the intricate speciation patterns driven by genetic introgression that could be present within the genus *Iberus*.

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