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A new extremophile species of *Pseudocandona* (Crustacea, Ostracoda) from a sulfidic cave in Albania

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Abstract

Subterranean ecosystems host highly specialized and often endemic crustacean faunas, yet ostracods inhabiting chemically extreme sulfidic caves remain poorly documented. In this study, a new stygobiont species of the genus *Pseudocandona* is described from a sulfidic cave in Albania using an integrative taxonomic approach combining detailed morphological analyses and molecular data. Subterranean ostracods exhibit a suite of morphological, physiological, and life-history adaptations to the energetic constraints of groundwater habitats, and species thriving in sulfidic systems additionally tolerate high concentrations of hydrogen sulfide and hypoxia. The new species, *Pseudocandona sulphurella* **sp. nov.**, belongs to the *rostrata* group, a clade otherwise dominated by surface-water taxa and with only one previously known stygobiont representative. Its valves and soft parts are frequently covered by sulfur-oxidizing bacterial epibionts, a feature shared with other crustaceans from sulfidic environments and potentially indicative of mutualistic interactions. Phylogenetic analyses based on COI and 28S sequences place the new species within a moderately supported clade of Western Palearctic *Pseudocandona*, while also highlighting the likely polyphyly of the genus. The discovery of *P. sulphurella* **sp. nov.** represents the first record of a stygobiont ostracod in Albania and expands current knowledge of biodiversity in chemoautotrophic subterranean ecosystems. This finding underscores the evolutionary significance of sulfidic caves as natural laboratories for studying adaptation, speciation, and the ecological dynamics of groundwater crustaceans.

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Keywords
Candonidae, chemoautotrophy, groundwater biodiversity, integrative taxonomy, non-marine ostracods, phylogenetic analysis, stygobionts

Introduction

Ostracoda are a class of small crustaceans enclosed within a bivalved calcified carapace, a feature that has contributed to their exceptional fossil record and long evolutionary history (Horne 2003; Smith et al. 2015). Although most of the ostracod diversity occurs in marine and surface freshwater environments, several lineages have independently colonized subterranean waters, constituting a distinctive and ecologically important component of groundwater biodiversity (Danielopol and Hartmann 1986).

Subterranean ostracods thrive in a wide range of groundwater habitats, including alluvial aquifers, stream hyporrheos, karstic fissures, and caves (Mori et al. 2025), and often exhibit elevated rates of speciation and endemism driven by spatial and hydrological isolation as well as persistent selective pressures (Rossetti et al. 2022). The perpetual darkness, limited and patchily distributed resources, and environmental stability of subterranean systems favour a suite of potential morphological, physiological, and behavioural adaptations, such as reduction or loss of eyes, depigmentation, and elongation of sensory setae that enhance chemo- and mechanoreception (Karanovic 2012; Pipan and Culver 2012). Stygobitic species often exhibit reduced metabolic rates, enhanced tolerance to hypoxia, and life history traits such as slow growth, delayed reproduction, and low fecundity (Rouch and Danielopol 1997; Moldovan 2018). These traits reflect the energetic constraints of most of the groundwater ecosystems, where food resources are scarce and often derived from allochthonous organic matter or microbial production (Gibert et al. 1994; Gibert and Deharveng 2002; Porter et al. 2009). Within these systems, ostracods contribute to nutrient cycling through grazing on biofilms, detritus, and microbial assemblages, and they may serve as indicators of groundwater quality and hydrological connectivity (Danielopol et al. 2003).

Among the diverse subterranean habitats colonized by ostracods, sulfidic caves represent one of the most chemically extreme and biologically specialized environments. These caves are characterized by the presence of hydrogen sulfide which may originate from geothermal sources, oxidation of sulfide-bearing minerals, or microbial sulfate reduction (Hose et al. 2000). When hydrogen sulfide–rich waters mix with oxygenated waters or cave air, steep redox gradients arise, fostering the development of abundant microbial biofilms dominated by sulfur-oxidizing bacteria (Sarbu et al. 1996; Tobler et al. 2016). These chemoautotrophy-based communities constitute the primary trophic resource of the ecosystem, sustaining life in conditions where photosynthetically derived organic matter is nearly absent.

Species inhabiting sulfidic caves have often evolved physiological tolerance to sulfide, which is toxic to most metazoans at high concentrations, as well as to hypoxia (Sarbu et al. 1996). Their presence suggests either long-term evolutionary adaptation

or repeated colonization events followed by rapid selection for sulfide tolerance (Hose et al. 2000). Only a few non-marine ostracod species have so far been documented from these environments (Peterson et al. 2013; Iepure et al. 2023; Rossetti et al. 2025), yet they offer valuable insights into the limits of crustacean adaptation.

This paper describes a new species of ostracod in the genus *Pseudocandona* Kaufmann, 1900, discovered in a sulfidic cave in Albania, using an integrated taxonomic approach that combines detailed morphological descriptions of valves and soft parts with molecular sequence data.

Materials and methods

Study area

Ostracod specimens were observed and collected in "Breath of the Dragon Cave" in the Langarica Canyon, Albania (40°14'39.81"N, 20°26'19.16"E) (Suppl. material 2). A large thermal sulfidic stream (26 °C) and several stagnant sulfidic lakes occupy the lower section of this extensive hypogene karst system that comprises over 10 km of cave passages. The thick layer of bat guano that covers the bottom of the stream represents a rich allochthonous trophic resource for the stygobitic invertebrates inhabiting this cave (amphipods, as well as cyclopoid and harpacticoid copepods). An additional abundant food source in the cave ecosystem is supplied by chemoautotrophic sulfur-oxidizing bacteria inhabiting the stream. These microorganisms occur both as free-living cells in the water column (bacterioplankton) and as structured microbial biofilms that coat the streambed.

Ostracod sampling and morphological analysis

Ostracods were collected by S.M.S. during two surveys (26 April 2025 and 17 October 2025) using a 180 μm hand net and were preserved in 70% ethanol. Carapaces were measured using a calibrated ocular micrometer prior to dissection or from SEM images. Dissections of adult specimens were done under a stereomicroscope (Zeiss 47 50 22). Owing to their decalcified state, most of the valves were partially or entirely destroyed during dissection, and consequently only a limited number could be preserved dry on micropalaeontological slides. The dissected soft parts were mounted in glycerine on microscope glass slides and sealed using nail polish. Line drawings of soft parts were made with the help of a camera lucida-equipped microscope (Zeiss Standard 25). All the figured individuals are adults.

Digital images of valves, carapaces, and soft part details of adult specimens were taken with a Phenom Pro SEM (Thermo Fisher Scientific). For the preparation of soft parts, specimens were rehydrated and fixed in 3% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4), then dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, and 100%), transferred to mixed solutions of ethanol and

hexamethyldisilazane (HMDS) (3:1, 1:1, and 1:3), and finally placed in 100% HMDS. The samples were left overnight to allow chemical evaporation and complete drying, followed by mounting on aluminum stubs using conductive double-sided carbon adhesive tabs, and sputter-coating with gold for 60 s (SEM Coating Unit E5100). Specimens prepared for SEM analysis are labeled "PsLan" followed by a number and kept in the RM laboratory at the National Museum of Natural History, Bucharest, Romania.

Type material is deposited in the collection of the Museum of Naturalistic Historiography (MUST), University of Parma, Italy; the sequences 'MUST-GR' followed by a number indicate the accession code of the deposited specimens.

Molecular analyses

Six specimens of *Pseudocandona sulphurella* sp. nov. used for molecular analyses were preserved in 96% ethanol and stored at -20 °C until further processing.

DNA extraction, PCRs, sequencing and sequence editing were similar to the ones previously described (Rossetti et al. 2025). A different set of primers (Edgecombe and Giribet 2006) were used to amplify a fragment of the nuclear 28S rDNA: RD1A (5'-CCCSCGTAAYTTAGGCATAT-3') and RD4B (5'-CCTTGGTCCGTGTTTCAAGAC-3'). The 28S rDNA fragment was also amplified from specimens of *Mixtacandona idrisi* and *M. thessalica* (Rossetti et al. 2025) and longer fragments were obtained and deposited (GenBank accession numbers: PX861656 and PX861657).

The manually curated sequences were checked against GenBank (Sayers et al. 2022) using BLASTn (Altschul et al. 1990) and sequences of members of the family Candonidae, together with those of the Paracyprididae and *Darwinula stevensoni* (Brady & Robertson, 1870) used as outgroups, were retrieved from GenBank and incorporated into the analysis (Suppl. material 1). The COI alignment was trimmed to the length of the shortest GenBank sequence, i.e., 515 bp. Sequence alignments for the two sets (COI and 28S) were done on the MAFFT web server (https://mafft.cbrc.jp/alignment/server/index.html, accessed on 03 December 2025) using default parameters (Katoh et al. 2019) and a phylogenetic reconstruction was performed by Bayesian inference in BEAST v.2.7.7 (Bouckaert et al. 2019). A GTR+G substitution model was used for the COI dataset, a TN93+G substitution model was used for 28S, with a strict clock and a mean clock rate of 1, and the Yule speciation model was used for both genetic markers. The Markov Chain Monte Carlo length was set at 50 million with sampling every 5000 generations. The first 10% of samples were discarded as burn-in and results were checked in Tracer v.1.7.2 (Rambaut et al. 2018), making sure the Effective Sample Size values for all parameters were greater than 200. Maximum clade credibility trees with Common Ancestor heights and 25% burn-in were calculated in TreeAnnotator v.2.7.7 (Bouckaert et al. 2019) and the phylogenetic trees were visualized in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 23 March 2023). The substitution saturation for COI was visualized by plotting the number of transitions and transversions versus the F84 genetic distance as implemented in DAMBE v.7.3.32 (Xia 2017). Species delimitation analyses were done

using the distance-based method ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2021) and the tree-based methods bPTP (Bayesian Poisson Tree Processes; Zhang et al. 2013) and GMYC (Generalized Mixed-Yule Coalescent; Pons et al. 2006). ASAP and bPTP were performed locally as implemented in the iTaxoTools v0.1 toolkit (Vences et al. 2021) and GMYC was performed using the *splits* v1.0-20 package (https://r-forge.r-project.org/projects/splits, accessed on 05 May 2025) inside the R v.4.2.3 statistical computing environment (R Core Team 2023). The metadata associated with the sequences downloaded from GenBank were downloaded and transformed into a tabular format using the R package *dplyr* v.1.1.4 (Wickham et al. 2023) and provided in Suppl. material 1. Basic sequence statistics and haplotype identity analysis were performed in DnaSP v.6 (Rozas et al. 2017), the presence of indels or early stop codons (for COI), and p-distances between species were calculated in MEGA v.7 (Kumar et al. 2016).

Abbreviations used in the text and figures

Valves. **Cp**: carapace; **dv**: dorsal view; **ev**: external view; **H**: height; **iv**: internal view; **L**: length; **LV**: left valve; **lv**: lateral view; **RV**: right valve; **W**: width.

Soft parts. **Ba**: basis; **Cx**: coxa; **A1**: antennule; **A2**: antenna; **Md**: mandible; **Mx1**: maxillula; **T1**: first thoracopod (maxilliped); **T2**: second thoracopod (walking leg); **T3**: third thoracopod (cleaning leg); **CR**: caudal ramus; **ex**: exopodite on A2; **ya**: aesthetasc on A1; **t1-4** and **z1-3**: setae, claws or male bristles on A2; **Y** and **y1-3**: aesthetascs on A2; **G1-3**, **GM**, **Gm**: claws on A2; **d1**, **f**, **g**, **g1-2** 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; **a**, **b** and **h**: outer, inner and medial lobes of hemipenis; **M** and **bc**: M-process and bursa copulatrix of hemipenis.

Taxonomic account (according to Meisch et al. 2024)

Class Ostracoda Latreille, 1802
Subclass Podocopa G.O. Sars, 1866
Order Podocopida G.O. Sars, 1866
Suborder Cypridocopina Baird, 1845
Superfamily Cypridoidea Baird, 1845
Family Candonidae Kaufmann, 1900
Subfamily Candoninae Kaufmann, 1900

Genus *Pseudocandona* Kaufmann, 1900

Diagnosis of the genus. (modified after Namiotko and Danielopol 2004; Namiotko et al. 2014) Carapace variable in shape, from triangular to trapezoidal in lateral view. Surface of adult valves smooth or pitted and usually bearing long, stiff setae; presence

of dense, long, stiff and perpendicularly attached setae on the valves; male second antenna (A2) with penultimate podomere differentiated or not (when divided with male bristles); externo-distal seta (γ) on the penultimate podomere of the mandibular palp smooth (not plumose); branchial plate on maxilliped (T1) rudimentary and set with 2 setae; protopodite of the cleaning leg (T2) bearing 3 setae (d1, d2 and dp), penultimate segment lacking the medial seta (f) and terminal segment with 2 long and one short setae; Zenker's organ with 7 rosettes of chitinous spikes; hemipenis with flat and weakly sclerotized M-process.

***Pseudocandona sulphurella* sp. nov.**

https://zoobank.org/A05EBBBF-5F51-4031-AD71-35F145C72BC8

Figs 1–6

Diagnosis. Cp in lv with dorsal margin gently sloping towards anterior and a hump at the posterior third corresponding to the posterior cardinal angle; anterior and posterior margins evenly rounded, ventral margin gently arcuate. Valve surface smooth with long, stiff and perpendicularly attached setae. Hinge adont. Muscle scars typical of the genus. Vestibulum well developed on the anterior margin. Male A2: penultimate segment subdivided, bearing male bristles; z1 transformed into a long claw. Setal group of Md second podomere with three setae. T2 with two ventral setae (g1 and g2) on penultimate segment.

Female genital lobe with triangular ventral margin, apex forming a mammilliform protrusion. Hemipenis: lobe a with distal margin fan-shaped; lobe h folded distally and with lateral rounded rostrum; lobe b with anterior digitiform expansion and posterior undulate margin.

Remark. The living species of *Pseudocandona* are allocated to different species groups (see Discussion). *Pseudocandona sulphurella* sp. nov. belongs to the *rostrata*-group, which is characterized by carapaces that do not exhibit a distinctly trapezoidal or triangular outline in lateral view and setal group on the second podomere of the Md palp with three setae (Namiotko and Danielopol 2004).

Type material. *Holotype*: • adult ♂ (MUST-GR1072), soft parts dissected in glycerine and mounted on a sealed slide, valves stored dry in a micropalaeontological slide (LV slightly damaged). Collected from the type locality on 26 April 2025.

***Allotype*:** • adult ♀ (MUST-GR1120), soft parts dissected in glycerine and mounted on a sealed slide, damaged valves stored dry in a micropalaeontological slide. Collected from the type locality on 17 October 2025.

***Paratypes*:** • adult ♂ (MUST-GR1091), soft parts dissected in glycerine and mounted on a sealed slide, valves stored dry in a micropalaeontological slide (RV damaged); • adult ♂ (MUST-GR1092), soft parts dissected in glycerine and mounted on a sealed slide, valves severely damaged and not retained; • adult ♂ (MUST-GR1102), soft parts dissected in glycerine and mounted on a sealed slide, valves severely damaged and not retained; • adult ♂ (MUST-GR1109), soft parts dissected in glycerine and mounted on a sealed slide, valves stored dry in a micropalaeontological

slide (RV damaged); • adult ♀ (MUST-GR1101), soft parts dissected in glycerine and mounted on a sealed slide, valves slightly damaged stored dry in a micropalaeontological slide; • adult ♀ (MUST-GR1121), soft parts dissected in glycerine and mounted on a sealed slide, valves severely damaged and not retained; • three adult whole specimens (sex undetermined), each stored dry in a micropalaeontological slide (MUST-GR1122–1124). MUST-GR1072, MUST-GR1091, MUST-GR1092, MUST-GR1101, MUST-GR1102 and MUST-GR1122-1124 collected on 26 April 2025 from the type locality; MUST-GR1109; MUST-GR 1120 and MUST-GR1121 collected on 17 October 2025 from the type locality.

Note. All the collected specimens had decalcified, fragile valves, which were partially or completely damaged during dissection. The observed valve decalcification is likely induced by the acidic conditions typical of sulfidic environments (Engel 2007, 2010).

Other material examined. About 30 specimens (adults and juveniles) from the type locality, partly used for dissections and the remaining ones preserved in ethanol and stored in the ostracod collection of the first author. Eight adult specimens from the type locality used for SEM and stored in the R.M. laboratory at the National Museum of Natural History, Bucharest, Romania.

Type locality. Albania, Langarica Canyon, "Breath of the Dragon Cave", (40°14'39.81"N, 20°26'19.16"E), sulfidic subterranean stream, 26 April 2025 and 17 October 2025, S.M. Serbu leg., Albania.

Etymology. The specific name *sulphurella* derives from the Latin noun *sulphur* (also spelled sulfur in later Latin), meaning "sulphur". To this root is added the Latin diminutive suffix *-ella*, which conveys the sense of "small" or "associated with".

Description. Cp (Fig. 1A–F). No evident sexual dimorphism in valve size or shape; L 1.09–1.13 mm for ♂♂ (n = 5), 1.06–1.09 mm for ♀♀ (n = 4). LV overlapping RV anteriorly and ventrally. In lateral view, dorsal margin with a hump at the posterior third and tapering anteriorly with a second gentle convexity; posterior margin more inflated than anterior margin, both evenly rounded; ventral margin slightly undulate. Valve surface smooth with weak reticulate texture visible in transmitted light, covered by rimmed pores bearing long setae, denser along the margins; inner calcified lamella wide anteriorly, distinctly narrower posteriorly. Colour brownish.

A1 (Figs 1F, 2A, 4A, 6A). Seven-segmented. First segment enlarged and stout, with two setae on dorsal margin and two long setae in ventro-apical position. Second and third segments with one dorso-apical seta. Fourth and fifth segments with two long dorso-apical setae and one short ventro-apical seta. Sixth segment with four apical setae. Final segment distally bearing two long and one short setae and aesthetasc ya.

A2 (Figs 1F, 2B, C, 4A, 6B). Cx with two dorsal setae; Ba with a long ventro-apical seta; Ex with one long and two short setae, the innermost with a broadened base; first endopodal segment with tripartite aesthetasc Y along the dorsal margin and two unequal dorso-apical setae; in ♂, second endopodal segment subdivided, seta t1 long, setae t2 and t3 transformed into male bristles, seta t4 tiny; z1 a long claw, setae z2 and z3 short; claws G1 and G2 subequal and about half length of G3; terminal segment with claw GM about 1.8× the length of Gm. In ♀, second endopodal segment

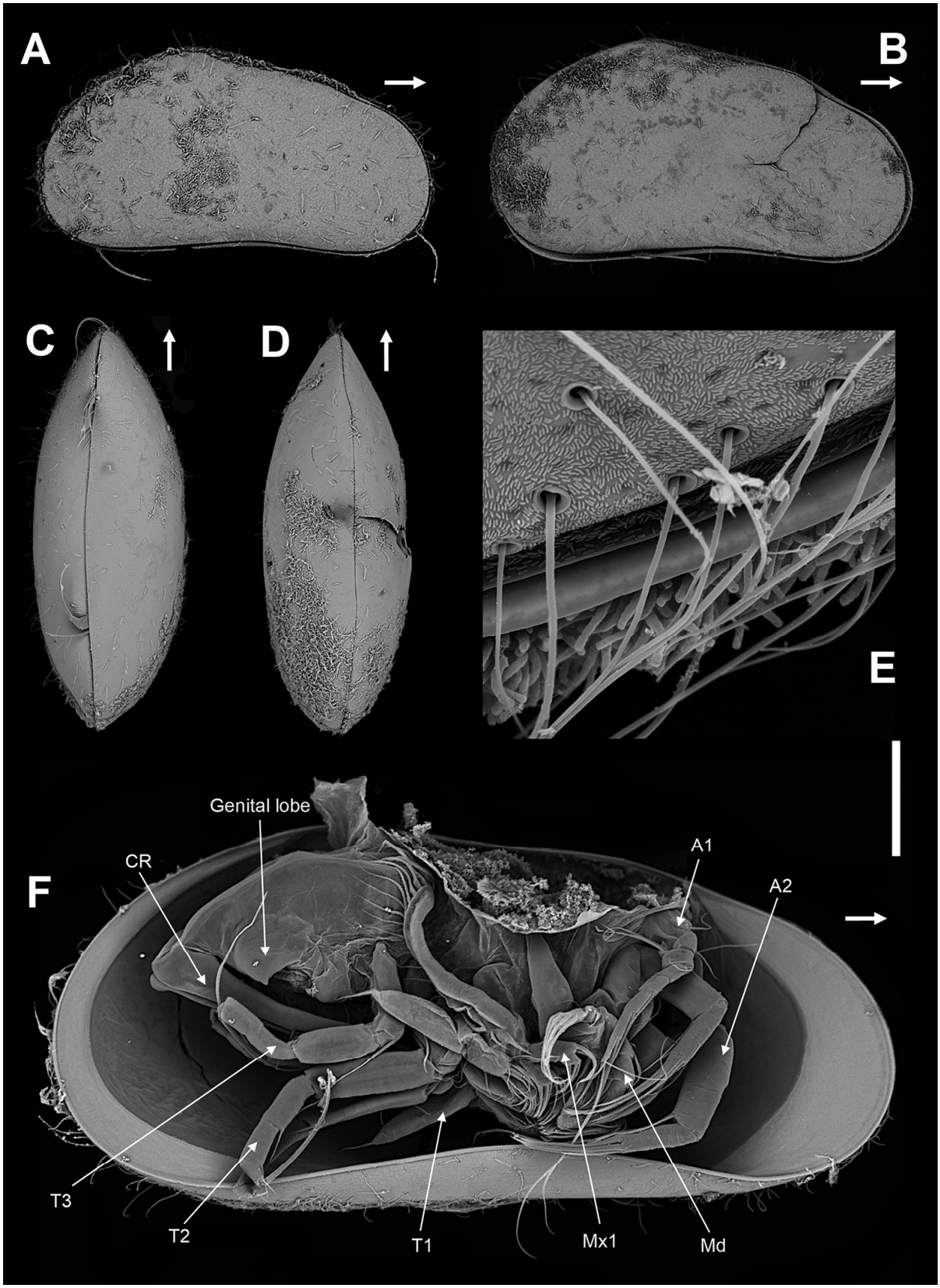


Figure 1. *Pseudocandona sulphurella* sp. nov. **A** ♀, Cp right lv (PsLan04) **B** ♂, Cp right lv (PsLan03) **C** ♀, Cp vv (PsLan05) **D** ♀, Cp dv (PsLan06) **E** ♀, Cp, detail rimmed pores and setae; note bacilli on the valve surface and filamentous, *Thiothrix*-like bacteria in background (PsLan04) **F** ♀, LV iv and whole body (PsLan01). Scale bars: 300 μm (**A–D**); 10 μm (**E**); 150 μm (**F**).

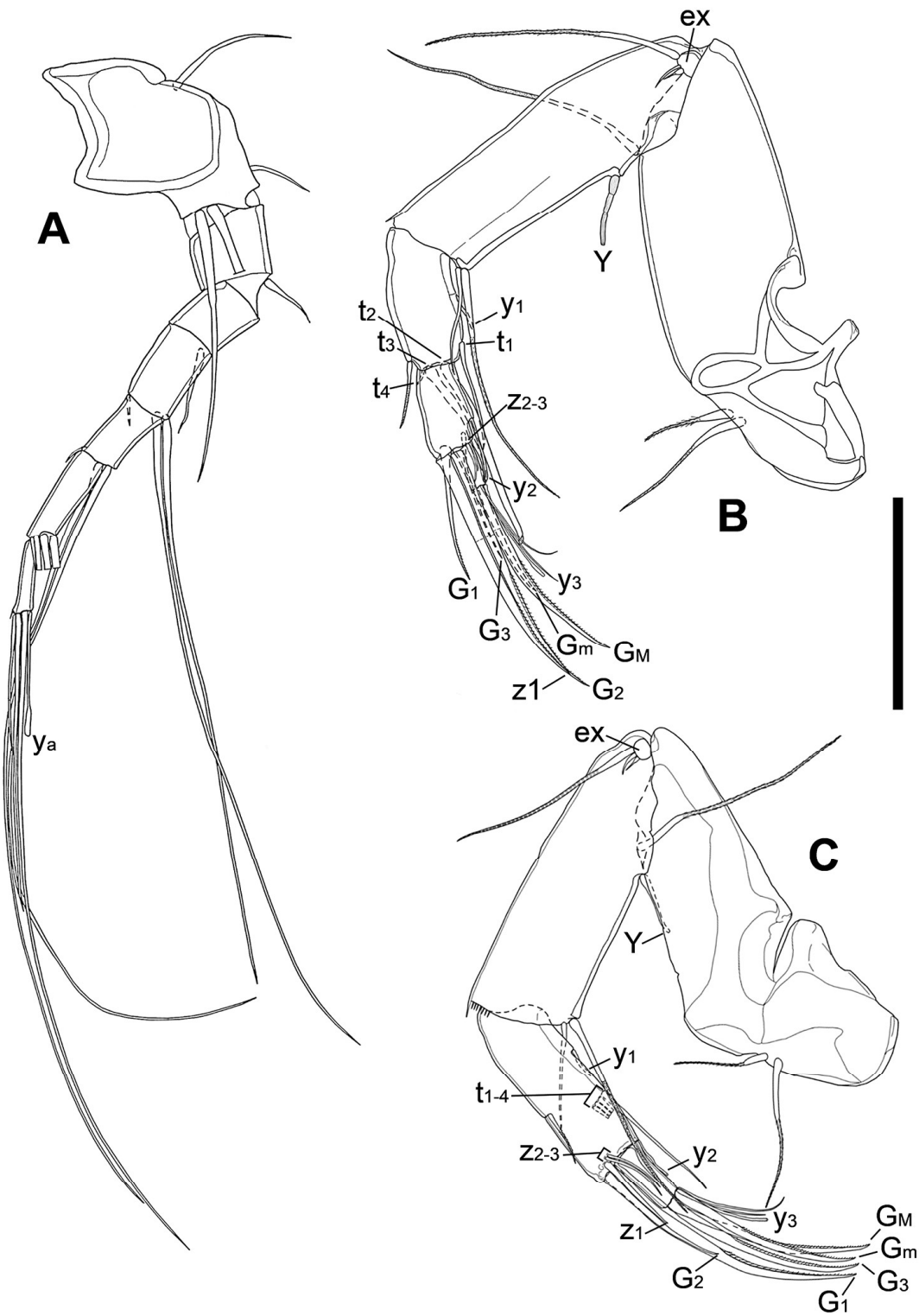


Figure 2. *Pseudocandona sulphurella* sp. nov. **A** ♂, A1 (MUST-GR1109) **B** ♂, A2 (MUST-GR1102) **C** ♀, A2 (MUST-GR1101). Scale bar: 100 μm (**A–C**).

undivided, setae z2 and z3 short and z1 claw-like with tip slightly exceeding the next segment, G2 about half-length of G1, GM and Gm similar in length and approximately three times longer than terminal segment.

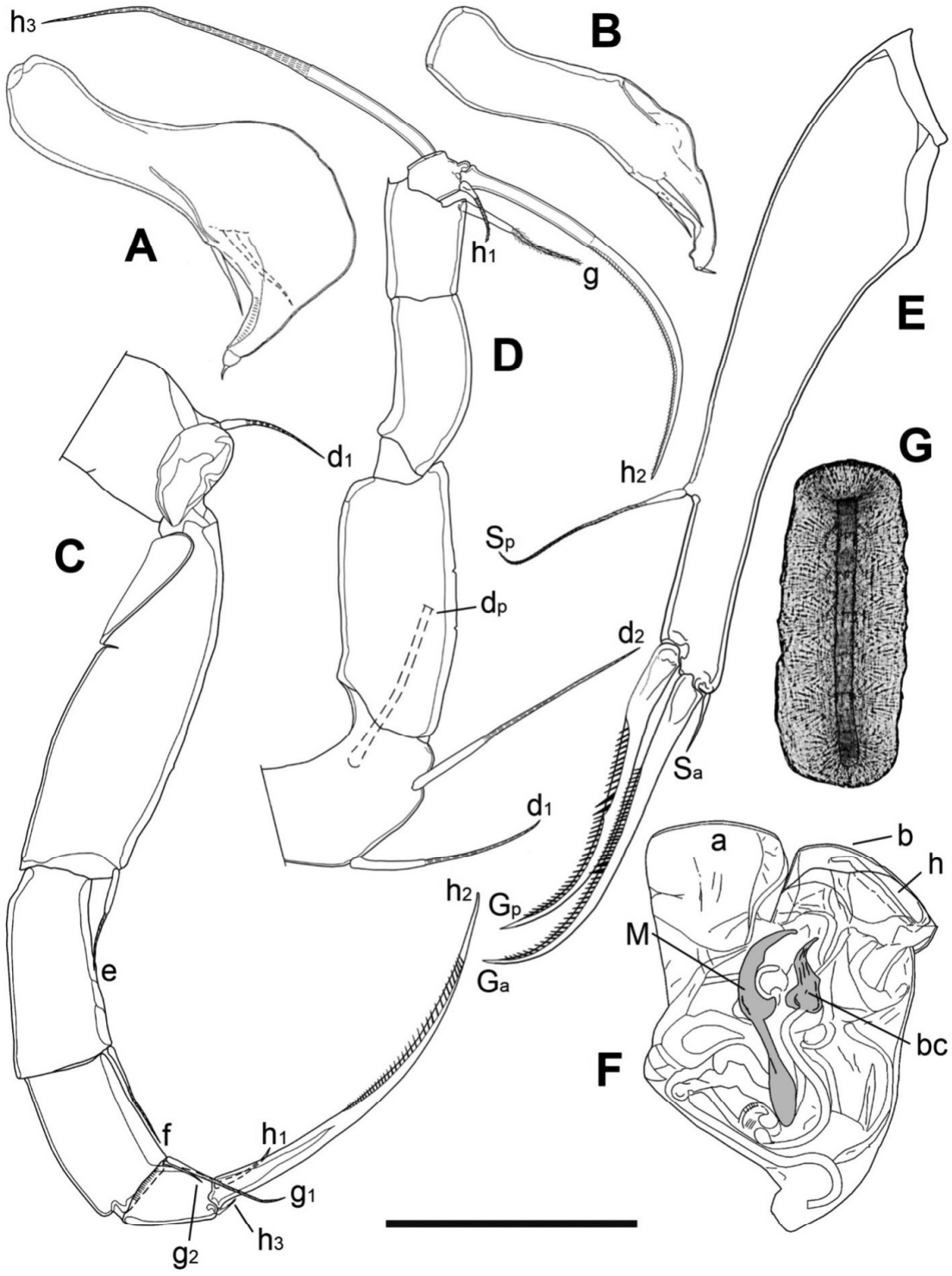


Figure 3. *Pseudocandona sulphurella* sp. nov. **A** ♂, right T1 (MUST-GR1102) **B** ♂, left T1 (MUST-GR1102) **C** ♂, T2 (MUST-GR1092) **D** ♂, T3 (MUST-GR1102) **E** ♂, CR (MUST-GR1109) **F** ♂, hemipenis (MUST-GR1092) **G** ♂, Zenker's organ (MUST-GR1109). Scale bar: 133 μm (**A, B**); 100 μm (**C–E**); 240 μm (**F**); 350 μm (**G**).

Md (Figs 1F, 4A–C) with a four-segmented palp, a coxa and a branchial plate as typical for the subfamily; second segment of palp bearing a group of three long setae as in the *rostrata*-group of *Pseudocandona*.

Mx1 (Figs 1F, 4A–C) as typical of subfamily, consisting of an undivided protopodite, a two-segmented palp (endopodite), three endites, and a branchial plate (exopodite). First segment of palp with three long dorso-apical setae and an additional ventro-apical seta at the insertion of the second segment; second segment short, rectangular, bearing six apical setae of unequal length.

T1 (Figs 1F, 3A, B, 4A–D, E, 6C). In ♂, palps strongly asymmetric; right palp swollen and rounded proximally with two robust ventral setae, tapering distally and ending with a minute seta; left palp sickle-shaped, bearing two ventral setae, distal part slender and with a tiny terminal seta. ♀ palp consisting of first two endites fused and short distal endite terminating with three setae.

T2 (Figs 1F, 3C, 4A, 5A). First segment with seta d1; second and third segments bearing respectively seta e and f in ventro-apical position; fourth segment ventro-apically with seta g1 surpassing the margin of the following segment and minute seta g2; terminal segment with seta h1 slightly shorter than segment itself, h2 a robust claw approximately equal in length to combined last three segments and armed in the distal half with a series of strong spines, except for a short terminal portion, seta h3 very reduced and about half the length of h1.

T3 (Figs 1F, 3D, 4A, 6D) five-segmented. First segment bearing long setae d1, d2 and dp; second and third segments without setae; fourth segment with seta g slightly longer than the segment itself; terminal segment with short h1, h2 a claw conspicuously setulate on the central third, h3 a long, robust seta, finely plumose on the distal two-thirds.

CR (Figs 1F, 3E, 4A, 5B) slightly curved and more expanded proximally; Sa and Sp in a 3:10 ratio; Ga and Gp subequal, about half the length of the ramus, with strong spines absent only on the basal portions and at the apices of claws.

Hemipenis (Figs 3F, 4A, 5C, D) very large. Outer lobe (a) elongate and widening distally, with slightly chitinized, fan-shaped margin; medial lobe (b) enveloping inner lobe (h), with a digitiform expansion directed toward lobe a and, on the opposite side, a slightly convex margin; lobe h with a sinuous distal margin, partially folded back, and producing a rounded lateral bulge. M-process elongate, slightly swollen at the proximal extremity and crescent-shaped in the distal part. Bursa copulatrix rounded proximally and beak-shaped distally.

Zenker's organ (Figs 3G, 4A) cylindrical, with seven whorls of spines.

Female genital lobe (Figs 1F, 6E) ventral margin approximately triangular in shape, bearing a papilliform protuberance.

Eye not visible.

Differential diagnosis. *Pseudocandona hartwigi* is the species of the *rostrata*-group whose valve morphology and dimensions most closely resemble those of *Pseudocandona sulphurella* sp. nov., although they differ chiefly in the dorsal margin. Moreover, *P. sulphurella* sp. nov. possesses a unique combination of morphological characters that readily distinguish it from all other known species of the genus, including the chaetotaxy of the last two segments of A2, the third endite of the female T1 palp not fused with the preceding endites, the presence of two ventro-apical setae on the fourth segment of T2, the distinctive morphology of the female genital segment, and, in particular, the structure of the hemipenis.

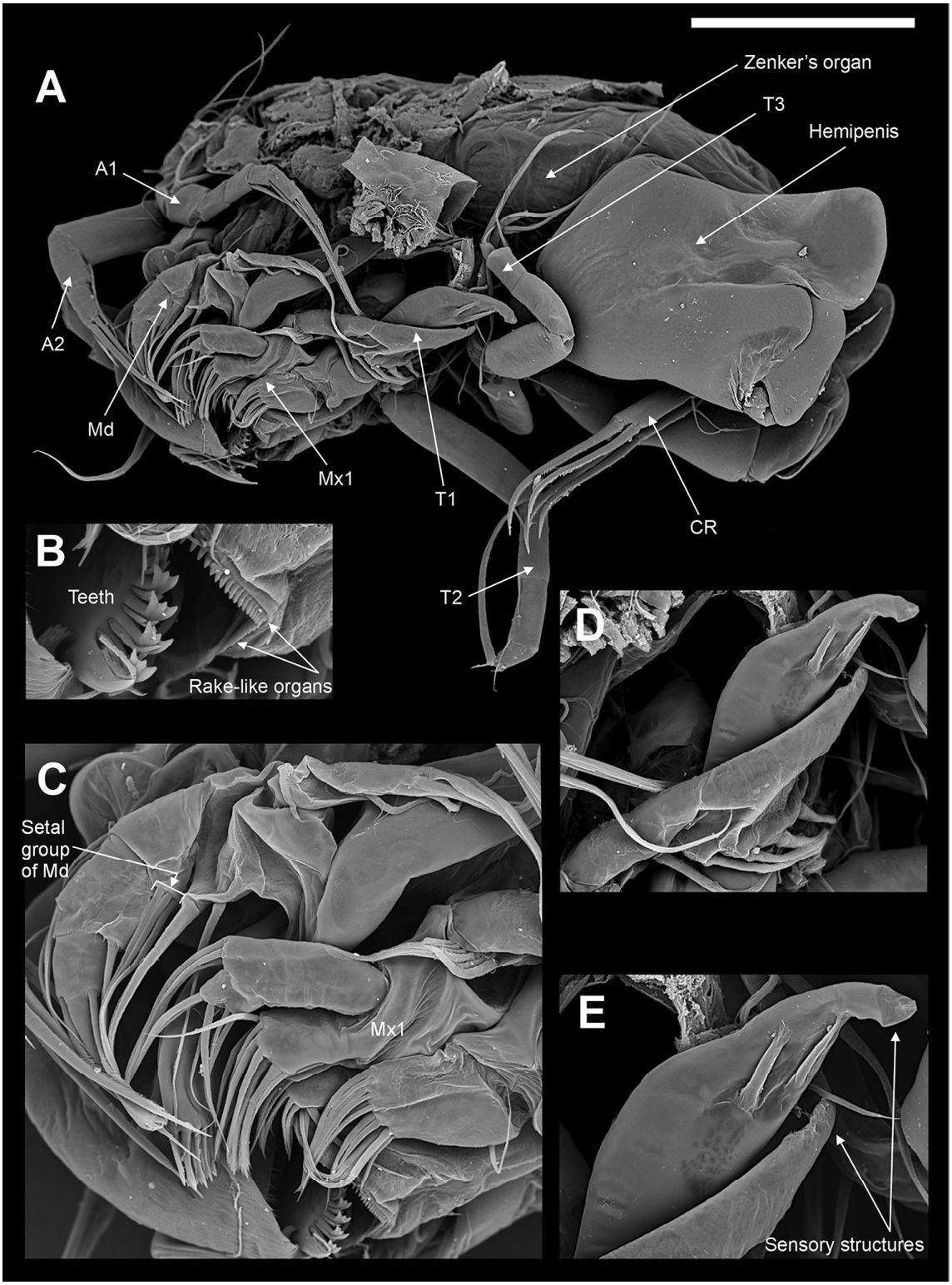


Figure 4. *Pseudocandona sulphurella* sp. nov. **A** ♂, whole body (PsLan02) **B** ♂, detail of mouth region (PsLan02) **C** ♂, Md and Mx1 (PsLan02) **D** ♂, right and left T1 (PsLan02) **E** ♂, detail of distal parts of right and left T1 (PsLan02). Scale bars: 200 μm (**A**); 55 μm (**B**); 82 μm (**C**); 100 μm (**D**); 62 μm (**E**).

Molecular analysis. Six sequences of *Pseudocandona sulphurella* sp. nov. were obtained for COI, with a length of 647 bp, all belonging to the same haplotype (GenBank accession number: PX867593) and three sequences were obtained for 28S rDNA, with a length of

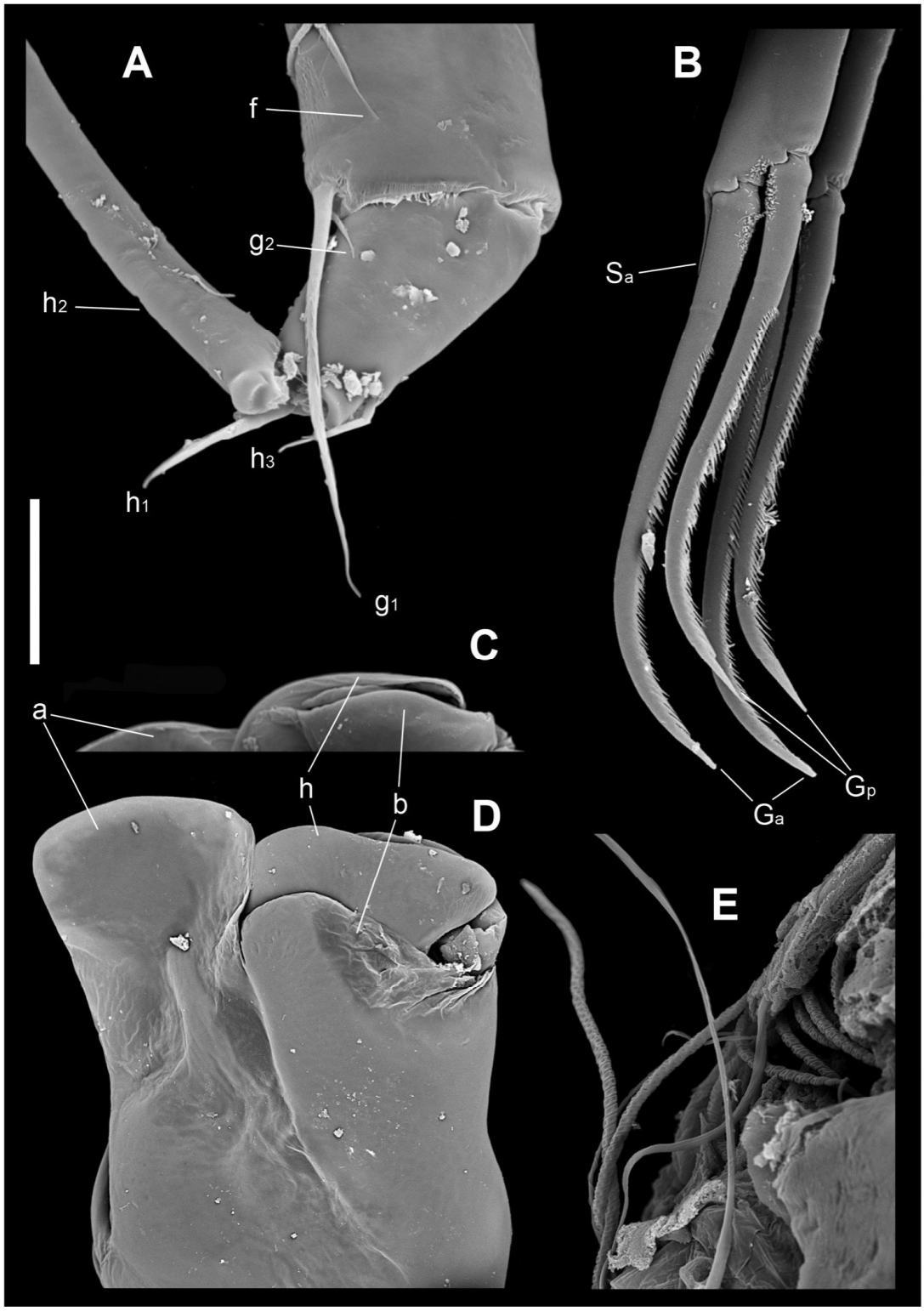


Figure 5. *Pseudocandona sulphurella* sp. nov. **A** ♂, detail of distal part of T2 (PsLan02) **B** ♂, detail of distal part of CR (PsLan02) **C** ♂, external view of hemipenis (PsLan02) **D** ♂, internal view of distal margin of hemipenis (PsLan02) **E** ♂, spermatozoa (PsLan02). Scale bars: 20 μm (**A**); 40 μm (**B**); 80 μm (**C, D**); 34 μm (**E**).

443 bp, all belonging to the same haplotype (GenBank accession number: PX866513). The COI phylogeny (Fig. 7) and p-distances suggest that *P. sulphurella* sp. nov. is closest to *P. marchica* (p-distance = 0.150) and *P. hartwigi* (p-distance = 0.151). For 28S, the

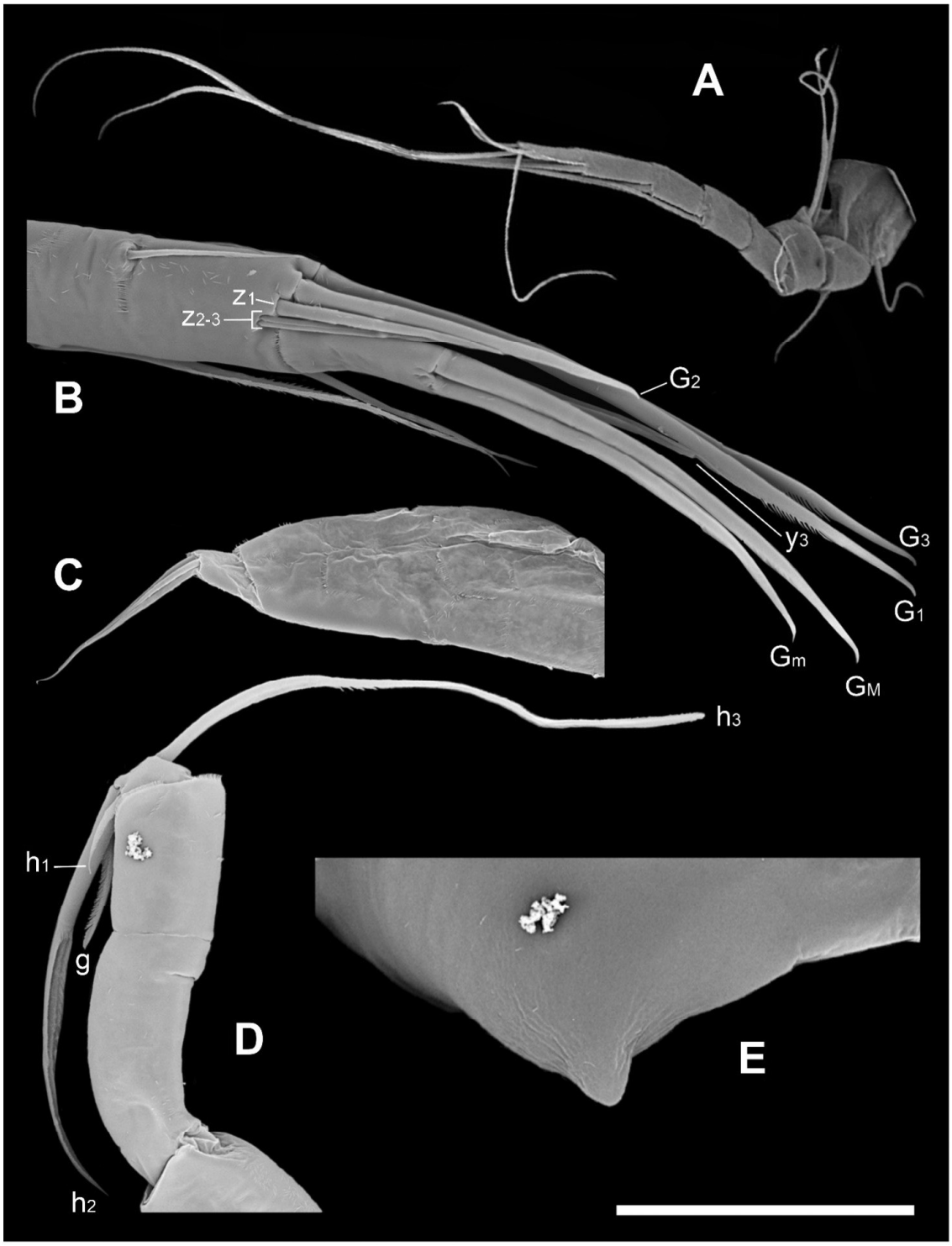


Figure 6. *Pseudocandona sulphurella* sp. nov. **A** ♀, A1 (PsLan01) **B** ♀, distal part of A2 (PsLan01) **C** ♀, detail of T1 (PsLan01) **D** ♀, detail of the last three segment of T3 (PsLan01) **E** ♀, detail of genital lobe (PsLan01). Scale bars: 156 μm (**A**); 60 μm (**B**); 75 μm (**C**); 80 μm (**D**); 42 μm (**E**).

lowest p-distance value was recorded between *P. sulphurella* sp. nov. and *Fabaeformiscandona kushiroensis* (p-distance = 0.062), but it clusters together with *Pseudocandona* sp. (p-distance = 0.091) on a moderately-supported clade in the 28S phylogeny (Fig. 8). All three species delimitation tests clearly distinguish *P. sulphurella* sp. nov. as a distinct species.

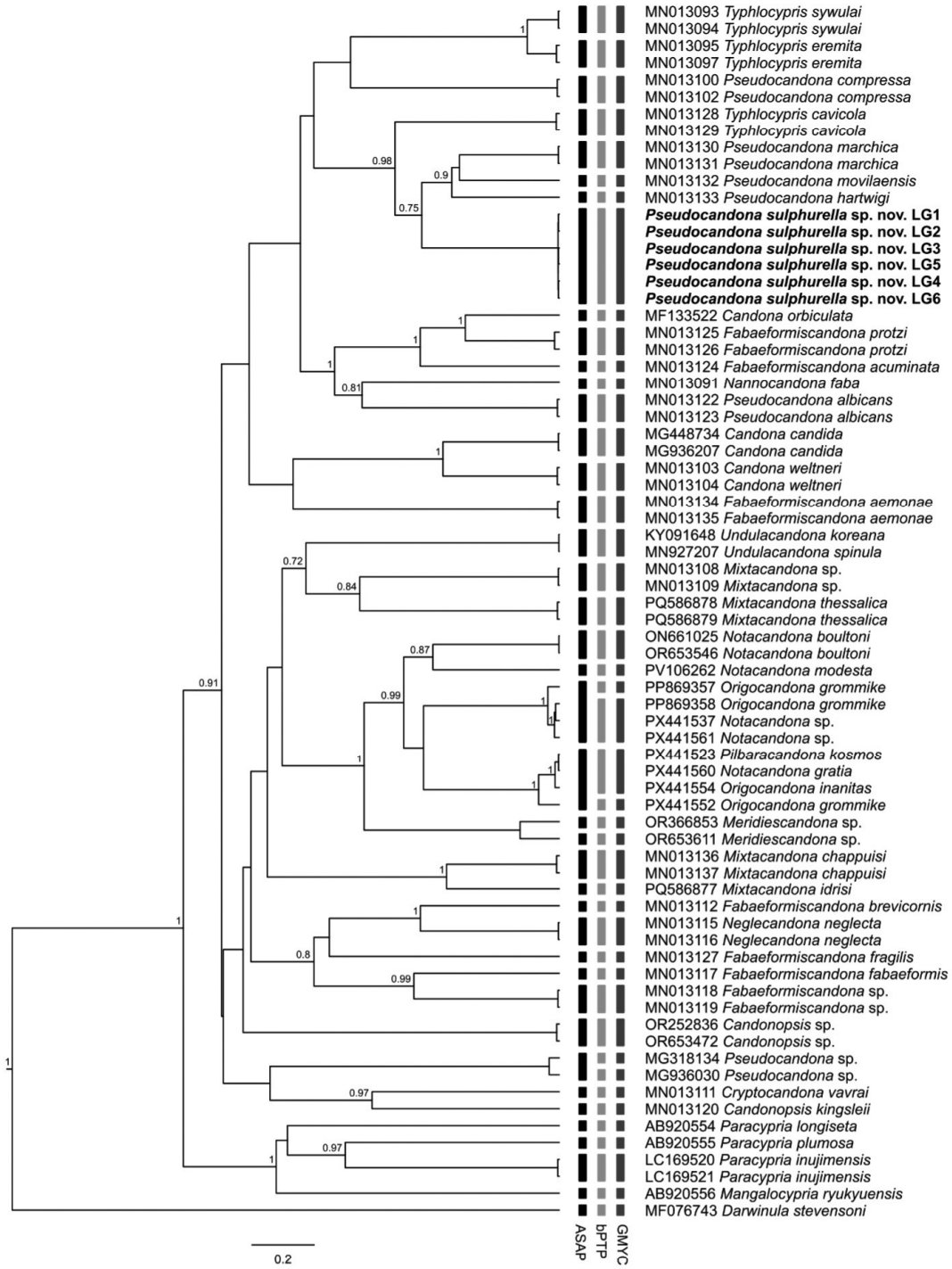


Figure 7. Bayesian-inference phylogeny of the family Candonidae reconstructed from COI sequences. *Darwinula stevensoni* (Brady & Robertson, 1870) and representatives of the family Paracyprididae were included as outgroups. Posterior probability values greater than 0.7 are shown next to the nodes. The species delimitation results are shown next to the tree, with each block denoting a distinct species.

Discussion

The long-standing taxonomic rearrangements within the subfamily Candoninae, together with the establishment of the genus *Pseudocandona*, have been thoroughly described by Higuti and Martens (2014) and Namiotko et al. (2014).

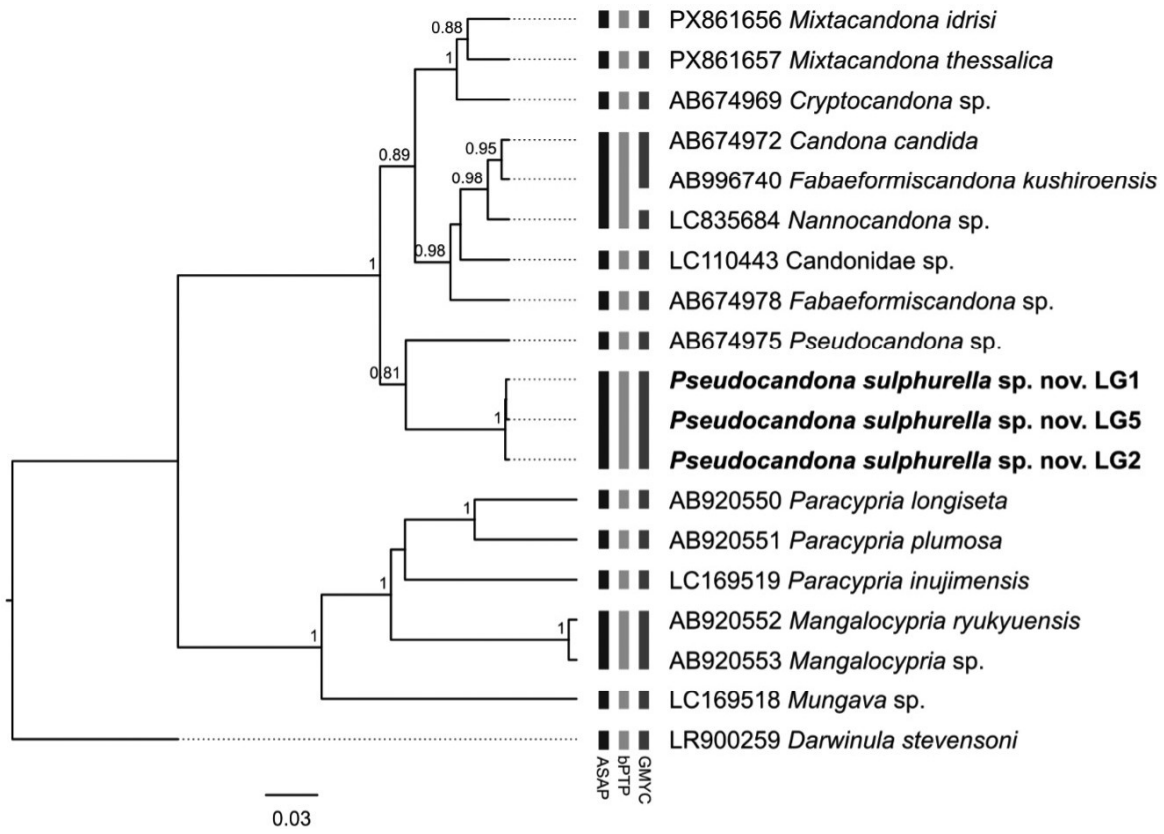


Figure 8. Bayesian inference phylogeny for 28S. *Darwinula stevensoni* (Brady & Robertson, 1870) and representatives of the family Paracyprididae were included as outgroups.

The genus *Pseudocandona* currently comprises more than 80 extant, formally described species (Meisch et al. 2024). According to Meisch (1996, 2000), these species are assigned to five groups, based primarily on carapace morphology and the number of setae in the setal group of the second segment of the mandibular palp: *caribbeana*, *compressa*, *eremita*, *rostrata*, and *zschokkei*. An additional group, the *prespica* one, was introduced by Karanovic (1999). Subsequent revisions have transferred species formerly placed in the *eremita* group to the genus *Typhlocypris* Vejdovský, 1882, while species of the *zschokkei* group have been reassigned to the genus *Marmocandona* Danielopol et al. 2012 (Karanovic 2005; Danielopol et al. 2012; Namiotko et al. 2014; Meisch et al. 2024). Furthermore, Higuti and Martens (2014) highlighted that the *Pseudocandona* species endemic to Lake Baikal, which constitute a substantial fraction of the genus diversity, will likely require transfer to a new genus pending further revision.

Pseudocandona sulphurella sp. nov. is placed in the *rostrata* group. This species group also includes *P. atmeta* Smith & Kamiya, 2015; *P. becca* Smith & Kamiya, 2015; *P. falcula* Smith & Kamiya, 2015; *P. hartwigi* (G. W. Müller, 1900); *P. lobipes* (Hartwig, 1900); *P. marchica* (Hartwig, 1899); *P. movilaensis* Iepure et al., 2023; *P. rostrata* (Brady & Norman, 1889); *P. sarsi* (Hartwig, 1899); *P. semicognita* (Schäfer, 1934); *P. stagnalis* (Sars, 1890); *P. tenuirostris* Hiruta & Mawatari, 2013.

All species of the *rostrata* group have a Palearctic distribution; only *P. rostrata*, *P. semicognita* and *P. stagnalis* have also been recorded in the Nearctic (Meisch 2000;

Smith and Kamiya 2015). Most group members are associated with surface-water habitats, with only occasional occurrences in subterranean and groundwater-dependent ecosystems. Two notable exceptions are the strict stygobionts *Pseudocandona sulphurella* sp. nov. and *P. movilaensis*, both restricted to sulfidic cave environments (Iepure et al. 2023). Other species of the *rostrata* group tolerate acidic conditions, such as *P. stagnalis*, *P. semicognita* and *P. tenuirostris* (Meisch 2000; Smith and Kamiya 2015). It is important to emphasize that information on distribution and ecology remains limited for many of the above-mentioned species, and should therefore be regarded as provisional.

The external surfaces of the valves of *Pseudocandona sulphurella* sp. nov. are extensively covered by undetermined small bacilli and by filamentous, *Thiothrix*-like bacteria (Fig. 1E), which also occur on the body of the animal. These sulfur-oxidizing bacteria convert hydrogen sulfide into various sulfur compounds to obtain metabolic energy and are frequently reported as epibionts or ectosymbionts on other crustaceans, such as amphipods (Flot et al. 2014). Their association with ostracods has previously been documented in sulfidic groundwaters at Mangalia (Romania) (Boancă et al. 2022) and at methane seeps in Lake Baikal (Khalzov et al. 2021). Such bacteria-ostracod interactions may be advantageous to the bacteria, to the host, or to both: epibiotic bacteria gain dispersal opportunities and access to host-derived secretions as nutrient sources, while microbial epibiotic consortia can constitute a food resource for the host (Khalzov et al. 2021).

The study of living ostracods in Albania has been primarily advanced through comprehensive research on Lake Prespa (Macedonian, Albanian, and Greek border) and Lake Ohrid (Albanian-Macedonian border) and its adjacent waters, revealing an exceptionally rich and highly endemic ostracod fauna (Namiotko et al. 2011; Lorenschat and Schwalb 2013; Lorenschat et al. 2014). Among the species reported in these studies are *Pseudocandona compressa* (Koch, 1838), *P. slavei* Pekovski, 1969, and *P. elongata* Holmes, 1937, the latter having been proposed by Meisch (2000) as a synonym of *Fabeformiscandona levanderi* (Hirschmann, 1912). Consequently, the discovery of *Pseudocandona sulphurella* sp. nov. represents the first record of the *rostrata* group in Albania and, more importantly, the first documented occurrence of a stygobiont ostracod in the country.

Concordant with the morphological affinities, the COI phylogeny places *Pseudocandona sulphurella* sp. nov. in the *rostrata* group, together with the Western Palearctic species *P. marchica* (from Oslo, Norway), *P. movilaensis* (from the sulfidic waters in Movile Cave, Romania) and *P. hartwigi* (from Otomin, Poland), on a moderately-supported clade. While the *rostrata* species group appears to be monophyletic, members of the *compressa* group (*P. compressa* and *P. albicans*) cluster on separate branches suggesting a possible polyphyly. This evidence of polyphyly of the genus is also supported by two undetermined species of *Pseudocandona* from the Nearctic (GenBank accession numbers MG318134 and MG936030) which cluster on a separate branch. Species of the genus *Typhlocypris* are the closest to the *rostrata* group and Wysocka et al. (2019) provide evidence for the monophyletic status of the *rostrata* group, and also note the intricate taxonomic history of the morphogenera *Pseudocandona* and *Typhlocypris*. The

28S phylogeny places *P. sulphurella* sp. nov. on a clade with an undetermined species of *Pseudocandona* sampled in Japan (Hiruta et al. 2016; GenBank accession number AB674975). We were unable to test the potential polyphyly of *Pseudocandona* for 28S due to the short overlap in the alignment caused by different amplification strategies; however, Karanovic and Sitnikova (2017) also demonstrate that *Pseudocandona* is polyphyletic for 28S. Both mitochondrial and nuclear phylogenies support *Pseudocandona sulphurella* sp. nov. as a distinct species and the molecular data generated in this study might prove useful in future taxonomic studies of the family Candonidae.

In conclusion, the occurrence of ostracods in sulfidic caves broadens current knowledge of groundwater biodiversity and provides a valuable system for investigating evolutionary processes under extreme environmental constraints. Their distinctive morphological and physiological specializations, together with narrow and often localized distributions, render these taxa particularly suitable as models for studies of adaptation and speciation, as well as for elucidating the ecological dynamics of chemoautotrophic subterranean ecosystems.

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Supplementary material 1

Phylogenetic data

Authors: Giampaolo Rossetti, Andrei Ștefan, Rozalia Motoc, Serban M. Sarbu, Ilaria Mazzini

Data type: docx

Explanation note: **table S1.** Metadata associated with the sequences downloaded from GenBank.

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Link: https://doi.org/10.3897/subtbiol.56.188589.suppl1

Supplementary material 2

Image data

Authors: Giampaolo Rossetti, Andrei Ștefan, Rozalia Motoc, Serban M. Sarbu, Ilaria Mazzini

Data type: jpg

Explanation note: **fig. S1.** Bat guano deposits on the streambed and white filamentous sulfur-oxidizing microorganisms near the water surface in the lower level of Dragon's Breath Cave in the Langarica Canyon, Albania.

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Link: https://doi.org/10.3897/subtbiol.56.188589.suppl2