Article

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A new Hydrobiidae species of the genus *Corrosella* Boeters, 1970 from Andalusia (S Iberian Peninsula)

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A new species of freshwater snails of the genus *Corrosella* is described from the province of Cádiz, Andalusia (southern Spain). This is the westernmost species of the genus known so far in this region, enlarging the distribution area to more than 100 km to the west. Shell morphology, anatomy and molecular tools distinguish it safely from other species in the genus. The characteristic corrosion of upper whorls, after which the genus is named, seems caused by burrowing organisms, not by chemical action.

Keywords: freshwater, springs, *Corrosella*, new species, Baetic Mountains, hotspot, corrosion.

Una nova espècie d'Hydrobiidae del gènere *Corrosella* (Boeters, 1970) d'Andalusia (S península Ibèrica)

Es descriu una nova espècie del gènere de cargols aquàtics *Corrosella* per a Andalusia, a la província de Cadis (Espanya). Es tracta de l'espècie coneguda del gènere més a l'oest d'aquest territori, amb la qual cosa augmenta la seva àrea de distribució més de 100 km cap a l'oest. La morfologia de la conquilla, la seva anatomia i les eines moleculars permeten distingir-la sense problemes d'altres espècies prèviament descrites en aquest gènere. La característica corrosió de les voltes superiors, que donen nom al gènere, sembla causada per organismes perforadors, no per causes químiques.

Paraules clau: aigua dolça, fonts, *Corrosella*, nova espècie, sistema Bètic, punt calent, corrosió.

The Hydrobiidae family ranks among the richest in species within all freshwater gastropods, with more than a thousand species described worldwide. In the Palaearctic region, it includes 148 genera and more than 740 species (Glöer, 2022), with a very high rate of new descriptions in the last decades. They inhabit different types of habitats, but are particularly abundant in springs. Some areas seem to have served as climatic refuges in the past glacial periods, and consequently the family has hotspots of biodiversity in the Iberian, Italian, and Balkanic peninsulas (Delicado *et al*., 2013).

The genus *Pseudamnicola* Paulucci, 1878 was described to differentiate it from similar snails from the American genus *Amnicola* Gould & Haldeman, 1840 (Delicado *et al*., 2015), and has many representatives in Europe (Radea *et al*., 2016). Recently, this genus was split into three genera, by raising the subgenus *Corrosella* Boeters, 1970 to genus level, and by the inclusion of a new genus, *Josefus* Arconada & Ramos, 2006, to accommodate a single species. This distinction is clearly supported by morphological, anatomical, and genetic data (Delicado *et al*., 2015; Radea *et al*., 2016).

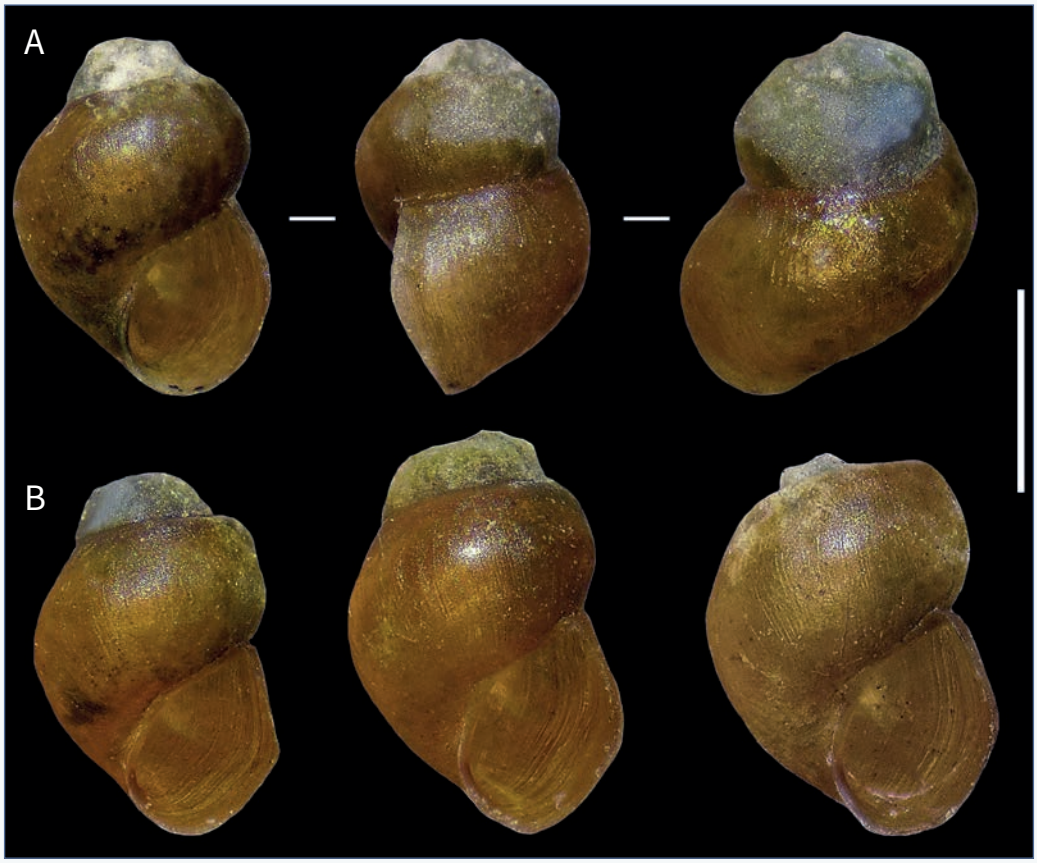

FIGURE 1. Pictures of type specimens of *Corrosella piti* sp. nov. from Algodonales (Cádiz, Spain). **A:** Holotype RMNH.MOL.350844 in different views; **B:** Different paratypes from the type locality. Scale: 1 mm.

Imatges de diversos espècimens de la col·lecció tipus de *Corrosella piti* sp. nov. de la localitat de Algodonales (Cadis, Espanya). **A:** Holotip RMNH.MOL.350844 en diferents vistes; **B:** Diversos paratips de la localitat tipus. Escala: 1 mm.

The genus *Corrosella* is represented only in the westernmost part of the Mediterranean basin, with one described species in southern France, sixteen in Spain, and six in the north of Morocco (Boulaassafer *et al*., 2021). This genus is particularly abundant in the upper part of river basins, in areas at some altitude. A hotspot for the genus is eastern Andalusia (provinces of Jaén and Córdoba), comprising several species in a narrow strip (Delicado *et al*., 2015; Boulaassafer *et al*., 2021; Miller, 2021) (Fig. 1).

In the present article, we describe a new species of the genus *Corrosella* from the province of Cádiz in Andalusia, located several kilometres to the west of the known records of this genus. The microscopic pictures of the corroded area in the upper whorls suggest that the characteristic corrosion present in this genus may be caused by burrowing organisms.

Material and methods

Specimen collection and morphological analysis

The type locality was visited in June 2023. To obtain the material, stones were lifted and sediment was collected from the spring and later washed with the use of sieves of different mesh (2.0, 1.0 and 0.25 mm). Shells were separated with the help of a brush, and cleaned with tap water.

Animals were dissected by submerging them in 80 % ethanol. The shell was carefully broken with the help of a microscope slide. The mantle was withdrawn with the help of two entomology pins in order to expose

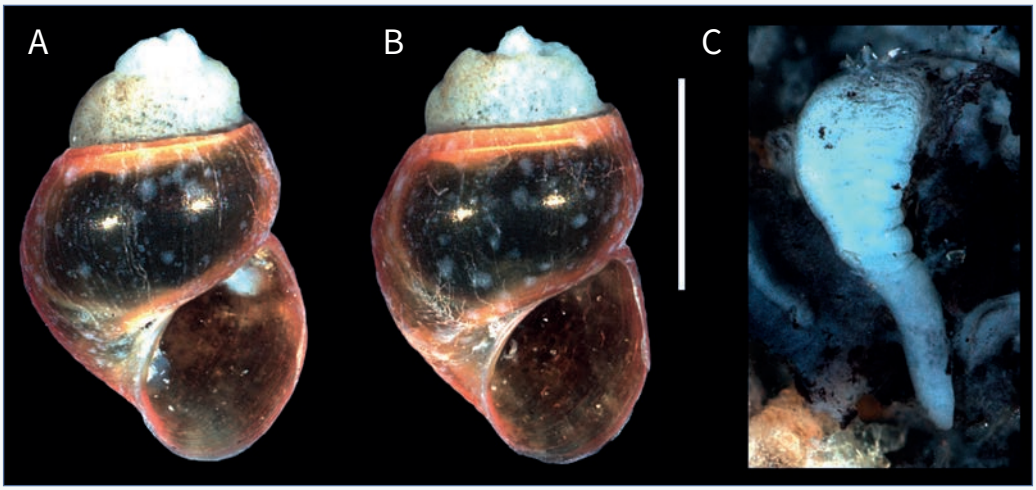

FIGURE 2. A-B: Anatomized specimens of *Corrosella piti* sp. nov. conserved in ethanol. **C:** Detail of the penis. Scale: 1 mm (A-B).

A-B: Espècimens anatomitzats de *Corrosella piti* sp. nov. conservats en etanol. **C:** Detall del penis. Escala: 1 mm (A-B),

the penis. Anatomical parts and anatomized specimen pictures were obtained with a Leica M205C microscope, with a Leica DMC5400 camera attached.

Type specimens were photographed under a trinocular Nexius Zoom NM1903–S stereomicroscope with a Euromex CMEX–10PRO camera. Empty shells representing the type material were mounted on an aluminium stub for scanning electronic microscopy (SEM) to reveal their microsculpture. SEM images were made without coating in a JEOL JSM–6480 LV using low vacuum, 10 KV voltage and 30 Pa pressure.

Abbreviations: Naturalis: Naturalis Biodiversity Center (Leiden); SEM: Scanning Electron Microscopy; a.s.l.: above sea level; s: shell.

DNA extraction, PCR amplification and sequencing

DNA was isolated from the foot tissue of two individuals of the new springsnails preserved in ethanol, using E.Z.N., A Mollusc DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. DNA sample integrity was checked by a horizontal electrophoresis in a 1% agarose gel, and subsequently stored at -20º C. The mitochondrial cytochrome c oxidase subunit I (COI) gene fragment was amplified by polymerase chain reaction (PCR) in a total volume of 40 µL, using the universal primers LCO1490 (Folmer et al., 1994) and COR722 (Davis *et al.*, 1998). The reaction mixture contained 2.5 µL template DNA, 2.5 µl of 25 mM MgCl2, 4 µl of 2.5 mM dNTPs, 1 µl of 10 µM primers, 0.15 µL Taq polymerase (GoTaq® G2 Flexi DNA Polymerase 5U/µl) and 8 µL of 5×Buffer GoTaq® Promega (1x final concentration). PCR conditions consisted of an initial denaturation step of 95° C for 4 min, followed by 35 amplification cycles (95° C for 45 s, 48° C for 45 s, 72° C for 30 s), and a final elongation step at 72° C for 7 min. A horizontal electrophoresis in a 2% agarose gel with 0.05 µl/ml of SimplySafe™ (EURx Ltd. 80–297 Gdańsk Poland) was performed with the PCR products, and later purified with Agarose-Out DNA Purification Kit (EURx Ltd. 80–297 Gdańsk Poland), following the manufacturer's instructions. Finally, the samples were sent for forward and reverse sequencing to MACROGEN (Amsterdam, The Netherlands), using standard Sanger sequencing method (Sanger & Coulson, 1975).

Genetic analysis

The forward and reverse sequences obtained by Sanger sequencing were edited for quality trimming, primer removal and manual correction to check any possible wrong base calling using Geneious Prime 2022.2.2 (https://www.geneious.com) and then aligned using ClustalW under default parameters. After alignment and corrections, a consensus sequence was generated with the default parameters. A preliminary genetic species identification was attempted using nBlast implemented in Geneious Prime using the default

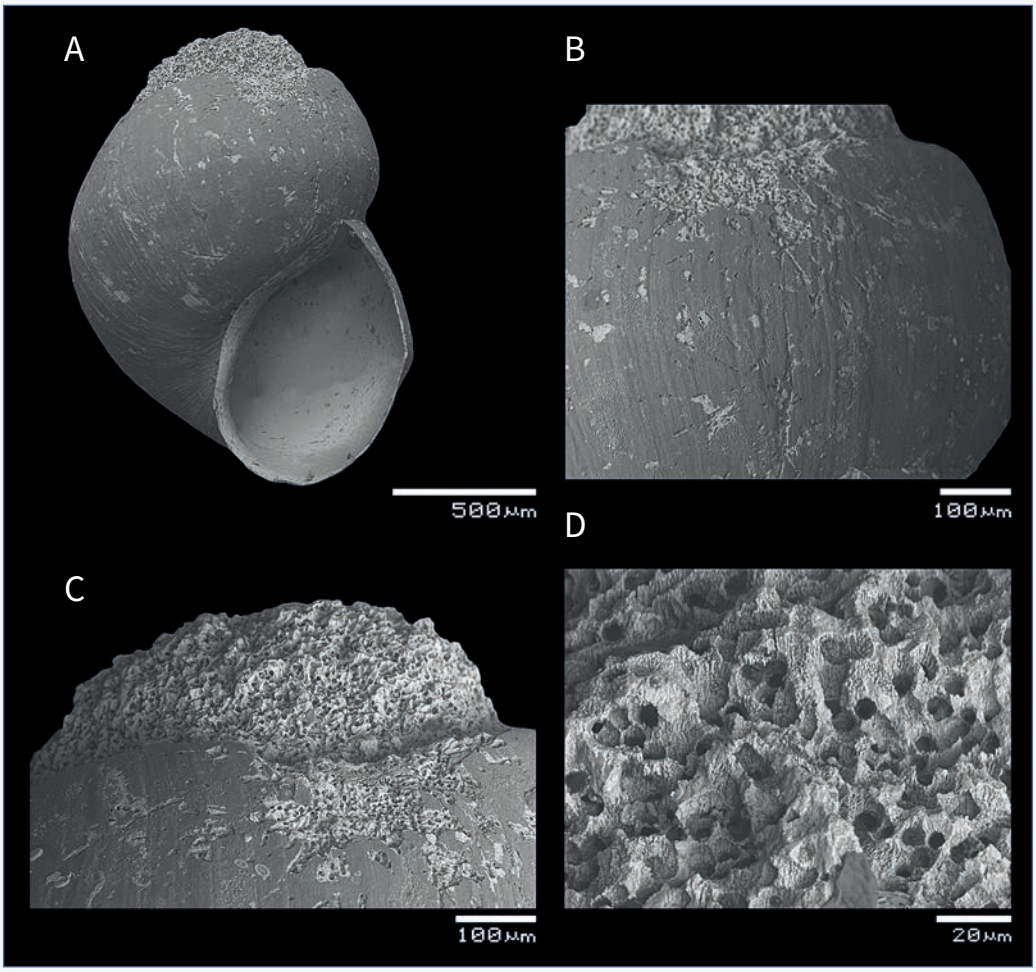

FIGURE 3. SEM pictures of *Corrosella piti* sp. nov. shell from Algodonales (Cádiz, Spain). RMNH.MOL.350845. **A:** Shell; **B:** Teleoconch detail; **C-D:** Detailed aspect of the corroded area at the protoconch.

Imatges de conquilles de *Corrosella piti* sp. nov. de la localitat d'Algodonales (Cadis, Espanya) sota el microscopi electrònic d'escaneig. RMNH.MOL.350845. **A:** Conquilla; **B:** Detall de la teleoconquilla; **C-D:** Aspecte detallat de la part corroïda de la protoconquilla.

values to search in GenBank databases. A phylogenetic analysis was conducted using two new sequences of *Corrosella piti* sp. nov. and other 95 sequences from GenBank of the available Iberian and French *Corrosella* species plus one sequence of *Pseudamnicola subproductus* (Paladilhe, 1869) used as outgroup. Sequences were deposited in GenBank with the accession numbers PP667386 and PP667387.

The test software IQ-TREE v2.3.1. (Minh *et al*., 2020) was used to predict the nucleotide substitution model showing the best BIC scores deploying ModelFinder options (Kalyaanamoorthy *et al*., 2017). A Maximum Likelihood tree was performed in IQ-TREE v2.0 using Ultrafast Bootstrap options (100,000 bootstrap replicates) (Minh *et al*., 2013) and a search was conducted for the best scoring tree using the Hasegawa-Kishino-Yano model (HKY+F+G4).

Results

Systematics

Family Hydrobiidae Stimpson, 1865

Genus *Corrosella* Boeters, 1970

Type species: *Corrosella falkneri* Boeters, 1970 (by original designation)

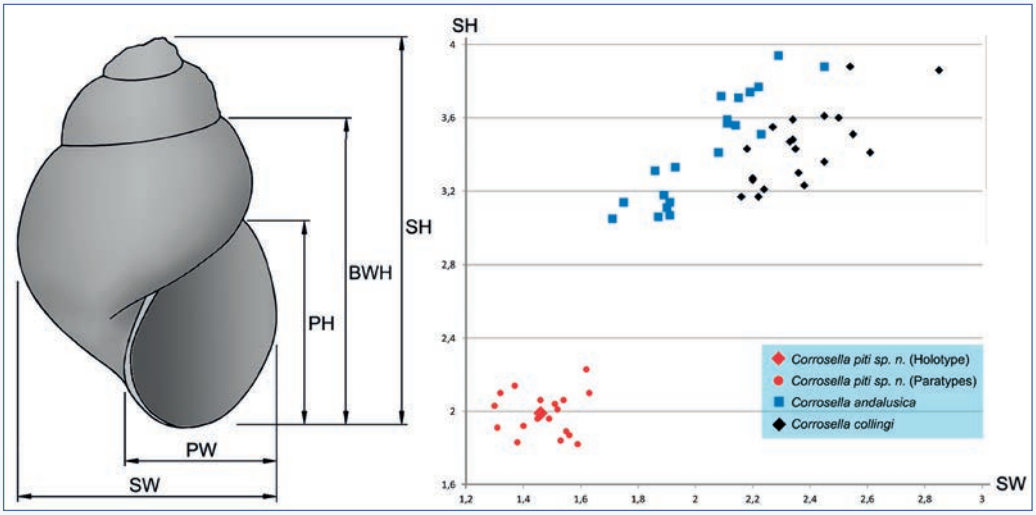

FIGURE 4. A: Schematic shell measurements used in this work. **B:** Graphic showing shell measurements (SH vs SW) of *Corrosella piti* sp. nov. compared to the closest representatives in the genus: *Corrosella collingi* from the type locality, and *Corrosella andalusica* from Fuente del Piojo (Almenidilla, Córdoba). A total of 20 specimens were measured for each species. SH: shell height; SW: shell width; BWH: body whorl height; PH: aperture height; PW: aperture width.

A: Representació esquemàtica de les mesures de la conquilla emprades en aquest treball. **B:** Gràfic mostrant les mesures de les conquilles (amplada vs. alçada) de *Corrosella piti* sp. nov. comparada amb les espècies més properes del gènere: *Corrosella collingi* de la localitat tipus, i *Corrosella andalusica* de la Fuente del Piojo (Almenidilla, Córdoba). Es van mesurar un total de 20 espècimens de cada espècie. SH: altura de la conquilla; SW: amplada de la conquilla; BWH; altura de la darrera volta; PH: altura de l'apertura; PW: amplada de l'apertura.

Corrosella piti sp. nov.

(Figs. 1-3)

Material. Holotype RMNH.MOL.350844, Naturalis. Paratypes: 3 s. RMNH.MOL.350845. 5 s. SQS coll. 5 s. PG coll. 12 s. JFMA 1056-20230615 coll.

Type locality. Fuente Cabera, Algodonales, Arroyo del Batán, Guadalete River basin (Cádiz province, Spain), 405 m a.s.l. 30S X286871 Y4084628 JFMA *leg.* 4/6/2023 (Fig. 4).

Etymology. Refers to the name of the mythological witch "*Piti*", who is burnt during the Carnival of Cádiz as the final act of this festival.

Description. The ovate shell is reddish-brown with 2–4½ slightly convex, regularly growing whorls, and deep sutures. The surface is glossy. Teleoconch microsculpture formed by growth lines (Fig. 3B). The aperture is pyriform-angled at the top, and the outer lip is straight from lateral view, slightly sinuated at the top (Fig. 3A). The umbilicus is closed. In most of the specimens, only the 1½ last whorls are conserved, as the protoconch and initial whorls are largely to totally corroded (Fig. 3). The microsculpture of the corroded area is formed by numerous small holes (Figs. 3C-3D). Animal has a dark brown mantle with a pattern of clear dots (Fig. 2).

Anatomy. The penis is long with a broad basis and a slender distal half (Fig. 2C).

Dimensions. Shell height of 1.82–2.23 mm, and diameter of 1.30–1.63 mm. The aperture has dimensions of 0.96–1.27 mm height and 0.74–0.91 mm in diameter. See Table 1 and Fig. 4.

Habitat. The species was found in a spring that runs without any human-mediated structure. There are two points where the water flows out of the calcareous rocks: one under a fig-tree, and another within the vegetation, just 15 metres apart, both barely visible (Fig. 5). Animals were found only at these two points and in the small stream formed where the waters from the two points join. Downstream, in a concrete canal where the water is used for field irrigation, no shells were found. Search in the vicinities (Batán main stream) also yielded negative results for the species. All specimens were found on rocks and under small stones in the initial part of the spring (Figs. 5C-D).

Differentiating characteristics. *Corrosella andalusica* (Delicado, Machordom & Ramos, 2012), *Corrosella bareai*

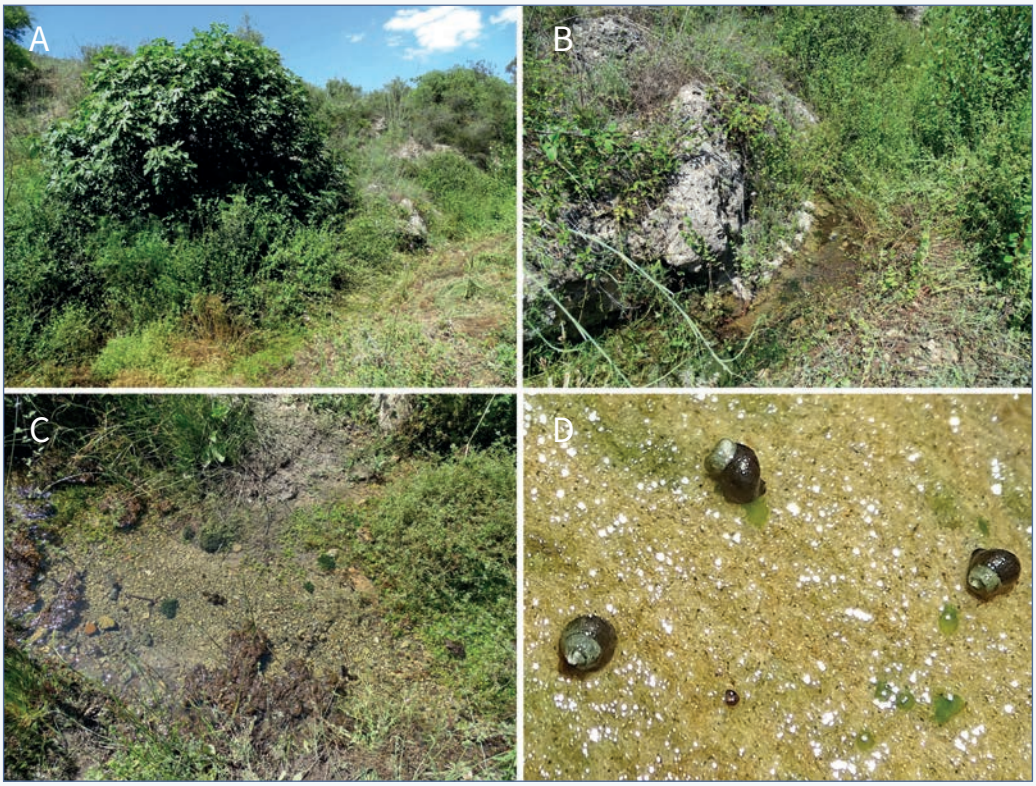

FIGURE 5. Habitats where *Corrosella piti* sp. nov. was found. **A-C:** Different views of the type locality; **D:** Live specimens on top of a rock.

Hàbitats on es va localitzar *Corrosella piti* sp. nov. **A-C:** Diferents vistes de la localitat tipus; **D:** Espècimens vius sobre una roca.

		S. Height SH	S. Width SW	BWH BWH	Peristome height PH	Peristome width PW
Corrosella piti sp. nov. (n=20)	**HOLOTYPE**	**1.99**	**1.46**	**1.84**	**1.11**	**0.83**
	min	1.82	1.30	1.67	0.96	0.74
	max	2.23	1.63	2.09	1.27	0.91
	mean	1.89	1.47	1.82	1.12	0.83
	st.dev.	0.114	0.100	0.096	0.074	0.049

TABLE 1. Shell measurements of *Corrosella piti* sp. nov. from Algodonales (Cádiz, Spain). SH: shell height; SW: shell width; BWH: body whorl height; PH: aperture height; PW: aperture width. A total of 20 specimens were measured.

Mesures de les conquilles de *Corrosella piti* sp. nov. de la localitat d'Algodonales (Cadis, Espanya). SH: altura de la conquilla; SW: amplada de la conquilla; BWH; altura de la darrera volta; PH: altura de l'apertura; PW: amplada de l'apertura. Es van mesurar un total de 20 espècimens.

(Delicado, Machordom & Ramos, 2012), *Corrosella collingi* (Boeters, Girardi & Knebelsberger, 2015), *Corrosella falkneri* Boeters, 1970, *Corrosella hydrobiopsis* (Boeters, 1999), *Corrosella iruritai* (Delicado, Machordom & Ramos, 2012), *Corrosella luisi* (Boeters, 1984), *Corrosella manueli* (Delicado, Machordom & Ramos, 2012), and *Corrosella marisolae* (Delicado, Machordom & Ramos, 2012) which also occur in southern Spain, are all typically larger, about 3 mm in height. In addition, other species are elongated/conical, or nearly cylindrical, like *C. falkneri*. On average, the degree of corrosion in the initial whorls is much more developed in the new species.

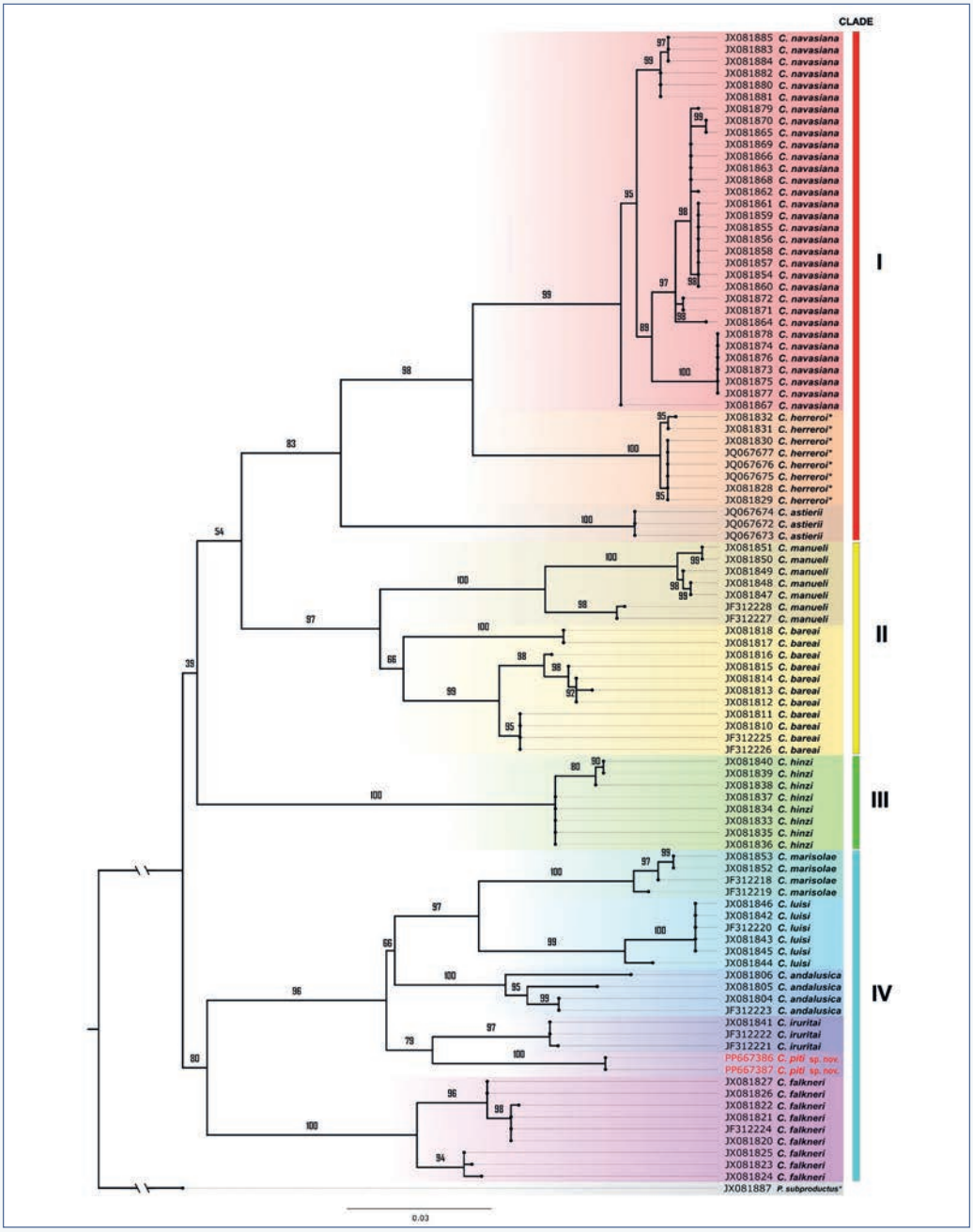

FIGURE 6. Maximum likelihood phylogeny inferred using the COI dataset in IQ-TREE. Ultrafast bootstrap values are provided at relevant nodes. GenBank accessions numbers are listed adjacent to each scientific name. The new sequences are highlighted in red. Names marked with (*) refer to names that have been updated according to the current accepted taxonomy.

Filogènia de màxima versemblança inferida usant una base de seqüències de COI en IQ-TREE. Valors de ultrafast bootstrap donats per nodes rellevants. Es llisten els números d'accés de GenBank al costat de cada nom científic. Les noves seqüències són ressaltades en vermell. Els noms marcats amb asterisc (*) es refereixen a noms que han estat actualitzats d'acord amb la taxonomia més actualitzada.

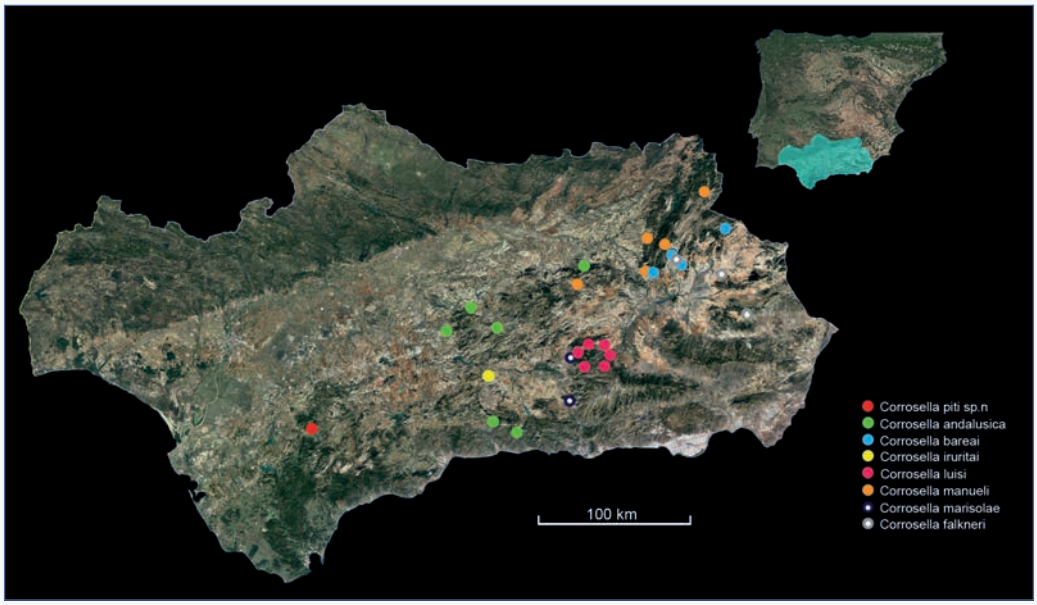

FIGURE 7. Map of Andalusia showing all the known localities for the different species of the genus *Corrosella* in this region, including *Corrosella piti* sp. nov.

Mapa d'Andalusia mostrant les localitats conegudes per les diferents espècies del gènere *Corrosella*, incloent *Corrosella piti* sp. nov.

In frontal view in most of the specimens of *Corrosella piti* sp. nov. a marked angulation of nearly 135° can be seen at the outer lip (Fig. 1B), which has not been reported in any other species of the genus.

Genetic results. The phylogenetic analysis revealed a similar topology and clades previously reported by Boulaassafer *et al.* (2021). Samples assigned to *C. piti* sp. nov. based on morphology formed a well-supported group with a high bootstrap support (i.e. 79 %) located within the Clade IV (Fig. 6). This clade was composed of *C. marisolae, C. luisi, C. andalusica, C. iruritai, C. falkneri* and *C. piti* sp. nov., all of them distributed throughout Andalusia (Fig. 7). The sequence divergence of COI for *C. piti* sp. nov. and the other Iberian congeners ranged between 10.89 % with *C. herreroi* and 4.45 % with *C. iruritai*, being this last one the genetically closest species to *C. piti* sp. nov. (Table 2).

Discussion

Corrosella Boeters, 1970 is a genus within the family Hydrobiidae, with representatives only in western Mediterranean areas, with a maximum of species diversity in eastern Andalusia (Fig. 7). It was separated from the closely-related genus *Pseudamnicola* Paulucci, 1878 on the basis of some differential traits, mainly of shell morphology and anatomy of the genitalia (Boeters, 1970), later confirmed by molecular analyses (Delicado *et al.*, 2015). One of the main characteristics is the corroded upper part of the shell, usually resulting in loss of the protoconch and part of the initial whorls of the teleoconch. Remarkably, this characteristic is not mentioned in Boeters' diagnosis of the genus. Boeters *et al.* (2015) noted that the corrosion does not seem to be related to environmental parameters, as shells from other prosobranch genera and families living sympatrically with *Corrosella* do not show such corrosion. *Corrosella piti* sp. nov. displays this characteristic perfectly, in an extreme way, as typically the uppermost whorls have disappeared, with one heavily corroded teleoconch whorl remaining and external corrosion present even on the other 1–1.5 teleoconch whorl(s).

Corrosion of calcareous shells in springs within limestone rocks is not easily explained. Falniowski *et al.* (2021) suggested that the corrosion in some representatives of this family (such as *Achaiohydrobia moreana* Hofman & Grego, 2021) may be due to sulphide components in the water. However, this cannot explain the

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*P. subproductus*	-	0	0	0	0	0	0	0	0	0	0	0	0
2	*C. navasiana*	14.35	-	0	0	0	0	0	0	0	0	0	0	0
3	*C. marisolae*	14.93	11.34	-	0	0	0	0	0	0	0	0	0	0
4	*C. manueli*	14.59	9.14	10.30	-	0	0	0	0	0	0	0	0	0
5	*C. luisi*	15.55	11.63	6.17	11.36	-	0	0	0	0	0	0	0	0
6	*C. iruritai*	14.29	9.87	6.50	9.36	6.59	-	0	0	0	0	0	0	0
7	*C. hinzi*	14.63	8.79	10.15	9.34	10.73	8.81	-	0	0	0	0	0	0
8	*C. herreroi*	14.54	6.18	10.69	9.06	11.93	9.44	9.08	-	0	0	0	0	0
9	*C. falkneri*	14.27	9.35	9.53	8.68	9.96	7.82	8.36	8.86	-	0	0	0	0
10	*C. bareai*	14.29	8.81	9.71	6.63	9.96	7.99	8.77	8.08	8.34	-	0	0	0
11	*C. andalusica*	13.94	10.26	6.71	9.98	6.86	5.48	8.93	10.55	8.54	9.03	-	0	0
12	*C. astierii*	14.74	8.74	10.44	9.60	10.69	8.11	9.75	7.45	8.99	8.13	9.31	-	0
13	*C. piti* sp.nov.	14.44	10.66	7.61	10.29	7.17	4.45	10.54	10.89	8.65	9.07	5.82	9.63	-

TABLE 2. Genetic divergence matrix for the species examined based on the COI gene sequence.

Matriu de divergència genètica per les espècies examinades, basada en la seqüència del gen COI.

situation at Algodonales as other species in the same spring show no corrosion at all. The same applies to other localities in Iberia. Indeed, the microscopic morphology revealed in our shells (Fig. 3C-D) does not match with that observed by Falniowsky *et al.* (2021), pointing to a peculiarity of the genus *Corrosella* that has thus far not been understood.

Our SEM images (Fig. 3) clearly show corrosion in *Corrosella piti* sp. nov. to be caused by burrowing organisms. In the upper whorls the burrowing is so intense that most of the shell has completely disappeared or has no outer surface remaining (Fig. 3C). Small parts of the final whorl are similarly affected, albeit over most of the surface only burrows of individual organisms are seen (Fig. 3B). The boreholes are slightly less than 10 microns wide (Fig. 3D), but burrows of hundreds of individuals are present within a single shell. It is not evident what organism may have created the burrows. Further investigation is ongoing as other species of *Corrosella* have similar boreholes, called "erosion" in Delicado *et al.* (2012), and "detailed microsculpture of protoconch" in Boulaassafer *et al.* (2012).

Corrosella and *Pseudamnicola* seem to have different biological and biogeographical features. The first genus is restricted to headwaters at high altitudes (usually over 400 m a.s.l.). This has probably limited their dispersal capabilities, as the species live in habitats with rather stable conditions and some degree of isolation. In some areas the limited genetic connection between populations may have favoured the high degree of speciation by allopatry. Contrary to this, *Pseudamnicola* usually lives in the lower part of hydrological basins, in more unstable habitats, but much larger and more interconnected, favouring long-distance dispersion, generally resulting in wider distribution ranges (Delicado *et al*., 2015; Miller, 2021).

The genus *Corrosella* includes a total of 23 recognized species according to MolluscaBase, 16 of them in the Iberian Peninsula, with a hotspot in eastern Andalusia, in the provinces of Jaén, Málaga, and Granada (Delicado *et al*., 2012 & 2015; Boeters *et al*., 2015; Boulaassafer *et al.*, 2021). The discovery of *Corrosella piti* sp. nov. further enlarges the distribution range in Andalusia to the west, as the closest known populations of the genus are about 120–140 km to the east (Fig. 6), and also adds a new hydrological basin, the Guadalete River. Given the high degree of endemism in this genus, and the richness of Hydrobiidae species in the Iberian Peninsula, future research will probably provide new surprises in this genus, as suitable habitats are abundant in the western part of the Baetic Mountains.

Since *Corrosella* species mainly inhabit mountain-top springs, they are less likely to disperse, and more prone to extinction compared with those inhabiting lower elevations (Miller, 2021), so their habitats should be a priority for protection efforts, when considering

management of endemic species. Habitat loss due to water overexploitation and climatic change, added to the arrival of invasive species, can also push this and similar species to extinction.

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