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# New records of ostracods (Crustacea, Ostracoda) from groundwater habitats in Italy, with faunistic and biogeographical notes

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## Abstract

Groundwater ecosystems host highly specialised and frequently endemic faunas, yet their biodiversity remains poorly explored. Although Italy is recognised as a hotspot of subterranean diversity, knowledge of its stygobitic ostracods (i.e., species that are obligate inhabitants of subterranean waters) remains fragmentary. Here we report new data on ostracods collected from 15 natural caves, a mine and an aquifer across mainland Italy and Sicily between 2009 and 2018, with the aim of documenting new occurrence records from poorly investigated subterranean sites, discussing their taxonomic implications, and reassessing the diversity and endemism of Italian stygobitic ostracods in a broader European context. Nineteen taxa belonging to 12 genera and five families were identified, several of which are regarded as strictly stygobitic. These include representatives of *Mixtacandona* (Candonidae), among which several putative undescribed species of the *laisi-chappuisi* group were detected. Particularly noteworthy is the occurrence of *Mixtacandona* cf. *botosaneanui* in southern Italy: if its identity with the nominal species is confirmed, this record would considerably extend its known geographical range and provide the first description of the male, thereby adding important taxonomic information for a poorly known subterranean lineage. We also report a new record of *Typhlocypris* cf. *eremita* and specimens referable to *Pseudolimnocythere*, further expanding the known distribution of these genera within Italian subterranean habitats. Although some specimens could be assigned only tentatively because well-preserved adults were scarce, the new records substantially refine current knowledge of Italian groundwater ostracod diversity and distribution patterns at both regional and European scales. An updated checklist raises the number of Italian stygobitic ostracods to more than 30 taxa, representing approximately 30% of the currently known European groundwater ostracod diversity. This proportion is remarkable given the limited extent of Italian territory, and the high frequency of endemic and potentially undescribed species further highlights the Italian peninsula and its islands as important centres of diversification for subterranean ostracods. These results emphasize the need for continued biospeleological surveys and integrative taxonomic approaches combining morphology and molecular data to resolve species boundaries, phylogenetic relationships and colonization histories in subterranean lineages.

**Key words:** groundwater biodiversity; subterranean ecosystems; stygofauna; endemism; range extension.

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## Introduction

Unexplored, diverse, dark and definitely ecologically unique, terms commonly used to describe the deep sea, the planet's largest biome (Ramirez-Llodra *et al*., 2010), apply equally well to groundwater ecosystems, the largest aquatic domain beneath Earth's continents (Ficetola *et al*., 2019). Both deep sea and subterranean aquatic habitats remain among the least-explored realms on the planet, and substantial gaps persist in our understanding of their biodiversity, biogeography and ecology**,** particularly for meiofaunal groups.

Subterranean waters comprise a mosaic of aquatic ecosystems whose physical, chemical, and hydrogeological characteristics vary with substrate type, depth, recharge regime, and local climate (Gibert *et al*., 1994; Griebler and Avramov, 2015; Culver and Pipan, 2019). These systems sustain and connect surface waters while supporting a rich assemblage of specialised organisms. Groundwater ecosystems are increasingly recognised as complex and ecologically significant habitats rather than mere conduits for water flow. Nevertheless, subterranean habitats, groundwater-dependent ecosystems (GDEs), and their typically low-dispersal faunas face widespread threats (Boulton, 2020). Conservation efforts remain largely localized and limited to a few initiatives, leaving many regions and taxa insufficiently protected and conservation actions poorly coordinated at regional and global scales (Mammola *et al*., 2019, 2024; Iannella *et al*., 2021; Rohde *et al*., 2024; Saccò *et al*., 2024). The limited dispersal ability and high levels of endemism typical of groundwater organisms further increase their vulnerability to habitat degradation, groundwater abstraction, pollution and climate change.

Groundwater organisms, collectively referred to as stygofauna, include stygobitic species restricted to groundwater, stygophilic species occurring in both surface and groundwater habitats, and stygoxenic species that enter groundwater only occasionally (Gibert *et al*., 1994). Subterranean habitats, and caves in particular, are widely recognised as natural laboratories for investigating adaptive processes under extreme ecological constraints (Mammola, 2019). Aquatic subterranean organisms often display marked adaptations to life in darkness, including reduced visual systems, depigmentation and enhanced chemo- and mechanoreception (Moldovan, 2018; Hanken, 2025). Many groundwater organisms are characterized by slow growth and long life cycles (Becher *et al*., 2022), as well as by the ability to persist in nutrient-poor environments (Howarth and Moldovan, 2018). In some crustaceans, a high tolerance to hypoxic conditions has also been documented (Malard and Hervant, 1999). The strong genetic and geographic isolation typical of subterranean habitats further promotes speciation processes (Juan *et al*., 2010; Pipan and Culver, 2012), contributing to the remarkably high levels of endemism observed in many stygobitic lineages (Danielopol *et al*., 2000). Consequently, subterranean ecosystems provide valuable systems for investigating patterns of diversification, historical biogeography and lineage persistence in fragmented landscapes.

Among subterranean invertebrates, crustaceans represent the most diverse and extensively studied groups. Substantial ecological, taxonomic, and biogeographic information has been accumulated for taxa that frequently dominate groundwater assemblages, such as amphipods, copepods, and isopods. In contrast, knowledge of ostracods remains comparatively limited despite their frequent occurrence in subterranean habitats and their recognised value as model organisms in evolutionary, ecological, and biogeographical research (Martens and Horne, 2000). Although extensive radiations of hypogean ostracods have been documented (Halse, 2018; Hotèkpo *et al*., 2024; Issartel and Marmonier, 2025), recent syntheses and regional surveys continue to reveal major gaps in distributional data, taxonomic resolution, and ecological information across many regions (Knight and Mori, 2022; Mori *et al*., 2025). In particular, the distribution limits of several genera remain poorly defined, and new faunistic data frequently result in significant range extensions or the recognition of previously overlooked lineages.

Approximately one fifth of the Italian territory is composed of soluble rocks, within which numerous natural caves have developed. More than 50,000 natural cavities have been documented across the peninsula and its surrounding islands (Sanna *et al*., 2023). In addition to caves, extensive drainage networks are present, supporting a wide range of subterranean systems and GDEs. These habitats sustain rich assemblages of stygofauna, making Italy a recognised hotspot of subterranean biodiversity (Pesce, 1985; Stoch, 2001; Malard *et al*., 2009). Within this setting, several studies have documented the occurrence of recent ostracods in the hyporheic zones of rivers (Bruno *et al*., 2012; Rossetti *et al*., 2025b), springs (Rossetti *et al*., 2005, 2020, 2022; Pieri *et al*., 2007; Bottazzi *et al*., 2008, 2011; Stoch *et al*., 2011), wells (Karanovic and Pesce, 2000, 2001), continental caves (Klie, 1938; Wagenleitner, 1990; Stoch, 1993a, 1993b, 1997, 2008; Stoch and Dolce, 1994; Peterson *et al*., 2013; Mazzini *et al*., 2017) and in various groundwater habitats, such as unsaturated and saturated zones of karstic aquifers (Stoch, 2004, 2017; Galassi *et al*., 2009). However, the available information remains geographically uneven, and several karst districts and aquifer systems still lack detailed ostracod surveys.

This study provides new data on the occurrence of ostracods in Italian subterranean environments, mainly natural caves, and addresses their biogeographic significance. It also presents an updated checklist of the ostracod species regarded as stygobionts in the country. Specifically, we aim to: i) document new occurrence records from poorly investigated subterranean sites; ii) discuss their taxonomic implications; iii) evaluate their contribution to current knowledge of distribution patterns; and iv) reassess the diversity and endemism of Italian stygobitic ostracods in a broader European context.

## Methods

Sixty-five samples containing ostracods were collected from 15 natural caves, a mine and an aquifer (Figs. 1 and 2) during several sampling campaigns conducted between 2009 and 2018, using hand nets or syringes. All the ostracods analysed in this study were collected from lentic or slow-flowing waterbodies, such as cave pools, including rimstone pools, except for those from the Teatro greco-romano, which were obtained from shallow groundwater seepage

**Fig. 1.** Map of Italy showing location of sampling sites (site codes as in Tab. 2): 01 Grotta Cava di Papipano; 02 Grotta Grava X; 03 Grotta Pertosa; 04 Grotta Grave Grubbo; 05 Grotta Palummaro; 06 Grotta Abisso del Gatto; 07 Grotta Conza; 08 Grotta Entella; 09 Grotta Totino; 10 Grotta Monte Conca; 11 Miniera di Streppenosa; 12 Grotta Sette Calzette; 13 Grotta Bremi; 14 Grotta Burritta; 15 Grotta Monte Santa Venera; 16 Grotta Villasmundo; 17 Teatro greco-romano.

emerging in one of the lateral access passages (western parodos), probably related to the subterranean course of the Amenano River. Details of the sampling sites are provided in the Supplementary Material (Tab. S1).

After sorting, ostracods were preserved in approximately 70% ethanol. Soft parts were dissected in glycerine and mounted on sealed slides, whereas valves were stored dry in micropaleontological slides. Both valves and soft parts were examined for identification, to the species level whenever feasible, especially when adults or late-stage individuals were present in numbers sufficient for detailed taxonomic analysis. Severely damaged material and early larval stages were classified as unidentified ostracods. For selected taxa, valves and carapaces were further examined with a ZEISS EVO MA10 scanning electron microscope (SEM) or photographed under a compound microscope. Soft parts were illustrated with the aid of a camera lucida or captured through photographic imaging. The chaetotaxy of the soft parts follows Meisch (2000), and the taxonomic arrangement is based on Meisch *et al.* (2024). Each dissected specimen was assigned an alphanumeric code consisting of the letters (GR) followed by a number. All material

**Fig. 2.** Images of selected groundwater habitats sampled in this study. **A)** Grotta Grave Grubbo. **B)** Grotta Palummaro (courtesy of G. Ragone). **C)** Grotta Conza. **D)** Grotta Entella. **E)** Grotta Totino. **F)** Miniera di Streppenosa. **G)** Grotta Sette Calzette. **H)** Grotta Bremi. **I)** Teatro greco-romano.

examined in this study is stored at the Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy.

Abbreviations used in the text and figures are as follows:

*Valves*

Cp: carapace; RV: right valve; LV: left valve; L: length, H: height; iv: internal view; ev: external view; lv: lateral view, vv: ventral view.

*Soft parts*

A1: antennule; A2: antenna; Md: Mandible; Mx1: maxillule; T1: first thoracopod (maxilliped); T2: second thoracopod (walking leg); T3: third thoracopod (cleaning leg); CR: caudal ramus; exo: exopodite on A2; a: seta on A1; ya: aesthetasc on A1; t1-3 and z1-3: setae on A2; Y and y1-3: aesthetascs on A2; G1-3, GM, Gm: claws on A2; f, g and h1-3: setae and claws on T2 and T3; d1-2 and dp: setae on T3; Sa and Sp: anterior and posterior setae on CR; Ga and Gp: anterior and posterior claws on CR.

Valve and carapace outlines in lateral view were obtained from scanning electron microscopy (SEM) images and from published sources. Images were processed in GIMP (GNU Image Manipulation Program, https://www.gimp.org) to extract valve contours, which were then saved as TPS files using TPSutil32 v. 1.76 and digitized with TPSdig v. 2.20 (Rohlf, 2009). Superimposition of valve outlines was carried out with Morphomatica 1.6.0.1 (Linhart *et al*., 2007), a software specifically developed for the geometric approximation of ostracod specimens using an adapted B-splines algorithm, applying the normalized area mode to minimize size-related effects.

## Results

### Taxonomic account

A total of 19 ostracod taxa were identified, belonging to 12 genera in five families (four within the superfamily Cypridoidea and one within the superfamily Cytheroidea). The subfamilies Candoninae (8 taxa) and Trapezicandoninae (5 taxa) were the most diverse (Tab. 1). The maximum number of taxa recorded at a single site was four. The most frequently occurring species were *Cypria ophtalmica* (Jurine, 1820) (present at four sites) and *Neglecandona neglecta* (Sars, 1887) (present at three sites) (Tab. 2), both common and widely distributed species occurring in a broad range of aquatic habitats.

### Notes on the taxonomy of selected taxa

**Candoninae indet.** (Figs. 3 and 4)

### Material investigated

One adult ♀ (GR806, L = 495 µm), soft parts dissected in glycerine in a sealed slide, valves stored dry in a micropaleontological slide, RV used for SEM (LV slightly damaged), collected on 29 August 2015 by R.G. and M.T.S. from Grotta Palummaro; one adult ♀? (GR849, L = 490 µm), Cp used for SEM (with damaged soft parts), and 5 empty carapaces of juveniles preserved in alcohol, collected on 2 August 2010 by R.G., A.I. and M.T.S. from Grotta Bremi.

### *Diagnosis*

Cp rectangular in lv, valve surface conspicuously pitted, L c. 0.5 mm, greatest W situated about at the anterior third. A1 seven-segmented, A2 exo with one long and two short setae, apical claws on the terminal segment of Md-palp "normally" built, CR short and stout.

### *Remarks*

The combination of valve morphology and appendage chaetotaxy does not allow a confident assignment to any currently recognized genus within the Candoninae. Pending the availability of additional material, we therefore retain a conservative taxonomic assignment.

**Tab. 1.** Taxonomic arrangement of ostracod taxa found in this study.

- Class Ostracoda Latreille, 1802
- Subclass Podocopa Sars, 1866
- Order Podocopida Sars, 1866
- Suborder Cypridocopina Baird, 1845
  - Superfamily Cypridoidea Baird, 1845
    - Family Candonidae Kaufmann, 1900
    - Subfamily Candoninae Kaufmann, 1900
      - Genus *Fabaeformiscandona* Krstić, 1972
        - *Fabaeformiscandona* gr. *fabaeformis*
        - *Fabaeformiscandona* sp.
      - Genus *Neglecandona* Krstić, 2007
        - *Neglecandona neglecta* (Sars, 1887)
      - Genus *Pseudocandona* Kaufmann, 1900
        - *Pseudocandona* sp. 1
        - *Pseudocandona* sp. 2
        - *Pseudocandona sarsi* (Hartwig, 1899)
      - Genus *Typhlocypris* Vejdovský, 1882
        - *Typhlocypris* cf. *eremita* (Vejdovský, 1882)
      - Candoninae indet.
    - Subfamily Trapezicandoninae Karanovic, 2007
      - Genus *Mixtacandona* Klie, 1938
        - *Mixtacandona* cf. *botosaneanui* Danielopol, 1973
        - *Mixtacandona* gr. *laisi-chappuisi* sp. 1
        - *Mixtacandona* gr. *laisi-chappuisi* sp. 2
        - *Mixtacandona* gr. *laisi-chappuisi* sp. 3
        - *Mixtacandona* gr. *laisi-chappuisi* juv.
    - Family Cyclocyprididae Kaufmann, 1900
      - Genus *Cypria* Zenker, 1854
        - *Cypria ophtalmica* (Jurine, 1820)
    - Family Cyprididae Baird, 1845
    - Subfamily Cypridopsinae Kaufmann, 1900
      - Tribe Cypridopsini Kaufmann, 1900
        - Genus *Cypridopsis* Brady, 1867
          - *Cypridopsis vidua* (O.F. Müller, 1776)
      - Tribe Potamocypridini Ghetti and McKenzie, 1981
        - Genus *Potamocypris* Brady, 1870
          - *Potamocypris fulva* (Brady, 1868)
    - Subfamily Eucypridinae Bronstein, 1947
      - Genus *Trajancypris* Martens, 1989
        - *Trajancypris clavata* (Baird, 1838)
    - Family Notodromadidae Kaufmann, 1900
    - Subfamily Notodromadinae Kaufmann, 1900
      - Genus *Notodromas* Lilljeborg, 1853
        - *Notodromas persica* Gurney, 1921
- Suborder Cytherocopina Baird, 1850
  - Superfamily Cytheroidea Baird, 1850
    - Family Loxoconchidae Sars, 1925
      - Genus *Pseudolimnocythere* Klie, 1938
        - *Pseudolimnocythere* sp.

## Genus *Typhlocypris* Vejdovský, 1882

(see Namiotko *et al.* 2014 for a diagnosis of the genus)

### *Typhlocypris* cf. *eremita (Vejdovský, 1882)* (Fig. 5)

Material investigated: several specimens collected on 17 August 2018 by FS from Grotta Cava di Papipano, two of which were an adult ♂ (GR844, L = 775 µm) and an adult ♀ (GR866, L = 797 µm) with soft parts dissected in glycerine in a sealed slide and valves used for SEM.

## Genus *Mixtacandona* Klie, 1938

(see Rossetti *et al.* 2025a for a diagnosis of the genus)

### *Mixtacandona* cf. *botosaneanui Danielopol, 1973*

(Figs. 6 to 8, Fig. 9A)

Material investigated: >20 adult specimens, of which one adult ♂ (GR793, L = 556 µm) and one adult ♀ (GR794, L = 518 µm) with soft parts dissected in glycerine in a sealed slide and valves used for SEM, and one adult ♀ (GR1106, L = 525 µm) used for SEM, collected by S.I. and E.R. on 3 June 2016 from Grotta Pertosa.

**Tab. 2.** List of ostracod taxa (header row) recorded in the investigated subterranean habitats. The left column reports site code, site name, sampling date(s), and number of analysed samples in parentheses.

| | *Fabaeformiscandona* gr. *fabaeformis* | *Fabaeformiscandona* sp. | *Neglecandona neglecta* | *Pseudocandona sarsi* | *Pseudocandona* sp. 1 | *Pseudocandona* sp. 2 | *Typhlocypris* cf. *eremita* | *Mixtacandona* cf. *botosaneanui* | *Mixtacandona* gr. *laisi-chappuisi* sp. 1 | *Mixtacandona* gr. *laisi-chappuisi* sp. 2 | *Mixtacandona* gr. *laisi-chappuisi* sp. 3 | *Mixtacandona* gr. *laisi-chappuisi* juv. | Candoninae indet. | *Cypria ophtalmica* | *Cypridopsis vidua* | *Potamocypris fulva* | *Trajancypris clavata* | *Notodromas persica* | *Pseudolimnocythere* sp. | Unidentified ostracods |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 Grotta Cava di Papipano 17 August 2018 (1) | | | | | | | ● | | | | | | | | | | | | | |
| 02 Grotta Grava X 03 June 2016 (1) | | | | | | | | | | | | | | | | ● | | | | |
| 03 Grotta Pertosa 03 June 2016 (1) | | | | | | | | ● | ● | | | | | | | | | | | |
| 04 Grotta Grave Grubbo 28 August 2015 (2) | | | | | | | | | | | | ● | | | | | | | | ● |
| 05 Grotta Palummaro 29 August 2015 (11) | | | ● | | ● | | | | | | ● | | ● | | | | | | | ● |
| 06 Grotta Abisso del Gatto 10 June 2017 (1) | | | ● | | | | | | | | | | | | | | | | | |
| 07 Grotta Conza 23 November 2009 (1) | | | | | | | | | | | | | | | | | ● | | | |
| Grotta Conza 31 August 2010 (2) | | | | | | | | | | | | | | | | | ● | | | ● |
| Grotta Conza 10 January 2011 (2) | | | | | | | | | | | | | | | | | ● | | | ● |
| Grotta Conza 26 October 2011 (1) | | | | | | | | | | | | | | | | | ● | | | |
| Grotta Conza 05 March 2012 (1) | | | | | | | | | | | | | | | | | ● | | | |
| Grotta Conza 22 October 2012 (1) | | | | | | | | | | | | | | | | | ● | | | |
| Grotta Conza 31 January 2014 (1) | | | | | | | | | | | | | | | | | ● | | | |
| 08 Grotta Entella 05 December 2013 (1) | | | | | | | | | | | | | | | | | | | | ● |
| Grotta Entella 05 January 2014 (1) | | | | | | | | | | | | | | | ● | | | | | |
| Grotta Entella 01 February 2014 (1) | | | | | | | | | | | | | | | | | | | | ● |
| Grotta Entella 05 April 2014 (1) | | | | | | | | | | | | | | | ● | | | | | |
| Grotta Entella 01 August 2014 (1) | | | | | | | | | | | | | | | ● | | | | | |
| 09 Grotta Totino 20 February 2016 (4) | | | | | | | | | | ● | | | | | | | | | | |
| Grotta Totino 06 March 2016 (1) | | | | | | | | | | ● | | | | | | | | | | |
| Grotta Totino 06 September 2016 (1) | | | | | | | | | | ● | | | | | | | | | | |
| 10 Grotta Monte Conca 22 May 2016 (4) | | | ● | | | | | | | | | | | | | | | | | ● |
| Grotta Monte Conca 01 July 2017 (1) | | | ● | | | | | | | | | | | | | | | | | |
| Grotta Monte Conca 02 September 2017 (2) | ● | | | | | ● | | | | | | | | | | | | | | |
| Grotta Monte Conca 08 October 2017 (7) | | | ● | | | | | | | | | | | | | | | ● | | ● |
| 11 Miniera di Streppenosa 09 August 2010 (1) | | | | | | | | | | | | | | ● | | | | | | |
| 12 Grotta Sette Calzette 12 June 2016 (1) | | | | | | | | | | | | | | ● | | | | | | |
| 13 Grotta Bremi 02 August 2010 (1) | | | | | | | | | | | | | ● | | | | | | | |
| 14 Grotta Burritta 05 August 2010 (1) | | ● | | | | | | | | | | | | | | | | | | |
| Grotta Burritta 16 November 2015 (2) | | | | | | | | | | | | | | | | | | | | ● |
| 15 Grotta Monte Santa Venera 14 November 2015 (4) | ● | | | | | | | | | | | | | ● | | | | | | ● |
| 16 Grotta Villasmundo 02 October 2017 (1) | | | | ● | | | | | | | | | | | | | | | | |
| 17 Teatro greco-romano 28 January 2016 (3) | | | | | | | | | | | | | | ● | | | | | ● | |

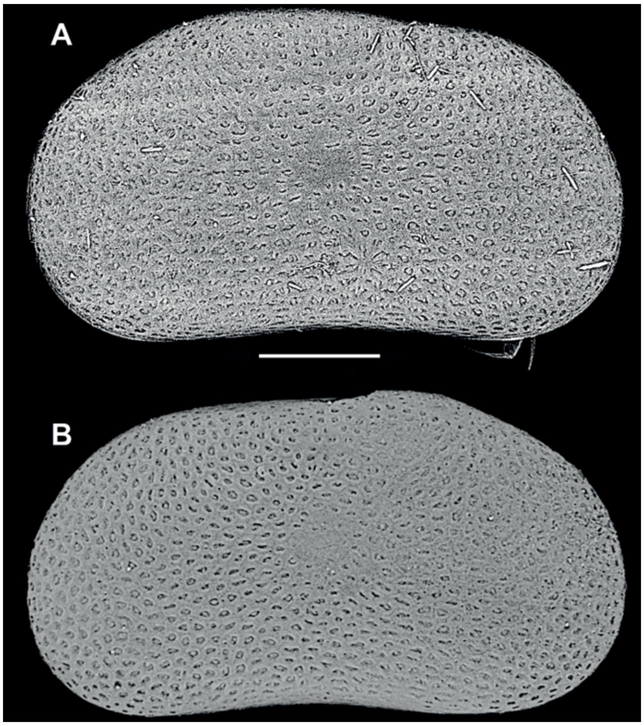


**Fig. 3.** Candoninae indet. **A)** Adult ♀? (GR849); Cp, left ev. **B)** Adult ♀ (GR806); RV ev. Scale bar: 100 µm.

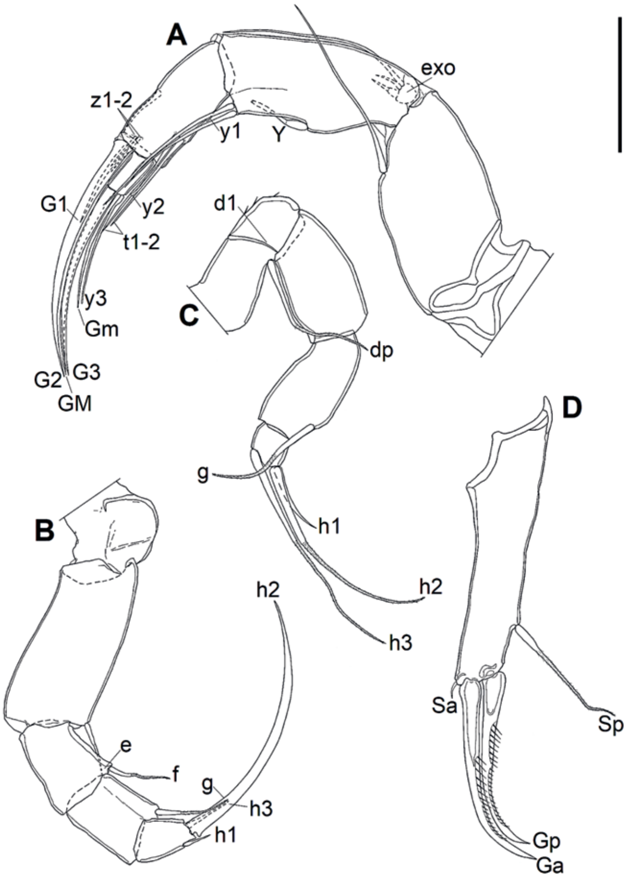


**Fig. 4.** Candoninae indet., adult ♀ (GR806). **A)** A2. **B)** T2. **C)** T3 (seta d2 possibly lost). **D)** CR. Scale bar: 50 µm.

## *Remarks*

The analysis of the soft parts and valves of ♀♀ of *Mixtacandona* cf. *botosaneanui* (Figs. 6 E-G,J, Fig. 8) confirms the close similarity of this species to *M. botosaneanui* Danielopol, 1973, originally described from a dozen specimens (adult females and juveniles) collected from a cave and a porous aquifer in Romania (Danielopol, 1973). This similarity is further supported by the marked overlap between the outline of an adult ♀ of *M. botosaneanui* from Danielopol (1982) and that of an adult ♀ of *M.* cf. *botosaneanui* from Grotta Pertosa (Fig. 9A).

## Abbreviated description of male

(Fig. 6 A-D,H,I, Fig. 7, Fig. 8)

Valve surface smooth; outline in lateral view subovate to reniform, dorsal margin slightly arched, ventral margin nearly straight; greatest height approximately at mid-length; anterior margin broadly rounded; posterior margin rounded, slightly narrower than anterior; inner lamella well developed; marginal zone clearly defined; no clear sexual dimorphism in valve outline; adductor muscle scar field a compact cluster. A2 exo with two short setae and one long seta reaching the distal margin of the first endopodal segment; first endopodal segment with a long, tri-

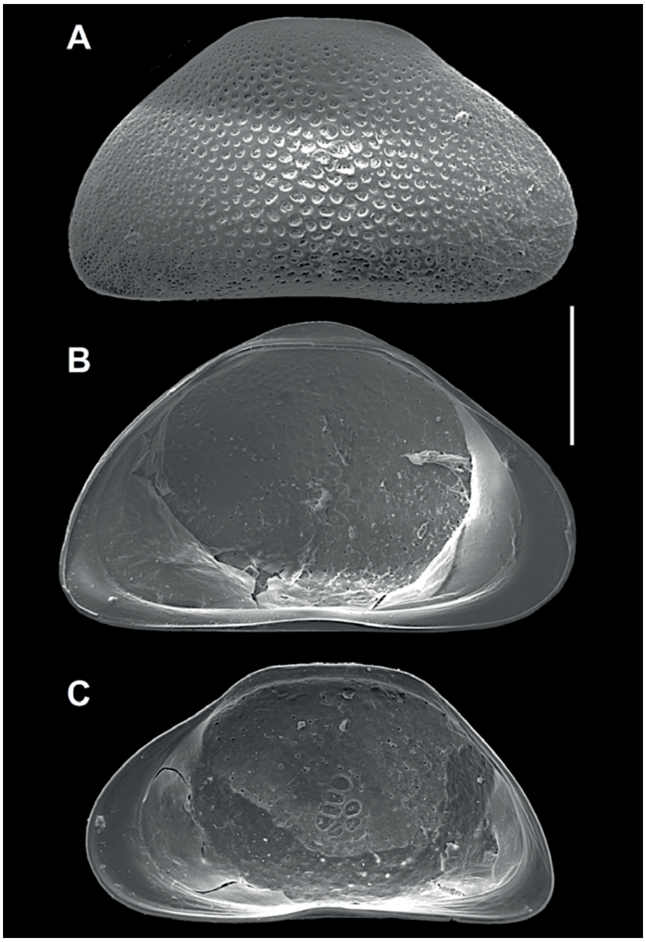


**Fig. 5.** *Typhlocypris* cf. *eremita*. **A,B)** Adult ♂ (GR844); RV ev. (**A**); LV iv (**B**). **C)** Adult ♀ (GR866); RV iv. Scale bar: 100 µm.

partite aesthetasc Y; second endopodal segment subdivided, with minute seta t1 and long seta t4, setae t2 and t3 transformed into bristles; setae z2 and z3 claw-like; seta z1 not observed; claws G1 and G3 subequal, long and robust; claw G2 slightly shorter than half the length of G1 and G3; terminal segment with claw GM reaching the tips of G1 and G3 and approximately 1.8× the length of Gm. T1 palps strongly asymmetrical, the right one more bumped and sinuous proximally than the left one, the latter with a weak expansion on the tapered distal part. T2 endopodite four-segmented; second and third segments each with an apical seta (f, g); terminal segment bearing a stout apical claw h2, approximately equal to the combined length of the first three segments, flanked by setae h1 and h3, the latter markedly reduced. T3 protopodite with setae d1 and d2 subequal, seta dp slightly longer; endopodite three-segmented, second segment partially subdivided; first endopodal segment without setae; second segment with a minute subapical seta g; third segment bearing three apical setae: h1 very small, h2 about as long as the distal segment, h3 roughly equal to the combined length of the first two endopodal segments. CR with seta Sp inserted at c. 3/4 of the posterior margin; seta Sa about half as long as Sp; claws Gp and Ga subequal in length. Zenker's organ elongate with 7 spinous whorls as typical for the genus. Inner margin of the hemipenis with straight median portion; lobe a slender, digitiform, slightly tapering distally; lobe b broadly developed, apical rim partially folded and sclerotized; lobe h with evenly rounded distal margin, also sclerotized. Eye not visible.

### *Mixtacandona* gr. *laisi-chappuisi* sp. 1 (Fig. 10 A-D, Fig. 11)

Material investigated: two adults ♂♂ (GR788, L = 532 µm; GR789, L = 496 µm) with soft parts dissected in glycerine in a sealed slide and valves stored dry in micropaleontological slides

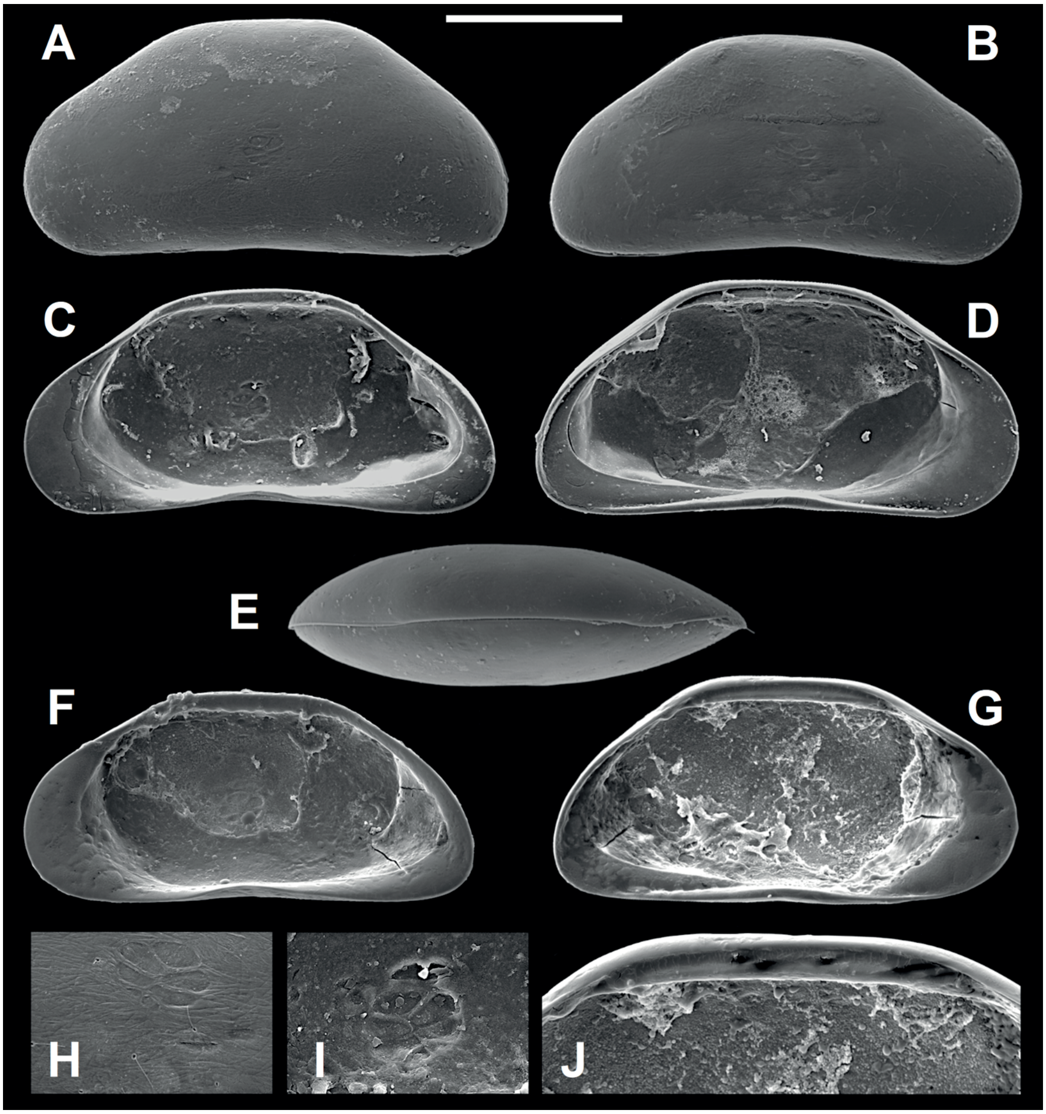


**Fig. 6.** *Mixtacandona* cf. *botosaneanui*. **A-D,H,I)** adult ♂ (GR793). **E)** adult ♀ (GR1106). **F,G,J)** adult ♀ (GR794). **A)** LV ev; **B)** RV ev; **C)** RV iv; **D)** LV iv; **E)** Cp dv; **F)** RV iv; **G)** RV iv; **H)** RV ev, detail muscle scar; **I)** RV iv, detail muscle scar; **J)** RV iv, detail hinge. Scale bars: A-G) 200 µm; H,I) 88 µm; J) 108 µm.

(GR788) or used for SEM (GR789), collected by S.I. on 3 June 2016 from Grotta Pertosa.

Comparative examination of the valve outline of the specimens from Grotta Pertosa against all illustrated representatives of the *laisi-chappuisi* group revealed no correspondence, supporting the hypothesis that they may belong to an undescribed species. However, morphological examination of the T1 palps and hemipenis in the only available specimen (Fig. 11), whose soft parts are incomplete, revealed characters essentially consistent with those observed in other congeners and provided no diagnostic features.

### ***Mixtacandona* gr. *laisi-chappuisi* sp. 2** (Fig. 10 E,F)

Material investigated: one adult ♀ (GR815, L = 521 μm) with soft parts dissected in glycerine in a sealed slide and valves used for SEM, collected from Grotta Totino on 20 February 2016 by R.G., A.I. and M.T.S. The remaining material from Grotta Totino comprised empty carapaces and disarticulated valves, many of which were decalcified.

As in the previous case, the distinctive valve outline of this species from Grotta Totino shows no correspondence with any other member of the *laisi-chappuisi* group, as indicated by comparative analyses of all illustrated valves reported in the literature. This suggests that it may represent an undescribed species.

### ***Mixtacandona* gr. *laisi-chappuisi* sp. 3** (Fig. 9B)

Material investigated: one ♀ (GR848, L = 550 μm), with a complete Cp and remains of soft parts, collected on 29 August 2015 by R.G. and M.T.S. from Grotta Palummaro.

As in the previous case, no further remarks are offered on this species because of the scarcity of available material. However, it is worth noting that the valve outline of *Mixtacandona* gr. *laisi-chappuisi* sp. 3 closely resembles that of *Mixtacandona* sp. described by Kalbe *et al.* (2016) from Middle Pleistocene deposits in central Syria (Fig. 9B).

### ***Mixtacandona* gr. *laisi-chappuisi* juv**

Material investigated: one immature specimen (GR862, L = 405 μm), collected on 28 August 2015 by R.G. and M.T.S. from Grotta Grave Grubbo.

Because the material consists of a single immature specimen, no additional details are provided for this taxon.

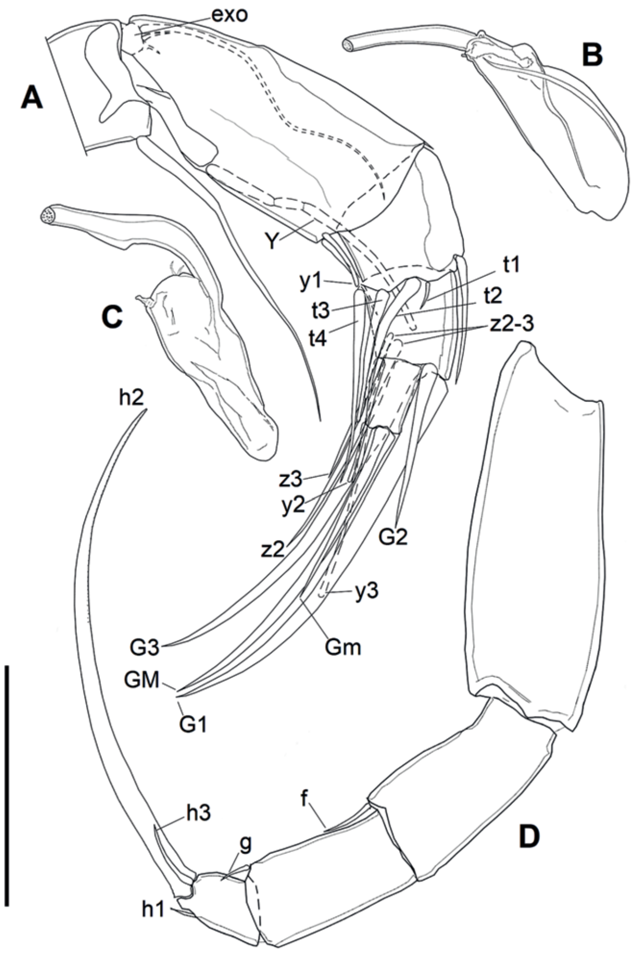


**Fig. 7.** *Mixtacandona* cf. *botosaneanui*, adult ♂ (GR793). **A)** A2. **B)** Right T1 prehensile palp. **C)** Left T1 prehensile palp. **D)** T1. Scale bar: 50 μm.

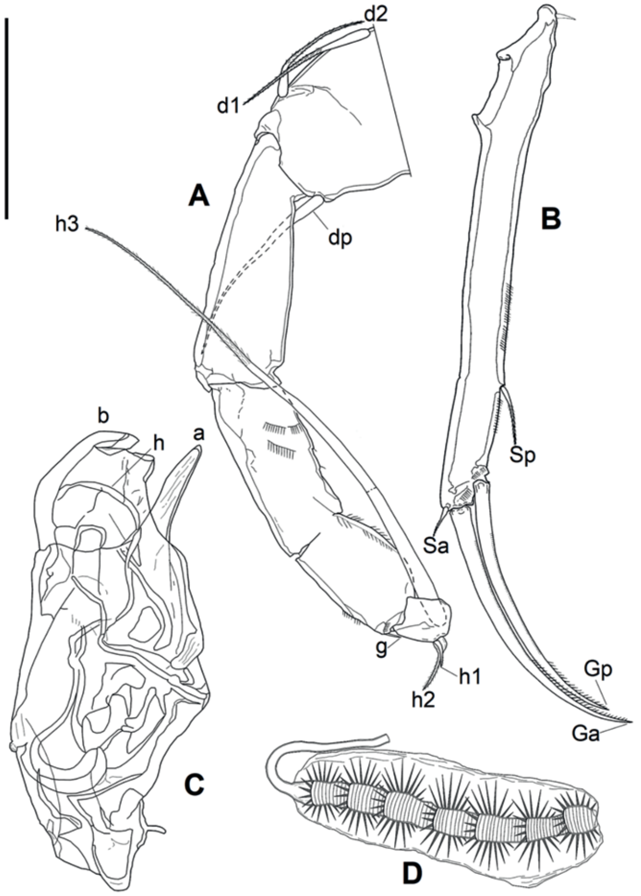
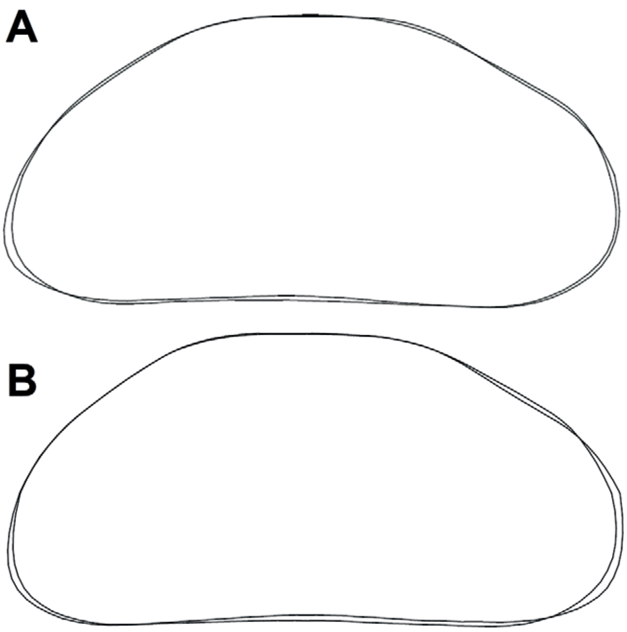


**Fig. 8.** *Mixtacandona* cf. *botosaneanui*, adult ♂ (GR793). **A)** T2. **B)** T3. **C)** Hemipenis. **D)** Zenker's organ. Scale bars: A-C) 50 μm; D) 100 μm.



### Genus *Pseudolimnocythere* Klie, 1938

(see Rossetti *et al.* 2022 for a diagnosis of the genus)

***Pseudolimnocythere* sp.** (Figs. 12 and 13)

Material investigated: three samples collected on 28 January 2016 by R.G. and M.T.S. from Teatro greco-romano, containing several empty carapaces (one, GR864, used for SEM), disarticulated valves, and one complete adult ♀ (GR818, L = 340 µm); soft parts of GR818 dissected in glycerine in a sealed slide, LV used for SEM (RV damaged).

Valve-outline analysis of *Pseudolimnocythere* sp. from Teatro greco-romano shows a strong similarity to *P.* sp. reported by Peterson *et al.* (2013) from the Frasassi cave system in central Italy, which is known only from subfossil carapaces and valves (Fig. 13A). Peterson *et al.* (2013) suggested a close affinity between the Frasassi specimens and *P. hypogaea* Klie 1938, originally described from groundwater in southern Italy and later recorded from caves

**Fig. 9. A)** Outline superimposition of adult ♀ RV ev of *Mixtacandona botosaneanui* (from Danielopol 1982) and of adult ♀ Cp in right lv of *Mixtacandona* cf. *botosaneanui* from Grotta Pertosa (GR1106). **B)** Outline superimposition of RV ev of *Mixtacandona* sp. (from Kalbe *et al*., 2016) and of adult ♀ Cp right lv of *Mixtacandona* gr. *laisi-chappuisi* sp. 3 from Grotta Palummaro (GR848).

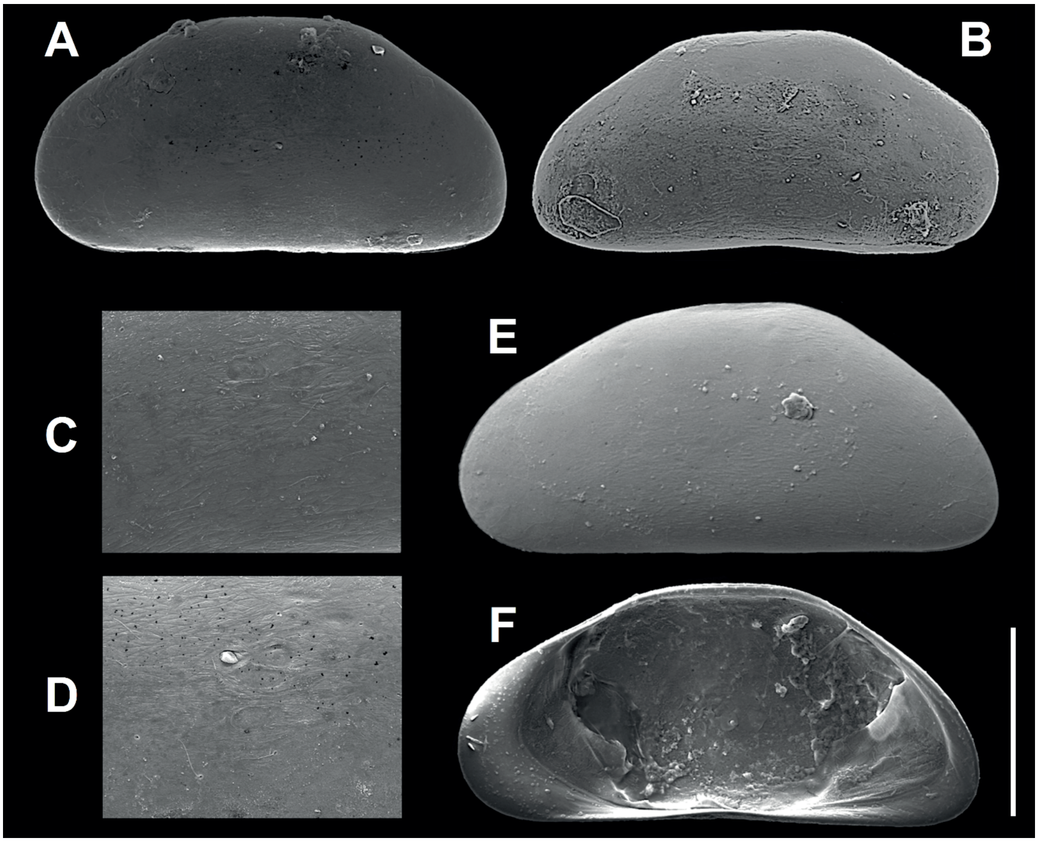


**Fig. 10. A-D)** *Mixtacandona* gr. *laisi-chappuisi* sp. 1, adult ♂ (GR789). **E,F)** *Mixtacandona* gr. *laisi-chappuisi* sp. 2, adult ♀ (GR815). **A)** LV ev. **B)** RV ev. **C)** Detail RV ev. **D)** Detail LV ev. **E)** LV ev. **F)** RV iv. Scale bars: A,B,E,F) 200 µm; C,D) 93 µm.

and wells in Puglia by Pesce *et al.* (1978), Pesce and Pagliani (1997), and Karanovic and Pesce (2001). However, the outline of *P. hypogaea* does not fully coincide with that of the Frasassi and Teatro greco-romano specimens (Fig. 13B).

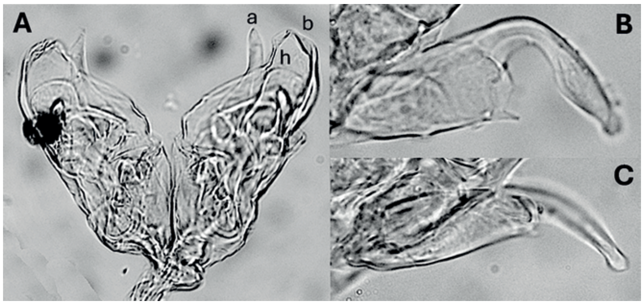


**Fig. 11. A)** *Mixtacandona* gr. *laisi-chappuisi* sp. 1, adult ♂ (GR788). **B,C)** *Mixtacandona* gr. *laisi-chappuisi* sp. 1, adult ♂ (GR789). **A)** Hemipenes. **B)** Right T1 prehensile palp organ. **C)** Left T1 prehensile palp. Not to scale.

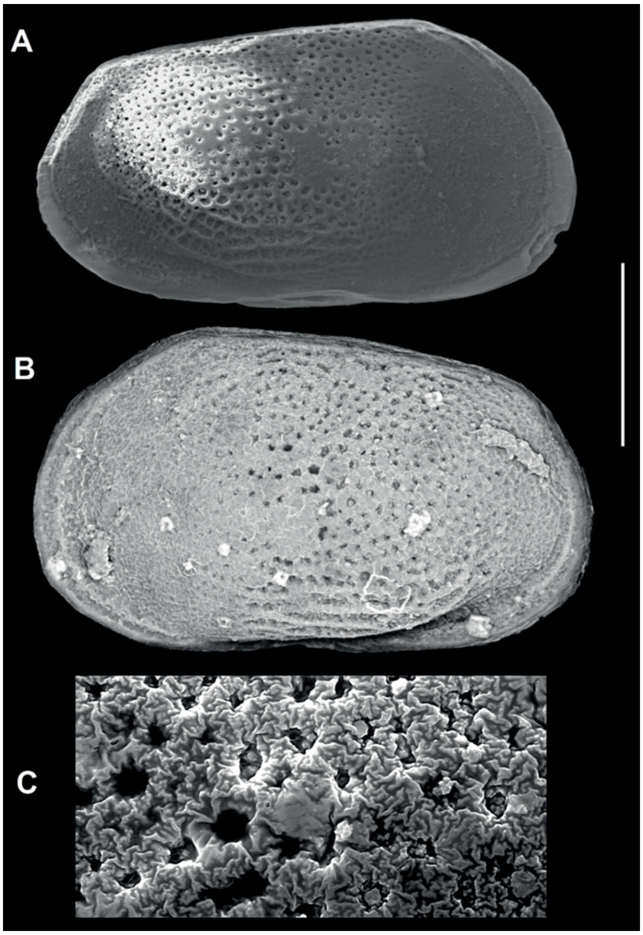


**Fig. 12. A)** *Pseudolimnocythere* sp., adult (sex undetermined) (GR864). **B,C)** *Pseudolimnocythere* sp., adult ♀ (GR818). **A)** Cp right ev. **B)** LV ev. C) Detail of LV ev. Scale bars: **A,B)** 100 µm; C) 35 µm.

The incomplete soft parts of the *Pseudolimnocythere* specimens examined here prevented detailed comparison with congeneric taxa; however, valve morphology suggests that they may represent a new species, to be described once additional material becomes available.

## Updated list of stygobitic ostracods from Italy

The ostracod taxa identified in this study that may be regarded as stygobionts are Candoninae indet., *Typhlocypris* cf. *eremita*, *Mixtacandona* cf. *botosaneanui*, *M.* gr. *laisi-chappuisi* sp. 1, *M.* gr. *laisi-chappuisi* sp. 2, *M.* gr. *laisi-chappuisi* sp. 3, *M.* gr. *laisi-chappuisi* juv. and *Pseudolimnocythere* sp. The remaining taxa are either stygophiles or, more commonly, occasional inhabitants of subterranean habitats.

The new records presented here, combined with those previously reported in the literature, raise the total number of stygobiont ostracod taxa documented in Italy to more than 30, although the precise taxonomic placement of some taxa remains to be confirmed (Tab. 3).

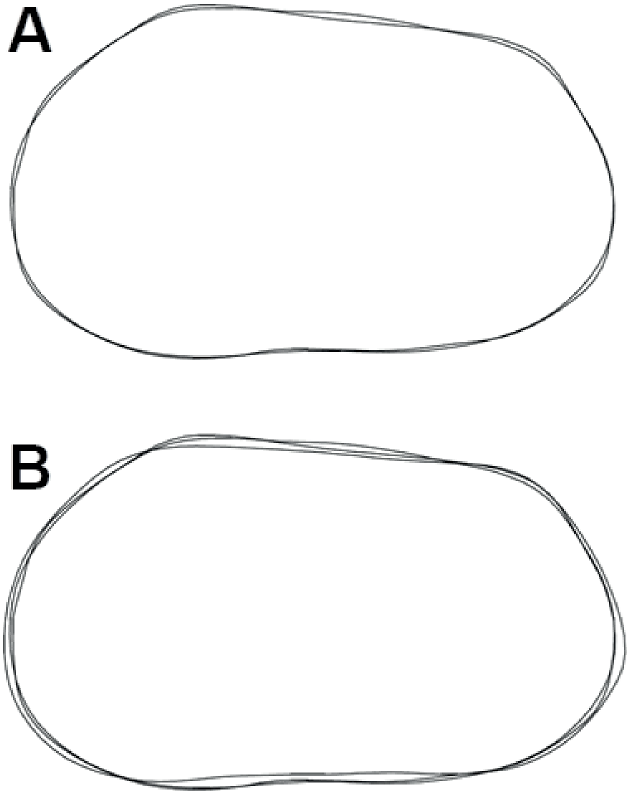


**Fig. 13. A)** Outline superimposition of RV iv of adult ♀♀ of *Pseudolimnocythere* sp. Peterson *et al.*, 2013 from Frasassi and *Pseudolimnocythere* sp. from Teatro greco-romano (GR818). **B)** Superimposition as A plus *Pseudolimnocythere hartmanni* Danielopol, 1979.

**Tab. 3.** List of Recent ostracod taxa reported from Italy and regarded as stygobitic according to the classification proposed by Mori *et al*. (2025). However, *Cyclocypris helocrenica* Fuhrmann & Pietrzeniuk, 1990, *Cyclocypris mediosetosa* Meisch, 1987, and *Cypria reptans* Bronstein, 1928, although classified as stygobitic by these authors, were not included in the list because they are more appropriately regarded as crenobiotic, as their records refer exclusively to springs. The list was obtained through a careful review of the available literature, including Colin & Danielopol (1980), Stoch & Dolce (1994), Crema *et al.* (1996), Peterson *et al.* (2013), Pieri *et al*. (2015, 2020), Mazzini *et al*. (2017), Stoch (2017), Rossetti (2021), and Rossetti *et al.* (2006, 2022, 2025b, 2026).

**Marmocandona zschokkei* (Wolf, 1920)
*Marmocandona* sp. aff. *M. zschokkei* (Wolf, 1920)
*Marmocandona valentinae* Rossetti, Stoch and Mazzini, 2026
*Marmocandona zanichellii* Rossetti, Stoch and Mazzini, 2026
§Candoninae indet.
*Fabaeformiscandona breuili* (Paris, 1920)
*Fabaeformiscandona tyrolensis* (Löffler, 1963)
*Fabaeformiscandona wegelini* (Petkovski, 1962)
*Pseudocandona* sp.
§*Typhlocypris* cf. *eremita* (Vejdovský, 1882)
**Typhlocypris trigonella* (Klie, 1931)
*Schellencandona antoniae* Rossetti *et al*., 2025
*Schellencandona* cf. *S. insueta* (Klie, 1938)
*Mixtacandona chappuisi* (Klie, 1943)
*Mixtacandona cottarellii* Danielopol, 1981
*Mixtacandona idrisi* Mazzini and Rossetti, 2017
*Mixtacandona laisi* (Klie, 1938)
§*Mixtacandona* cf. *botosaneanui* Danielopol, 1973
§*Mixtacandona* gr. *laisi-chappuisi* sp. 1
§*Mixtacandona* gr. *laisi-chappuisi* sp. 2
§*Mixtacandona* gr. *laisi-chappuisi* sp. 3
*Cypria cavernae* Wagenleitner, 1990
*Paralimnocythere relicta* (Lilljeborg, 1863)
*Sphaeromicola sphaeromidicola* Hubault, 1938
*Sphaeromicola stammeri* Klie, 1930
*Kovalevskiella* sp. fide Colin and Danielopol 1980
*Vestalenula boteai* (Danielopol, 1970)
*Pseudolimnocythere hypogaea* Klie, 1938
*Pseudolimnocythere abdita* Rossetti *et al*., 2022
*Pseudolimnocythere sofiae* Rossetti *et al*., 2022
§*Pseudolimnocythere* sp.
*Pseudolimnocythere* sp. Peterson *et al.,* 2013

*Doubtful record in Ghetti and McKenzie (1981); §taxa found in the present study.

## Discussion

In several cases, the limited availability of well-preserved adult individuals led us to restrict identifications to the genus or species-group level, or to exercise caution when assigning specimens to nominal species. A more definitive taxonomic assessment of Candoninae indet., as well as of specimens of *Mixtacandona* and *Pseudolimnocythere*, will therefore require additional material. Such limitations are common in subterranean surveys, where population densities are often low and sampling opportunities constrained by environmental conditions.

Some characters of the specimens reported here as Candoninae indet., especially the valve outline and the small size of the carapace, recall the genus *Nannocandona*. However, several diagnostic traits differ from those of species currently assigned to that genus, including the number of A1 segments, the presence of three setae on the A2 exopodite, and the absence of strongly developed claws on the terminal segment of the mandibular palp. Species with subparallel dorsal and ventral margins also occur in the genus *Pseudocandona*, for example *P. semicognita* (Schäfer, 1934), but the limb chaetotaxy of our material does not fit that genus well.

Nevertheless, although we provide a detailed description of the main diagnostic features of both valves and soft parts, the limited number of examined specimens makes it advisable to retain them as Candoninae indet., pending the availability of additional material for further analysis. It is worth noting that the two specimens described here were collected in two different regions. Such a pattern, despite the lack of a conclusive taxonomic assignment, could be indicative of an Italian endemic species whose range is not confined to a single locality. Given the limited knowledge of the stygofauna, an even wider distribution cannot be excluded.

The genus *Typhlocypris* has undergone numerous taxonomic revisions (Namiotko *et al*., 2014). This genus was previously reported for northeastern Italy by Ghetti and McKenzie (1981) as *Pseudocandona trigonella* Klie. To date, *Typhlocypris trigonella* (Klie, 1931) is known only from a single individual, measuring 0.55 mm in length, collected in the Postojna Cave, Slovenia (Klie, 1931). Since its discovery, living specimens of this species have not been recorded in samples from that cave or from any other locality (Namiotko *et al*., 2014). The record from Italy must be regarded with caution, even if it appears plausible from a biogeographical perspective, because of the absence of an exact geographical reference and, in particular, the lack of illustrations.

The occurrence of *Typhlocypris* cf. *eremita* in the Grotta Cava di Papipano represents the second record of this species in Italy, following the report by Peterson *et al*. (2013) of valves of *Pseudocandona* ex gr. *P. eremita* from sediments of a sulfidic spring in the Frasassi cave system (central Italy).

*Mixtacandona* is one of the most speciose genera among Recent Candoninae with a western Palearctic distribution. Meisch *et al*. (2024) report 21 formally established species in the genus, all of them considered to be stygobionts (Danielopol and Cvetkov, 1979, Meisch, 1996), to which *M. thessalica* Rossetti and Mazzini, 2025 from Greece must be added (Rossetti *et al*., 2025a) and two putative new species from Romania (Rossetti *et al*., in prep.). In the literature there are several records identified at the generic level, which suggests an even higher diversity within this genus.

The relatively high diversity within the genus can be explained by a diversification of these limnicoid species *sensu* Boutin and Coineau (1990) before they colonized groundwater, or by later speciation driven by isolation in subterranean habitats. On the other hand, it cannot be ruled out that some described species are in fact different morphotypes of the same species. Addressing this issue will require integrated taxonomy, which to date has been conducted for only a few species of the genus, also suggesting possible polyphyly for *Mixtacandona* (Rossetti *et al*., 2025a). According to these authors, the differences in chaetotaxy documented across species described with high taxonomic resolution could support splitting *Mixtacandona* into separate genera. Future phylogenetic analyses integrating molecular data will be essential to test whether the observed morphological diversity reflects deep evolutionary splits or phenotypic divergence within a single or a more limited number of lineages.

Most species in the genus *Mixtacandona* are currently regarded as endemic. Exceptions include *M. laisi*, the type species of the genus, which occurs in several European countries (Meisch 2000); *M. chappuisi*, first reported from Romania (Danielopol 1973) and later recorded from different sites in Slovenia (Mori and Meisch 2012) and Serbia (Petrov, 2013); *M. idrisi*, known from a cave in Sicily (Mazzini *et al*., 2017), may also occur with living specimens and as a fossil in Spain in a Late Pleistocene-Holocene transition (Iriarte *et al*., 2023). The record of this species in central Greece by Popa *et al.* (2019) refers to *M. thessalica* Rossetti *et al*. (2025a).

The record of *M.* cf. *botosaneanui*, if its identity with the nominal species collected from a well and a cave in Romania (Danielopol, 1982; Danielopol and Hartmann, 1986) is ultimately confirmed, would extend the known geographical range of this species and provide the first description of the male. *Mixtacandona* cf. *botosaneanui* from this study shows a pronounced sexual dimorphism in valve morphology that is seldom reported within the genus (see *M. thessalica*; Rossetti *et al.* 2025a), although it is particularly important as a diagnostic character in palaeontological research. The record of *M.* cf. *botosaneanui* from the Balearic Islands (IMOST database; Castillo-Escrivà *et al.* 2023), on the other hand, most likely represents a different species within the *laisi-chappuisi* group rather than *M. botosaneanui*, as supported by SEM-based comparison of valve morphology.

Although the similarity between *Mixtacandona* sp. 3 from Grotta Palummaro and the *Mixtacandona* sp. reported by Kalbe *et al.* (2016) from the Middle Pleistocene of Syria may evoke intriguing evolutionary and biogeographic scenarios, given that it is based on the comparison of only two specimens, any interpretation must, in light of current knowledge, be regarded as highly speculative.

All the aforementioned broadly distributed species belong to the *laisi-chappuisi* group (valves rectangular to trapezoidal in lateral view, small to medium-sized, with a smooth surface), one of five groups proposed by Danielopol (1977/78) for *Mixtacandona* based on carapace size, shape, and ornamentation. Mazzini *et al*. (2017) argued that these species-groups currently lack taxonomic value, but they are retained for practical convenience.

Notably, the Alburni karst massif in Campania hosts three species of the *laisi-chappuisi* group: the Grotta di Castelcivita is the locus typicus of *M. stammeri* Klie, 1938, which Pieri *et al*. (2015) synonymized with *M. laisi* (as previously also suggested by Meisch, 2000), and this study reported *M.* cf. *botosaneanui* and *M.* gr. *laisi-chappuisi* sp. 1 from Grotta Pertosa. To date, the only other documented instance of syntopy between two species of *Mixtacandona* is that reported by Rossetti *et al*. (*personal communication*) from a well in Romania. The rarity of syntopic occurrences may indicate strong ecological specialization or allopatric diversification among closely related taxa.

The living and sub-Recent representatives of the genus *Pseudolimnocythere* Klie, 1938 currently comprise four nominal species, along with several additional records identified only at the genus level. The *Pseudolimnocythere* sp. from Teatro greco-romano belongs to the morphological group defined by Rossetti *et al*. (2022) as species with "sloping valves." This group includes *P. abdita*, *P.* sp. sensu Schornikov, 2013 (Schornikov *et al.* 2014), *P.* sp. Danielopol (1980), *P.* sp. Peterson *et al.* (2013) (sub-Recent), and *P.* sp. Montanari *et al*. (2021), the latter two being sub-Recent forms.

Among the formally described living species, three (*P. hypogaea* Klie 1938, *P. abdita* Rossetti *et al.* 2022 and *P. sofiae* Rossetti *et al.* 2022) are endemic to Italy. The fourth, *P. hartmanni* Danielopol, 1979, has been reported from Euboea Island in Greece and as fossil in the Quaternary of southern Italy, although the conspecificity of these records requires confirmation (Rossetti *et al.,* 2022). If *P.* sp. from Teatro greco-romano proves to correspond to the sub-Recent *Pseudolimnocythere* sp. described by Peterson *et al.* (2013) from the Frasassi Cave, this would constitute the first documented case of a species of the genus exhibiting a broad geographical distribution. Furthermore, loose valves of *P. abdita* were recorded in the hyporheic zone of the Ceno River in the northern Apennines, belonging to a hydrographic basin different from that of the spring designated as the species' type locality, located approximately 30 km away in a straight line (Rossetti, *unpublished data*).

The locality where *Pseudolimnocythere* sp. was discovered lies close to the Middle Miocene shoreline of the Pontian-Mediterranean region, consistent with the known distribution of other extant and fossil species of the genus (see Fig. 9 in Rossetti *et al*., 2022). This distribution pattern points to an earlier dispersal phase along coastal habitats of this thalassoid genus, followed by multiple invasion routes, rather than a single colonization event with subsequent dispersal (Rossetti *et al*., 2022).

It is also worth noting that some ostracod species have been recorded from sulfidic cave environments, such as the gypsum karst systems of Monte Conca, Grave Grubbo and Totino (Tab. S1). The occurrence of ostracods under high hydrogen sulfide concentrations suggests that at least some taxa can be regarded as extremophiles. Comparable evidence comes from other sulfidic cave systems (e.g., Peterson *et al.,* 2013; Iepure *et al*., 2023; Rossetti *et al*., 2025a). Although ostracods are rarely dominant components of these ecosystems, their presence indicates ecological specialization and broadens the recognized adaptive spectrum of several subterranean lineages.

The stygobitic ostracod taxa recorded in Italy (Tab. 3) account for about 30% of those known from Europe and are represented in all families that include at least one exclusively subterranean species, except Kliellidae Schäfer, 1945 (Mori *et al*., 2025). Moreover, at least seven living stygobitic species (Candoninae indet., *Schellencandona antoniae* Rossetti *et al.* 2025b, *Marmocandona valentinae* Rossetti *et al*. 2026, *Marmocandona zanichellii* Rossetti *et al*., 2026, *Mixtacandona cottarellii* Danielopol, 1981, *Pseudolimnocythere abdita* Rossetti *et al*. 2022, and *P. sofiae* Rossetti *et al*. 2022) are currently considered endemic to Italy, and the true number is likely to be higher, as outlined above. This proportion is remarkable considering the limited extent of Italian territory compared to the European continent as a whole and reinforces the status of the Italian peninsula as a major centre of subterranean diversification.

## Conclusions

The results of this study expand the known geographic ranges of several stygobitic ostracod species and reveal additional putative new species for which the available material is too scarce or poorly preserved to allow a complete taxonomic assessment. These new faunistic data refine current knowledge of distribution patterns at both regional and European scales. At the same time, our findings reaffirm the importance of mainland Italy and its surrounding islands as hotspots of subterranean biodiversity, while also highlighting how limited our knowledge of stygobitic faunas, and of ostracods in particular, still remains. Continued exploration of subterranean environments is therefore essential to improve our understanding of non-marine ostracod diversity and to clarify their evolutionary history and systematic relationships.

Alongside traditional morphological approaches, the use of molecular markers will provide new opportunities to investigate the phylogenetic affinities, antiquity, and colonization dynamics of subterranean ostracods. In addition, advances in environmental DNA metabarcoding hold great promise for monitoring the still largely unexplored biodiversity of groundwater ecosystems. Such integrative efforts will ultimately contribute to a more comprehensive understanding of diversification processes in subterranean aquatic systems.

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*Online supplementary material:*

*Tab. S1. Details of the sampling sites.*

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