

Review of the taxonomic status of several taxa of the genus *Iberus* from the southwest of the Almería Province (Spain)

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SUMMARY

Geographical, morphological and genetic evidences are provided that allow to reconsider the current taxonomic status of the taxa *Iberus gualtieranus mariae* and *Iberus gualtieranus ornatissimus*, which are reclassified within a new species named *Iberus mariae*. A new local morphotype is also described.

INTRODUCTION

Iberus gualtieranus mariae and *Iberus gualtieranus ornatissimus* (*I. g. mariae* and *I. g. ornatissimus*, henceforth) were placed by Elejalde *et al.* (2008) in their clade G along with *I. gualtieranus*, corroborating the taxonomic status assigned by the original author (Cobos, 1979). The pairwise sequence divergence of Elejalde's clade G1 ranged up to 6.26%, which was not considered sufficient to define new species. Consequently, their classification as subspecies is currently considered valid (Bank & Luijten, 2015).

There is hardly any information on the distribution of both taxa throughout the middle and east of the Sierra de Gádor (Almería Province, Spain). In Elejalde's reference work no new information is provided in this regard. The only thing that seems clear is that *I. g. mariae* remains isolated in the coastal Punta Entinas Natural Area. It already was in 1979 when Cobos established that "*there is no other Iberus within many kilometres around*". Indeed, the isolation of *I. g. mariae* is expected to

be more intense today due to the unstoppable advance of greenhouse crops throughout the surrounding area. Having a detailed map of the contact among populations of *I. g. mariae* and *I. g. ornatissimus* and a morphometric study that quantifies how different their shells are, would help to delimit the taxonomic status of both subspecies with strong arguments.

In this work, we apply an integrative taxonomy approach consisting of i) an intensive exploration of the territory where the taxa of the *gualtieranus-mariae-ornatissimus* complex may come into contact, ii) a comparative morphometric analysis of a large number of shells and iii) new molecular data from specimens sampled in key locations. The body of evidence obtained will allow us to understand accurately the taxonomic status of *I. g. mariae* and *I. g. ornatissimus*.

MATERIAL AND METHODS

Plate 1 shows the shells of the three specimens from Almería Province whose tissues were genetically analysed:

- i) Specimen S1 was sampled in the Punta Entinas Natural Area and assigned to the classical morphotype of *I. g. mariae*.
- ii) Specimen S2 was collected close to the Santa Cruz hermitage in Dalías and identified *a priori* as *I. g. ornatissimus*. Although the shape of the shell and the lip expansion did not fit the classical description of the taxon, the shell ornamentation clearly reminded that of *I. g. ornatissimus*.
- iii) Specimen S3 was collected in Alcaudique (Berja) as an unknown morphotype that was tentatively classified as an extreme local variant of *I. g. mariae*.



Plate 1. Shells of alive specimens sampled to obtain biological tissues for genetic analyses. S1. *I. g. mariae* (classical morphotype); S2. *I. g. ornatissimus* (uncommon morphotype); S3. *I. g. mariae* (new morphotype).

For genetic characterization, the following three molecular markers were used: Large Ribosomal Subunit (LSU) as nuclear gene, and both 16S Ribosomal RNA (16S rRNA) and Cytochrome Oxidase I (COI) as mitochondrial genes.

The methodology developed to morphometrically characterize the shells of the taxa under study is the same shown in the article published by Liétor

et al. in this journal issue.

A sampling that systematically explored the entire potential distribution area of the target taxa consisting of 70 sample locations was carried out. Only the western part of the Sierra de Gádor was discarded due to the absence of lanes accessible by motor vehicle. Finally, 864 shells from 12 representative locations were measured.

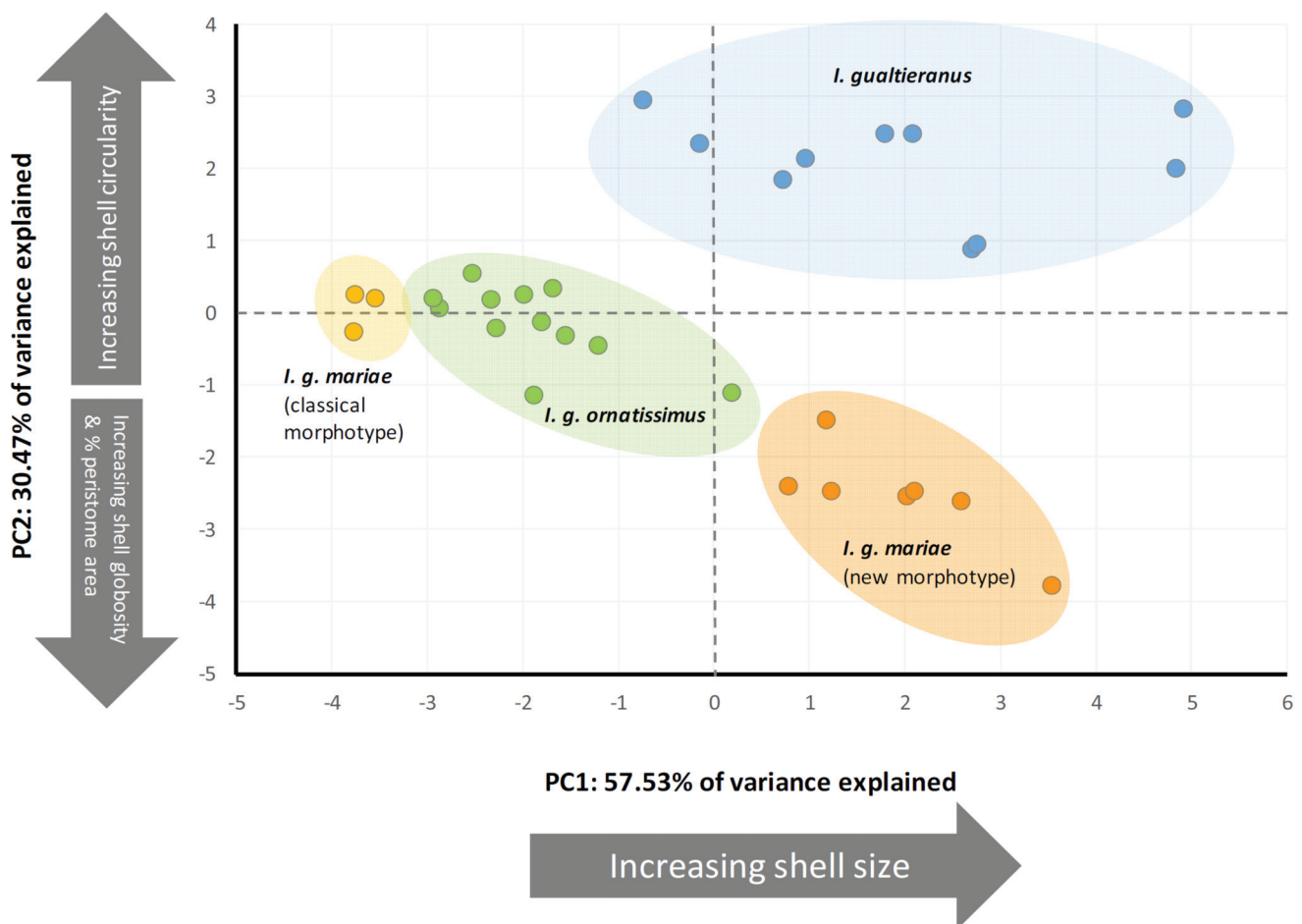


Figure 1. Distribution of the species under study across the bi-dimensional morphospace generated by the two first PC of a PCA analysis. Each point in the graph represents a single sampling locality.

RESULTS

1- Molecular evidence

Phylogenetic analyses of the genus *Iberus* (Galán-Luque, 2023) recovered a strongly supported relationship of samples S1 and S3 (grouped with *I. g. mariae* from GenBank, assignable to G1b subclade of Elejalde *et al.*, 2008) with its sister clade containing the sample S2 (grouped with *I. g. ornatissimus* from GenBank, assignable to Elejalde’s G1c subclade). This sister clade relationship was recov-

ered with mitochondrial data but the nuclear data revealed a strong non-differentiated clade. The S3 positioning close to S1 confirmed our preliminary identification of this specimen as *I. g. mariae*.

2- Morphological evidence

Figure 1 shows how the taxa under study are distributed throughout the morphospace provided by the first two principal components (PC) of a Principal Component Analysis (PCA) performed with the variables in Table 1.

Table 1. Morphometric comparison among the two morphotypes of *I. g. mariae*, *I. g. ornatissimus* and *I. gualtieranus*. Values are given with the mean \pm SD. Due to the data were not normally distributed for any of the morphometric parameters and ratios, we compared pairs of variables with a non-parametric Kruskal Wallis plus 2-tailed multiple comparison H test. Different letters indicate significant differences (p-value < 0.05). The number of shells measured for each taxon is indicated in parentheses in the headings.

Parameters and ratios	<i>I. g. mariae</i> classical morpho- type (n=246)	<i>I. g. mariae</i> new morpho- type (n=151)	<i>I. g. ornatissimus</i> (n=228)	<i>I. gualtieranus</i> (n=239)
Major Ø of the shell (mm)	27.28 \pm 1.76 c	36.09 \pm 2.22 a	29.60 \pm 2.21 b	36.70 \pm 4.25 a
Minor Ø of the shell (mm)	21.21 \pm 1.19 d	27.84 \pm 1.90 b	24.14 \pm 1.78 c	30.8 \pm 3.63 a
Shell height (mm)	15.58 \pm 1.20 c	21.00 \pm 1.80 a	16.60 \pm 1.31 b	16.90 \pm 2.22 b
Major external Ø of the peristome (mm)	17.13 \pm 1.24 c	24.04 \pm 1.78 a	18.33 \pm 1.85 b	23.37 \pm 2.97 a
Minor external Ø of the peristome (mm)	13.76 \pm 1.10 d	19.85 \pm 1.57 a	16.01 \pm 1.45 c	17.37 \pm 1.81 b
Shell height/Major Ø of the shell (ratio)	0.57 \pm 0.03 b	0.58 \pm 0.03 a	0.56 \pm 0.03 c	0.46 \pm 0.03 d
Shell area (mm ²)	455.80 \pm 53.36 c	792.17 \pm 102.06a	563.94 \pm 81.23 b	900.47 \pm 216.29 a
Peristome area (mm ²)	185.85 \pm 26.48 d	376.52 \pm 55.62 a	232.23 \pm 45.73 c	322.02 \pm 70.78 b
Major Ø/Minor Ø of the shell (ratio)	1.29 \pm 0.04 a	1.30 \pm 0.04 a	1.23 \pm 0.06 b	1.19 \pm 0.04 c
Major Ø/Minor Ø of the peristome (ratio)	1.25 \pm 0.08 b	1.21 \pm 0.06 c	1.15 \pm 0.06 d	1.35 \pm 0.13 a
Peristome area x100/Shell area (%)	40.84 \pm 3.75 b	47.56 \pm 3.79 a	41.19 \pm 5.50 b	36.06 \pm 3.66 c

PC1 (which groups populations according to shell size) determines the morphological clustering better than PC2 (which groups populations according to shell shape) because it accounts for almost twice the variance of the data (57.53 versus 30.47%, respectively). The shells of *I. g. mariae* occupy a position close to those of *I. g. ornatissimus*, without overlapping, being both subspecies well separated morphologically from *I. gualtieranus*. However, the new morphotype of *I. g. mariae* shows a size range similar to that of *I. gualtieranus*, although its average shell is more globose and elliptical and its peristome is proportionally wider (Table 1). It should be added that *I. gualtieranus* typically shows a marked keel and dense reticulation that never appear in the shells of *I. g. mariae* or *I. g. ornatissimus* (Plate 2).

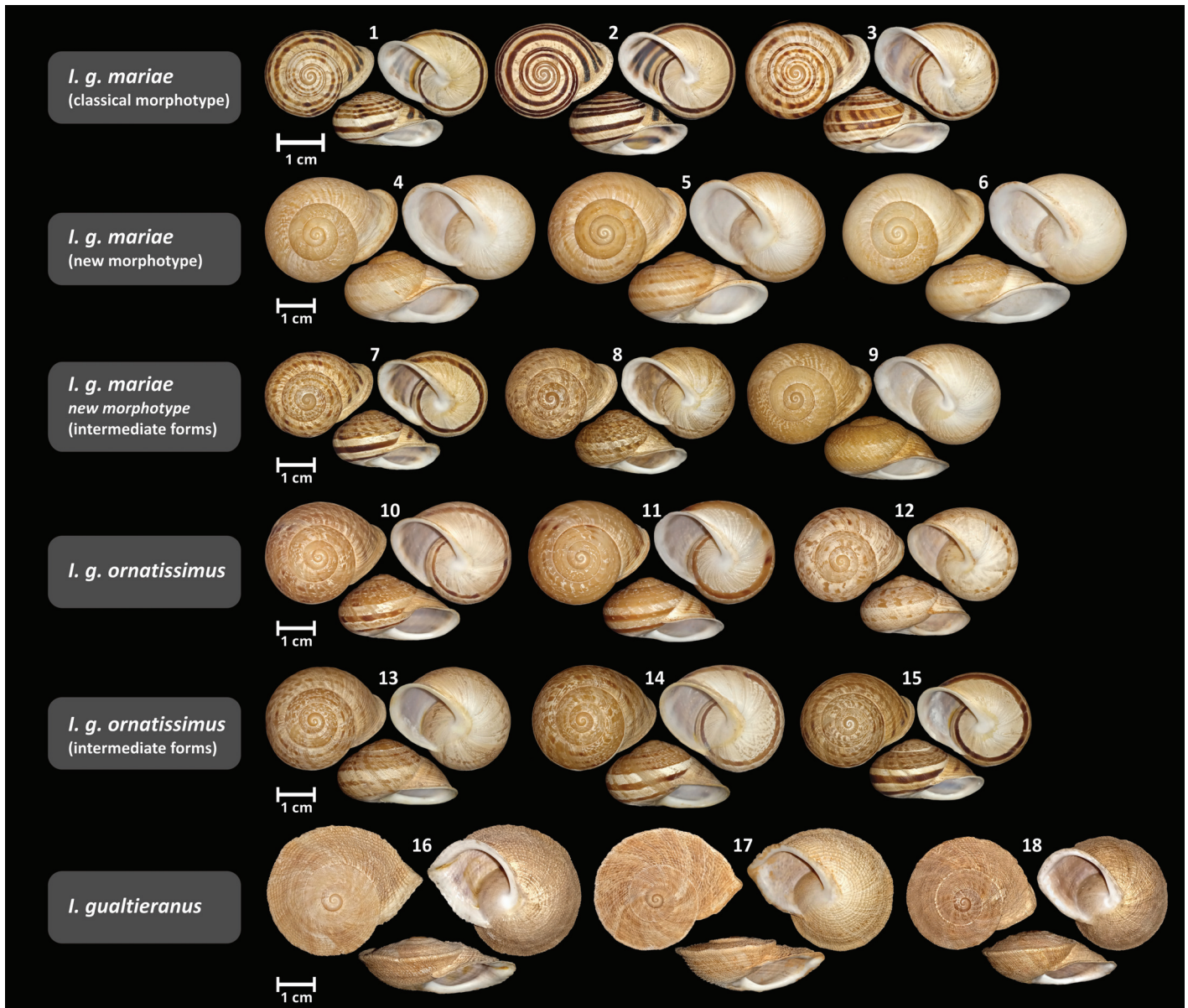


Plate 2. Some representative shells of the taxa involved in this work, including some intermediate with *I. alonensis*-like 02 (*sensu* Elejalde *et al.*, 2008). Note that the scale has been expanded for *I. g. mariae* to achieve a better view of the shells. The sampling locations (all of them within the Almería Province) are the following: 1 to 3- Paraje Natural Punta Entinas; 4 to 6- Alcaudique, Berja; 7 and 8- Balanegra; 9- Los Cerrillos, Berja; 10- Alicún; 11- Huécija; 12- Canjáyar; 13 and 14- Alicún; 15- El Marchal de Enix, Alhama de Almería; 16- El Palmer, Aguadulce, Roquetas de Mar; 17- La Molineta, Almería; 18- Vícar.

3- Geographic evidence

Figure 2 shows the distribution of the taxa under study in the surroundings of the Sierra de Gádor (Almería Province). *I. gualtieranus* inhabits the eastern edge of the Sierra de Gádor, from Vícar in the south to Alhama de Almería in the north, barely penetrating the eastern third of the mountain massif. *I. g. mariae* continues to occupy the narrow strip of rocky slope near the marsh of the Punta Entinas-Sabinar Natural Area described by Cobos in 1979, now completely surrounded by greenhouse crops. Hitherto, it was well known that *I. g. ornatissimus* borders the northern slope

of the Sierra de Gádor, from Alhama de Almería to Ohanes. Now, our sampling provides new evidence that *I. g. ornatissimus* crosses the easternmost part of Sierra de Gádor until connecting with the municipalities of Dalías and la Mojónera on the southern slope. Throughout this path, the populations of *I. g. ornatissimus* differ in their conchological characteristics from those originally described for the subspecies, since a genetic introgression of the most eastern populations of *I. alonensis*-like 02 probably occurs (Plate 3.A). Finally, the new morphotype of *I. g. mariae* inhabits the southwestern foothills of the Sierra de

Gádor (up to the municipalities of Berja to the north and Adra to the south). The rest of the populations of this new morphotype surrounding the municipal area of Berja seem to show intermediate traits, once again, with the eastern populations of *I. alonensis*-like 02 (Plate 3.B). The populations of the classical morphotype of *I. g. mariae* and those of the new morphotype are separated

by the dense greenhouse area of El Ejido. In a patch of degraded Mediterranean scrub immersed between greenhouses, close to the coastal population of *I. g. mariae*, subfossil shells of the new morphotype have been found, which suggest that there could have been somewhat continuity between both populations in the past, currently constituting a meta-population.



Figure 2. Geographic distribution throughout the southwest of the Almería Province (Spain) of the taxa of the genus *Iberus* addressed in this work and some intermediate forms from the contact areas with the easternmost populations of *I. alonensis*-like 02.



Plate 3. A. Intermediate specimen (middle) between *I. g. ornatissimus* (left) and *I. alonensis*-like 02 (right), all of them sampled in nearby locations within the municipality of Alicún (Almería Province). **B.** Intermediate specimen (middle) between the new morphotype of *I. g. mariae* (left) and *I. alonensis*-like 02 (right), all of them sampled in nearby locations within the municipality of Berja (Almería Province).

DISCUSSION

It is important to consider characteristics beyond genetic ones when reevaluating the taxonomy of a group, as these play a role in how a species adapts, inhabits and coexists with others. In the words of Elejalde *et al.* (2008), the decision of considering *I. g. mariae* and *I. g. ornatissimus* as valid subspecies was supported on the “*traditional consideration of them as different taxa* (Cobos, 1979; Alonso & Ibáñez, 1981) *together with their differences in morphology and genetic divergence*”. However, the approach of Elejalde’s team was exclusively molecular, without providing morphological analyses or deepening their geographical distribution. Indeed, they did not know the geographical continuity between both subspecies, as well as the limited (and probably random) spatial contact between them and *I. gualtieranus*.

Typical genetic distances for the COI used to define species in land snails are between 4 and 8% (Hebert *et al.*, 2003; Davison *et al.*, 2009; Köhler & Johnson, 2012). For instance, Criscione & Köhler (2014) considered a minimum distance of 5% for the 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%.

Despite considering them as subspecies, Elejalde *et al.* (2008) reported distances for the COI between *I. gualtieranus* and *I. g. mariae* ranging from 7.17 to 8.28%. Similarly, they reported distances from 8.12 to 8.44% between *I. gualtieranus* and *I. g. ornatissimus*. Genetic distances of this magnitude have been considered sufficient for other helicids to delimit new species. Colomba *et al.* (2015) set between 4 and 7.5% for the COI to differentiate among species of the genus *Ercella*; Holyoak *et al.* (2018) established a range from 8.2 to 12.1% to delineate species within the genus *Eremina*.

Our genetic analyses determine that the operational taxonomic units here called *mariae* and *ornatissimus* represent well-differentiated lineages. The lack of genetic divergence found in the nuclear data phylogenetic tree (Galán-Luque, 2023) suggests an ancestral origin of the two *mariae* sister clades with an ongoing speciation process, as revealed by the mitochondrial data. Morphological and geographical evidences are consistent with this result: i) these two lineages show many more morphological similarities to each other than regarding to *I. gualtieranus*; ii)

their distribution areas show a solution of continuity through Sierra de Gádor, with hardly any contact between them and the eastern populations of *I. gualtieranus*. In the case that this amount of evidence could be considered sufficient to define new subspecies, we propose the following taxonomic denominations:

Phylum MOLLUSCA Cuvier, 1795
Class GASTROPODA Cuvier, 1795
Subclass HETEROBRANCHIA Burmeister, 1837
Superorder EUPULMONATA Haszprunar & Huber, 1990
Order STYLOMMATOPHORA Schmidt, 1855
Suborder HELICINA Rafinesque, 1815
Superfamily HELICOIDEA Rafinesque, 1815
Family HELICIDAE Rafinesque, 1815
Subfamily HELICINAE Rafinesque, 1815
Tribe ALLOGNATHINI Westerlund, 1903
Genus *Iberus* Montfort, 1810
Iberus mariae mariae comb. nov.
Iberus mariae ornatissimus comb. nov.

Although *I. g. mariae* and *I. g. ornatissimus* were described by Cobos (1979) within the same publication, since *I. g. mariae* was listed first, it has prevalence as nominal species.

To the northwest of the classical morphotype of *I. mariae mariae* comb. nov., there is another big one with a typically extended lip documented for the first time in this work. More specifically, this new morphotype has a diameter 30% larger, and a shell surface 75% greater than the typical form of *I. mariae mariae* comb. nov. But the conchological character that best differentiates both is the peristome area, double for the new morphotype. That is why we propose that this new form could be recognized as *I. mariae mariae* comb. nov. *f. macrolabiatus*. The fact that samples S1 and S3 coincide in the same clade together with a strong bootstrap support from the mitochondrial data suggest that despite their morphological differences (Figure 1, Plate 2) the two morphotypes are not different taxa. We assign the population of Alcaudique (Berja) as a type locality for this local form of *I. mariae mariae* comb. nov.

Further samplings will be needed to determine if other populations of the *macrolabiatus* morphotype exist and understand whether the genetic introgression to which it is being subjected by *I. alonensis*-like 02 on every side could involve a decline of this rarity of the Iberian malacofauna.

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