

## Uncovering hidden diversity in Iberian freshwater fossil snails: an integrative approach to *Horatia schlosseri* (Royo-Gómez, 1922) (Gastropoda: Hydrobiidae)

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### INTRODUCTION & AIM

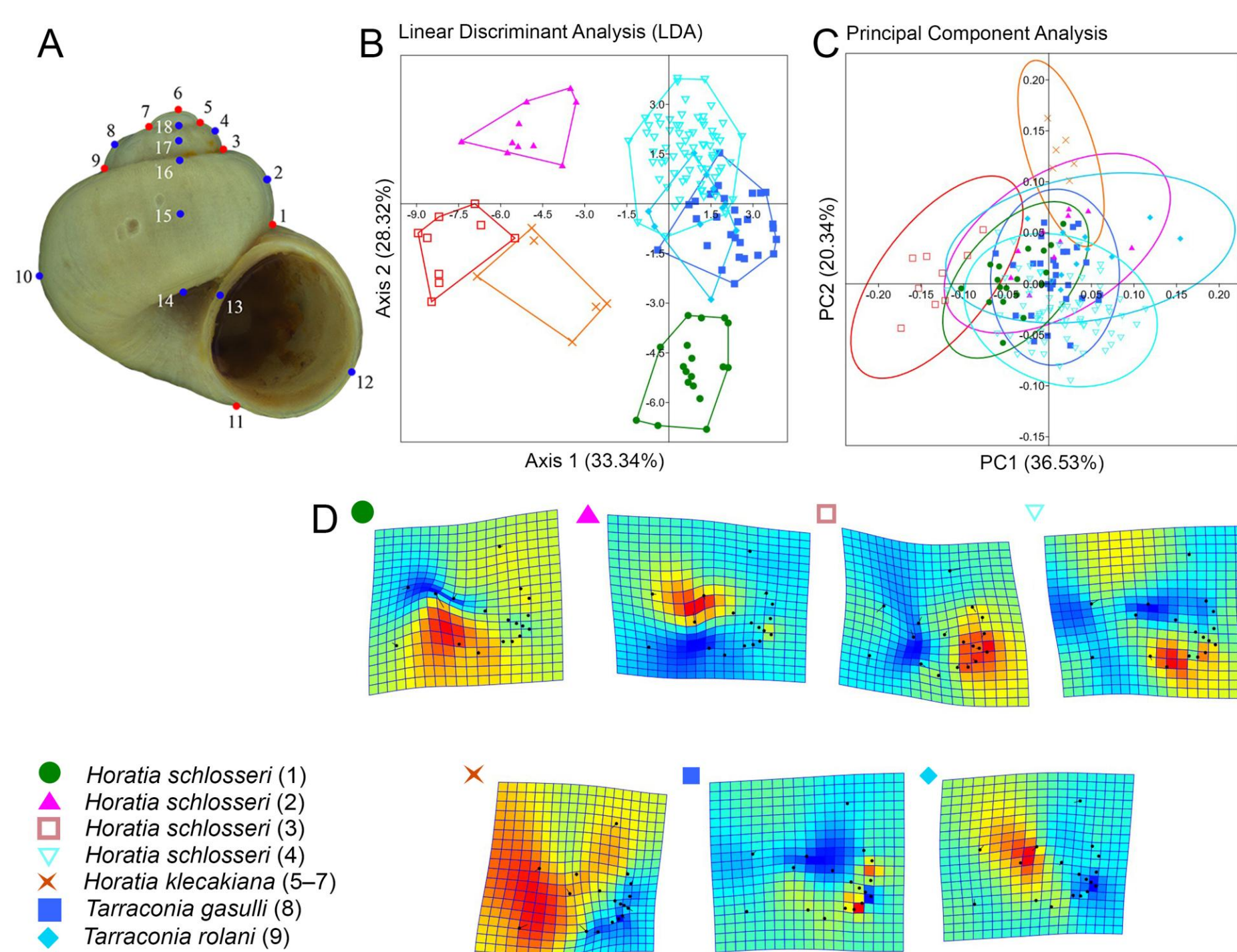
Freshwater gastropods are highly diverse, with ~4,000 valid species likely representing only a quarter of actual diversity, and occur worldwide across most aquatic habitats (Strong et al., 2008). Hydrobiidae is the most speciose family, including many narrow-range endemics, yet its taxonomy is hindered by minute size and simplified morphology. *Valvata schlosseri* Royo-Gómez, 1922, now treated as *Horatia schlosseri*, has undergone unstable generic placements; phylogenetic evidence restricts *Horatia* Bourguignat, 1887 to the Balkans, raising doubts about Iberian assignments and suggesting affinities with *Tarraconia* Ramos & Arconada, 2000. Given intraspecific shell-shape variability and potential cryptic diversity, we apply geometric morphometrics to compare *H. schlosseri* (including type material) with *Tarraconia* and *H. klecakiana* Bourguignat, 1887, evaluating species coherence and generic placement.

### METHOD

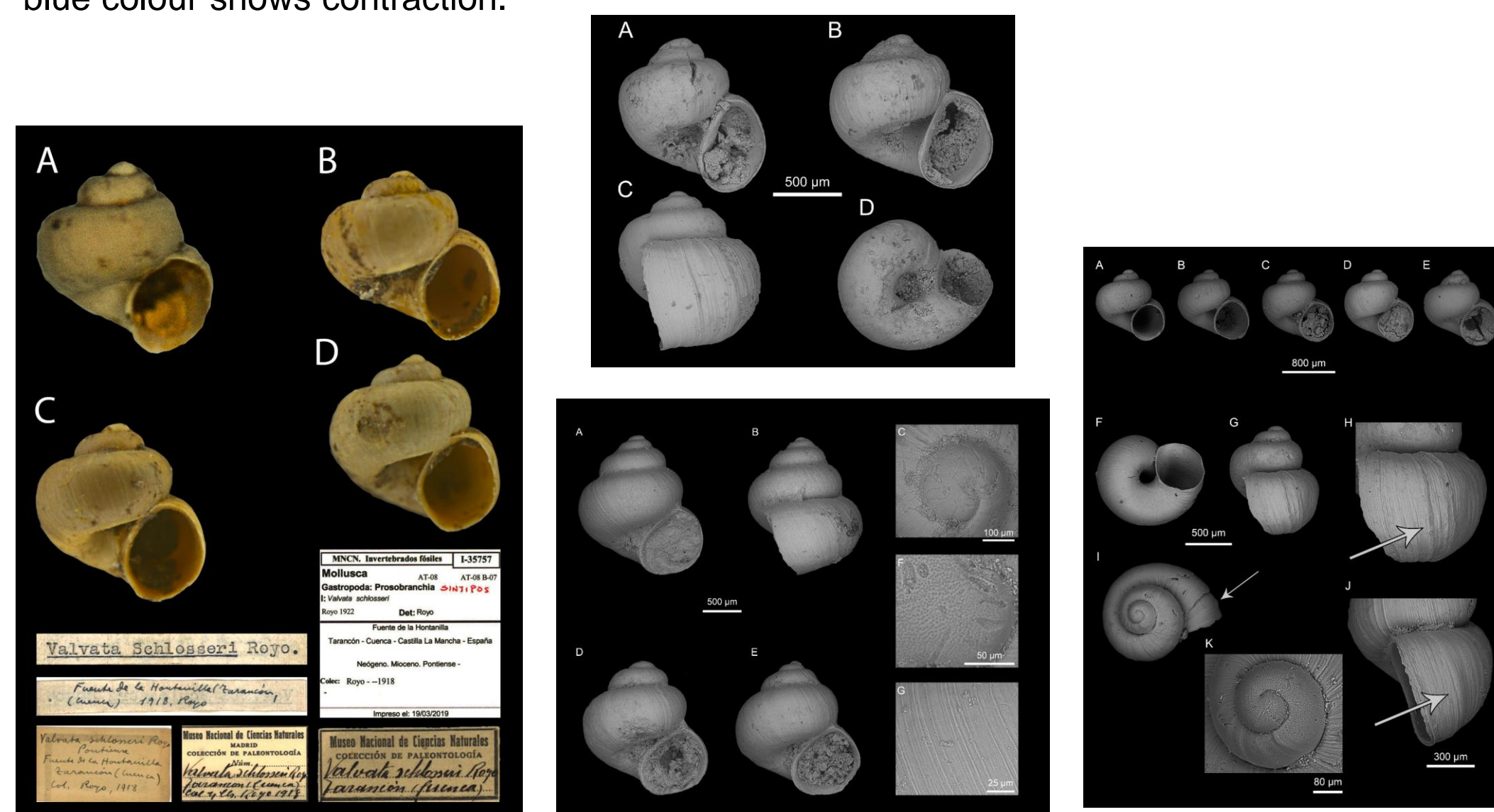
We analyzed *Horatia schlosseri* across seven populations (153 specimens), including Royo-Gómez localities, syntypes (MNCN-35757), and material from Alcalá de Júcar consistent in morphology, age, and stratigraphy. Given similarities to Iberian *Tarraconia*, we incorporated paratypes of *T. gasulli* (Boeters, 1981) (MNCN 15.05.32059) and *T. rolandi* Ramos, Arconada & D. Moreno, 2000 (MNCN 15.05.33131), plus type material of *Horatia klecakiana* (from Falniowski et al., 2021). Standardized frontal photographs were taken with Leica stereomicroscopes; microsculpture was imaged via ESEM. We digitized 18 landmarks and semilandmarks, performed GPA, assessed allometry, and applied PCA, LDA with LOOCV, PERMANOVA with pairwise tests, ANOSIM, and PERMDISP in R 4.3.2, following MolluscaBase/FreshGEN taxonomy and Hershler–Ponder terminology.

### RESULTS & DISCUSSION

After Generalized Procrustes Analysis, a significant allometric effect was detected ( $R^2 = 0.0655$ ,  $p = 0.0001$ ); subsequent analyses used residuals. PCA of size-corrected shapes explained 73.44% of variance (PC1 = 36.53%, PC2 = 20.34%, PC3 = 16.57%), revealing partially overlapping yet distinct morphospaces. PC1 tracked aperture expansion, spire height, and whorl convexity; PC2 captured apical angle and body-whorl inflation. LDA achieved 89.54% LOOCV accuracy, with LD1–LD2 explaining 61.66% between-group variance. ANOSIM ( $R = 0.5245$ ,  $p = 0.0001$ ) and PERMANOVA (pseudo- $F = 18.94$ ,  $R^2 = 0.4377$ ,  $p = 0.0001$ ) confirmed significant differences; PERMDISP indicated unequal dispersion, evidencing structured, non-allometric shape divergence (Figure 1).



**Figure 1.** Geometric morphometric analyses of the studied taxa. **A**, Shell morphometric configuration of variables, 18 landmarks (red dots) and semi-landmarks (blue dots) used to digitize the images into coordinates; **B**, Linear Discriminant Analysis (LDA) illustrating the morphological similarities among the studied taxa; **C**, Principal Component Analysis; **D**, Thin-Plate Spline plots depicting deformation of the consensus of each taxon against the mean shape; red colour shows expansion while blue colour shows contraction.



**Figure 2.** Type material of *H. schlosseri* as well as potential new species revealed by the GM analyses.

### CONCLUSION

This study demonstrates that geometric morphometrics robustly resolves shell-shape structure within Iberian Hydrobiidae, revealing significant, non-allometric differentiation among taxa and clarifying the *Horatia schlosseri* complex. Evidence from PCA/LDA, ANOSIM, and PERMANOVA supports a structured morphospace with diagnostic variation in spire height, whorl inflation, and aperture architecture. The recognition of four morphotypes within *H. schlosseri*, including forms aligning with *Tarraconia* and two novel *Horatia* species, indicates cryptic diversity and past taxonomic conflation (Figure 2). By designating a lectotype for *H. schlosseri* and standardizing landmark-based analyses, we refine species limits, stabilize nomenclature, and strengthen biogeographic inferences, underscoring the value of integrative taxonomy, quantitative frameworks for paleomalacological systematics.