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Description and Phylogenetic Position of *Thermocypris isabella* gen. et sp. nov. (Ostracoda: Cyprididae: Cyprinotinae) From a Hot Spring in Peru

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During a faunal survey in Baños del Inca hot spring (40–50°C), Cajamarca, Peru, we collected a cyprinotine ostracod species, the sixth example of a thermophilic ostracod inhabiting freshwater in temperatures above 40°C. Because this could not be assigned to any of nine genera in Cyprinotinae, we established the new genus *Thermocypris* for it and described it as *Thermocypris isabella* gen. et sp. nov., defined by the following combination of characters: (1) no dorsal hump on carapace, (2) no tubercles on either valve, (3) left valve slightly overlapping right valve along all margins, (4) no selvage on anterior part of right valve, (5) only seta d1 on first podomere of 6th limb, (6) only two shields (medial and lateral) on hemipenis, and (7) medial shield unilobed and lateral shield tooth-shaped. We determined partial sequences for the mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 18S and 28S rRNA genes from a specimen of the new species. In a maximum likelihood tree based on 18S + 28S, *T. isabella* was the sister taxon to *Heterocypris chetumalensis* (Yoo, Cohuo, Macario-Gonzalez, and Karanovic, 2017), with high branchial support. A clade including Cyprinotinae (*Cyprinotus* Brady, 1868 + *Heterocypris* Claus, 1892 + *Thermocypris*) and Eucypridinae (*Eucypris* Vavra, 1891) was fully supported; our phylogeny showed *Heterocypris* not to be monophyletic.

Key words: cox1, Cypridoidea, non-marine, seed shrimp, taxonomy

INTRODUCTION

One of 25 subfamilies in the ostracod family Cyprididae Baird, 1845, subfamily Cyprinotinae Bronstein, 1947 is distinguished from other subfamilies in having (1) a stout carapace, (2) (usually) asymmetrical right and left valves, (3) (usually) tubercles on the valve margins, (4) the 5th limb without a c-seta, and (5) a well-developed caudal ramus (also referred to as uropodal ramus; cf. Meisch, 2007; Smith, 2025) with two claws and two setae (Meisch, 2000; Karanovic, 2012; Halse and Martens, 2019; Meisch et al., 2024). Cyprinotinae comprises nine genera: *Billcypris* Halse and Martens, 2019; *Bilocypris* Halse and Martens, 2019; *Cyprinotus* Brady, 1868; *Hemicypris* Sars, 1903; *Heterocypris* Claus, 1892; *Homocypris* Sars, 1924; *Patcypris* Halse and Martens, 2019; *Riocypris* Klie, 1935; and *Trilocypris* Halse and Martens, 2019 (Halse and Martens, 2019; Meisch et al., 2024). The genera are differentiated by the presence or absence of (1) selvage on the right valve margin, (2) marginal tubercles on the valves, and (3) a dorsal hump on the carapace; in (4) having symmetrical or asymmetrical valves; and in (5) the number of setae on the first podomere of the 6th limb, and (6) the number and shape of shields on the hemipenis (Karanovic, 2012; Halse and Martens, 2019).

Few freshwater crustacean species have been reported from environments at temperatures as high as 47°C, the long-term heat tolerance limit for metazoans (Pörtner, 2002; Tomikawa et al., 2023). Ostracoda is one of the groups with species found from such extreme environments, with the following five species representing three families reported: *Heterocypris balnearia* (Moniez, 1893) (≥ 51.5°C); *H. sabirae* Gülen, 1985 (≥ 50°C); *Ilyocypris gibba* (Ramdohr, 1808) (38–42°C); *Potamocypris* sp. (28–53.5°C); and *Thermopsis thermophila* Külköylüoğlu, Meisch, and Rust, 2003 (37–48°C)

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(Moniez, 1893; Klie, 1939; Gülen, 1977, 1985; Wickstrom and Castenholz, 1985; Külköylüoğlu and Vinyard, 2000; Külköylüoğlu et al., 2003, 2020). They include species known only from thermal waters (e.g., *Thermop. thermophila*) and ones found also from cooler environments (e.g., *I. gibba*) (Külköylüoğlu and Vinyard, 2000; Külköylüoğlu et al., 2003).

During a faunal survey of the Baños del Inca hot spring in Peru, we collected a cyprinotine ostracod species that we could not assign morphologically to any known cyprinotine genus. Here we describe it as new, and establish a new genus for it. We also test whether *Cardinium*, a group of "reproduction-manipulating" endosymbiotic bacteria (Ma and Schwander, 2017), infects this species, present partial nucleotide sequences for its mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 18S rRNA (18S) and 28S rRNA (28S) genes, and infer its phylogenetic position based on an 18S + 28S dataset.

MATERIALS AND METHODS

Sampling

Ostracoda were collected by hand net along with substrates such as fallen leaves and cobble on 24 November 2022 at two sites in the Baños del Inca hot spring, Cajamarca, Peru (Fig. 1A): in a corner of the cooling pool (Fig. 1C, D) and in the channel flowing out of that pool (Fig. 1E). The depth of the cooling pool and the channel were ca. 60 cm and from ca. 10 to 15 cm, respectively. There were some fine layers of algae on the bottom of the pool; 13 algae species have been reported from this hot spring (Aldave, 1973). The water temperature was 40–50°C. This hot spring had a small stone wall built in the 1950s to protect the water. In the late 1990s, the current cement wall was constructed (Saldaña, 2018).

Morphological observations

Ostracods were fixed and preserved in 100% ethanol. The methods used for dissection, preparation of slides, light microscopy, scanning electron microscopy (SEM), and drawing were as described by Munakata et al. (2021). The material studied has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, under catalog numbers ICHUM-8924 to 8932.

The following abbreviations are used in the text: Ca, carapace; LV, left valve; RV, right valve; H, height; L, length; W, width; An1, antennula; An2, antenna; Md, mandible; Mx, maxillula; L5–7, fifth to seventh limbs; CR, caudal ramus; ls, lateral shield; ms, medial shield. The appendage chaetotaxy follows Broodbakker and Danielopol (1982) for An1, Md, and Mx, Martens (1987) for An2, Meisch (2000) for L5–7, and Meisch (2007) for CR. The following measurements were made from digital images by using ImageJ (Schneider et al., 2012): L and H of the LV and RV (LV-L, LV-H, RV-L, and RV-H), W of the carapace (Ca-W), and L and W of CR (CR-L and CR-W).

Molecular analysis

Total DNA was extracted from the soft parts of a specimen of the Peruvian species and an ethanol-fixed specimen of *Cyprinotus* sp. (ICHUM-8932) collected on 24 June 2023 in Miyazaki Prefecture, Japan (32.0121°N, 130.8208°E) by using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany) following the manufacturer's protocol. DNA from *Cyprinotus* sp. and a total DNA sample from a paratype (ICHUM-6135) of *Heterocypris spadix* Munakata, Tanaka, and Kakui, 2021 were included in the study for the determination of 28 and/or 18S sequences. The primers used for PCR amplification and sequencing were listed in Hiruta et al. (2016) for 28S, and

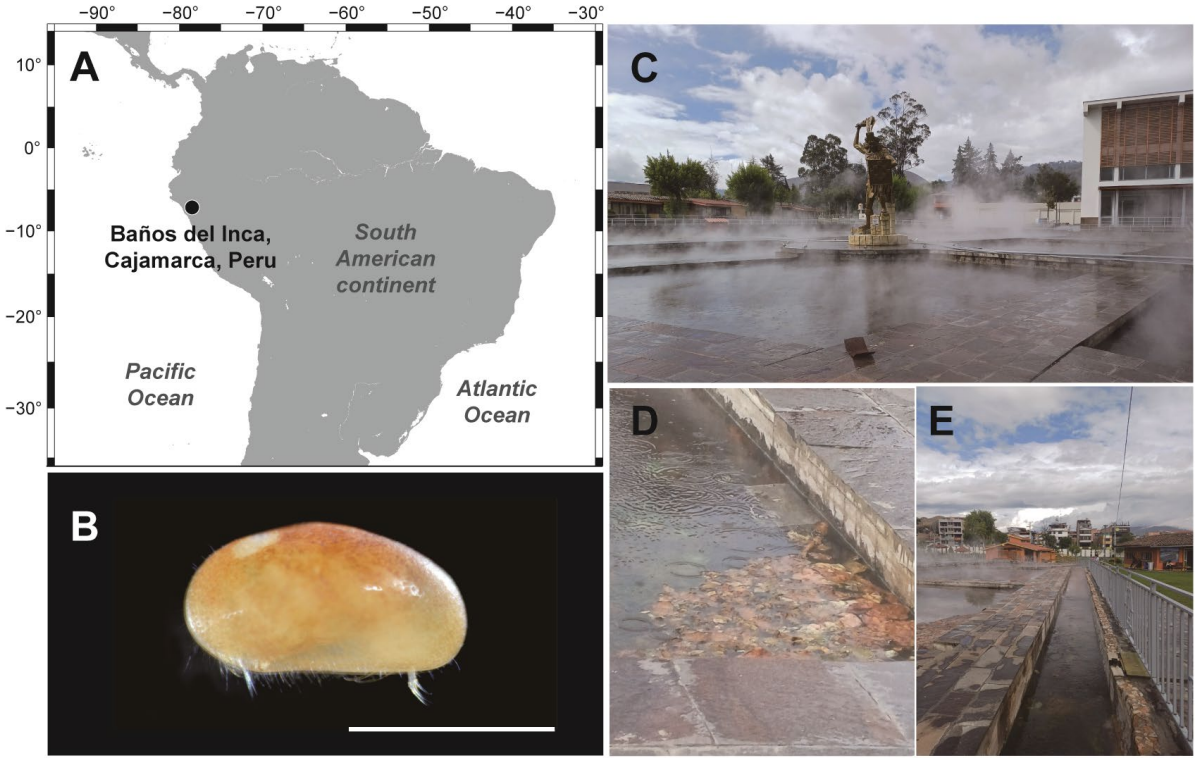


Fig. 1. Type locality, local habitat, and exterior morphology of *Thermocypris isabella* gen. et sp. nov. **(A)** Map of South America, with circle indicating the type locality (map generated with GMT6; Wessel et al., 2019). **(B)** Fixed specimen, left lateral view; scale bar, 0.5 mm. **(C)** Cooling pool derived from the hot spring. **(D)** Corner of the pool. **(E)** Channel flowing from the pool.

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Munakata et al. (2021) for COI, 18S, and *Cardinium* 16S. For cycle sequencing of 28S, we also used the newly designed primer 2KF_MM (GGTCTCCGAGGTGAACAGC). PCR amplification conditions for COI and *Cardinium* 16S with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) and for 18S with KOD FX Neo (Toyobo Life Science, Japan) were as described by Munakata et al. (2021). Amplification conditions for 28S with KOD FX Neo were 94°C for 2 min; 45 cycles of 98°C for 10 s, 50°C for 30 s, and 68°C for 90 s; and 68°C for 3 min. All nucleotide sequences were determined by direct sequencing using a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA 7 (Kumar et al., 2016). BLAST (Altschul et al., 1990) was used to search the International Nucleotide Sequence Database (INSD, 2024) for sequences most similar to ours.

Phylogeny

We assembled a concatenated 18S + 28S dataset that included sequences for 31 cypridoid species and one outgroup taxon, the pontocyprid *Pontocypris mytiloides* (Norman, 1862) (see Supplementary Table S1). The 18S and 28S sequences were aligned independently by using the "Q-INS-i" strategy (Katoh and Toh, 2008) in MAFFT ver. 7 (Katoh and Standley, 2013), aligned further by eye according to the secondary structure of the honeybee 18S and 28S sequences (Gillespie et al., 2006), and then trimmed with MEGA7 to match the shortest length for each gene (see Supplementary File S1). Alignment-ambiguous sites were removed with Gblocks ver. 0.91b (Castresana, 2000) in NGPhylogeny.fr (Lemoine et al., 2019) under the "relaxed" parameters described in Talavera and Castresana (2007). The final dataset contained 3556 aligned positions (1985 positions for 28S and 1571 positions for 18S; see Supplementary File S2). Methods for selecting the optimal substitution model (GTR + F + G + R3 for 28S; GTR + F + G + R2 for 18S), the maximum likelihood (ML) analysis, estimating branchial support (1000 pseudoreplicates for both Shimodaira–Hasegawa-like approximate likelihood ratio tests [SH-aLRT] and ultrafast bootstraps [uBS]), and drawing the tree were as described by Shimada et al. (2023).

RESULTS

Taxonomy

Family **Cyprididae** Baird, 1845
Subfamily **Cyprinotinae** Bronstein, 1947
Genus ***Thermocypris*** Munakata, gen. nov.

Diagnosis. No dorsal hump on carapace; no tubercles on RV or LV; LV overlapping RV entire margins with slight ventral expansion; no selvage on anterior part of RV; only seta d1 on first podomere of L6; two shields (medial and lateral) on hemipenis; unilobed ms and tooth-shaped ls.

Type species. *Thermocypris isabella* Munakata, gen. et sp. nov., by original designation.

Etymology. *Thermocypris* is from the Ancient Greek adjective 'θερμός' (*thermós*; hot) and 'cypris' (a common suffix for ostracods in Cyprididae). Gender: feminine.

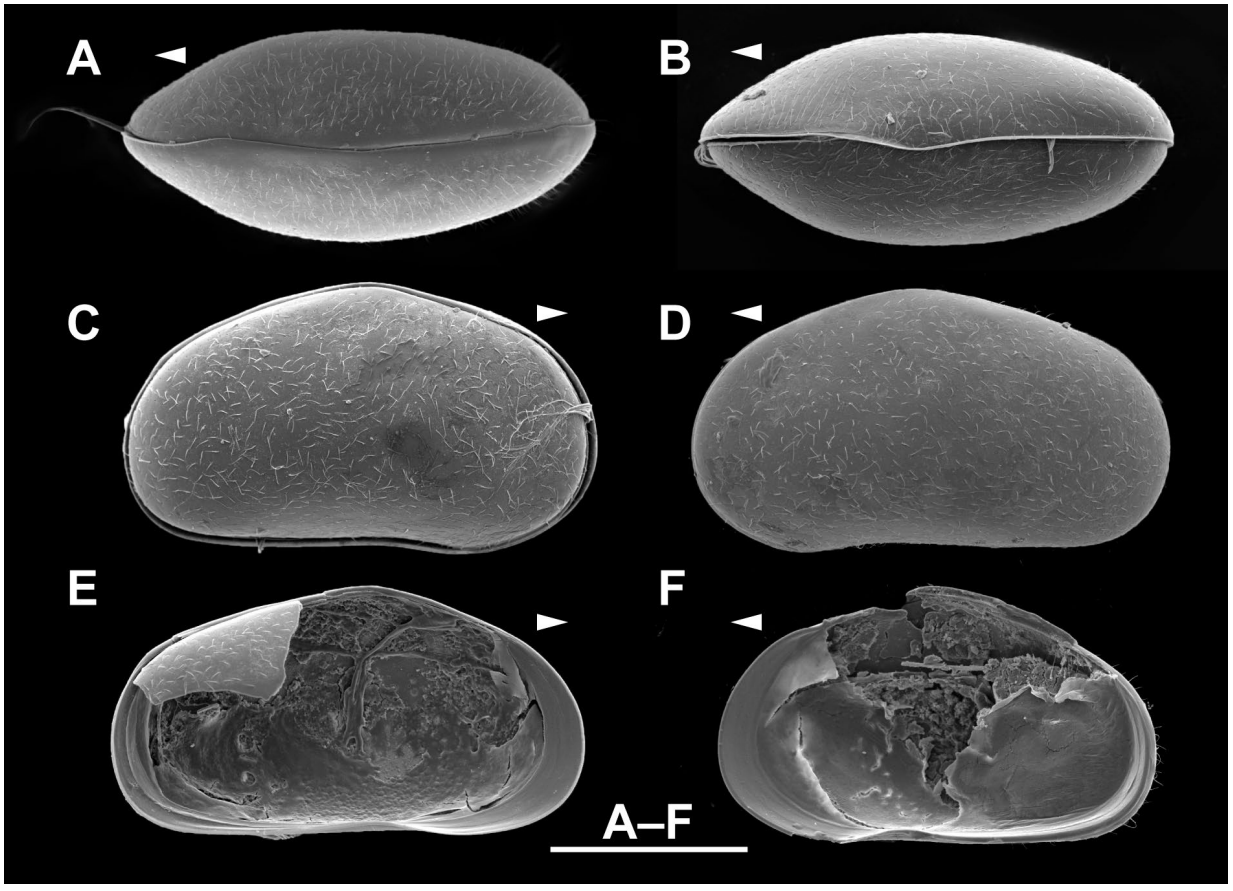


Fig. 2. SEM images of carapaces and valves of female *Thermocypris isabella* gen. et sp. nov.; **(A–D)** paratype (ICHUM-8924), **(E, F)** paratype (ICHUM-8931). **(A–D)** Dorsal, ventral, right lateral, and left lateral exterior views of whole carapace. **(E, F)** Interior views of left and right valves. Arrowheads indicate anterior direction. Scale bar: 0.3 mm.

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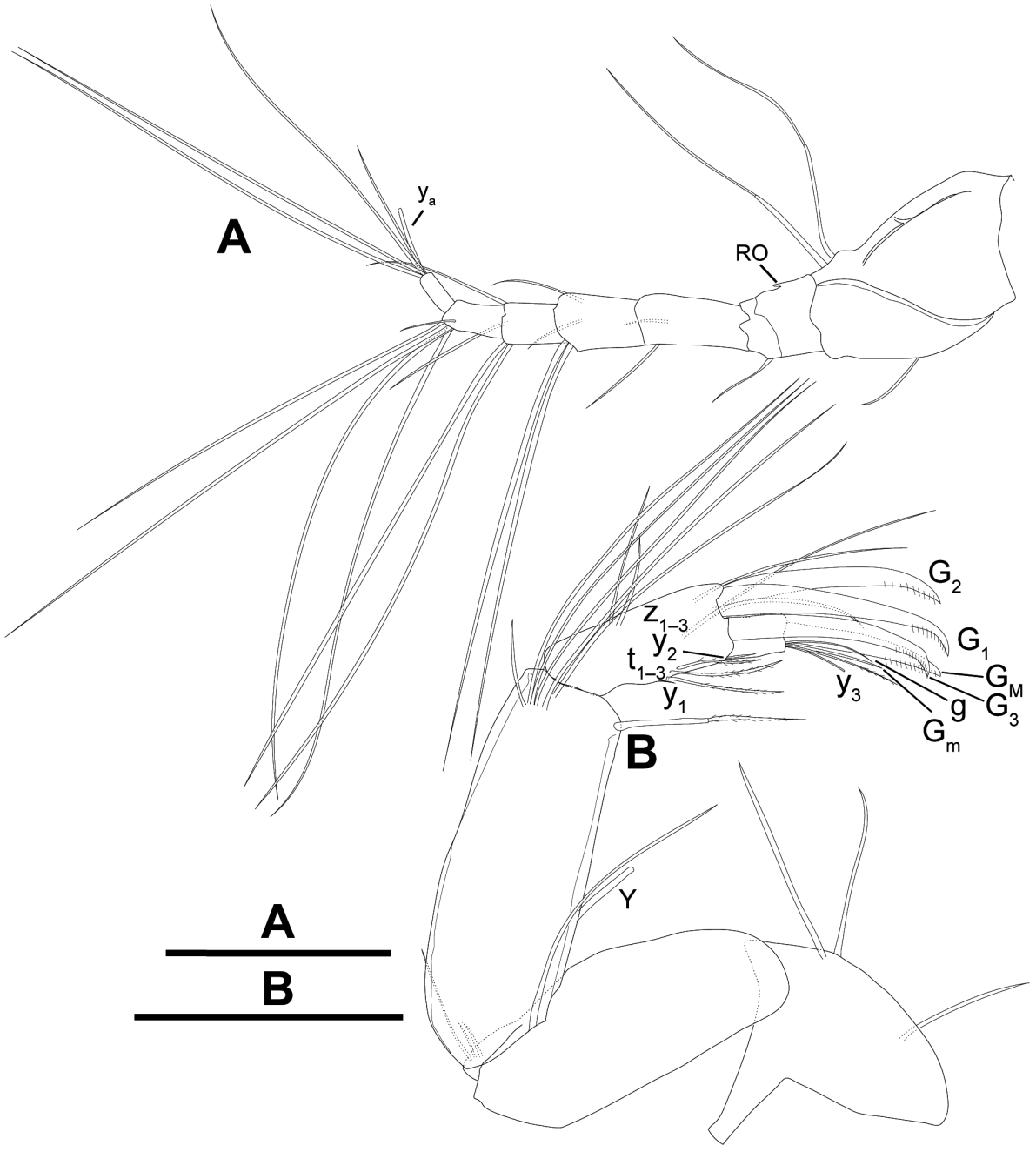


Fig. 3. *Thermocypris isabella* gen. et sp. nov., holotype, female (ICHUM-8925). **(A)** Antennula. **(B)** Antenna, inner view. Abbreviation: RO, Rome organ. Scale bars: 0.1 mm.

Thermocypris isabella Munakata, gen. et sp. nov.
(Figs. 2–6)

Diagnosis. Same as the generic diagnosis.

Type locality. Baños del Inca, a hot spring in Cajamarca, Peru (7.1635°S, 78.4709°W).

Material examined. Holotype: ICHUM-8925, female, two slides. Paratypes: ICHUM-8928–8930, two females and male, two slides for each; ICHUM-8931, females, one SEM stub; ICHUM-8924, female, one SEM stub; ICHUM-8926, 8927, sex unknown, undissected, one vial for each. All individuals were collected at the type locality by Nilton Deza on 24 November 2022.

Etymology. The specific name is a noun in the nominative case and named after an author's granddaughter, Isabella.

Description of female. For measurements of carapace and valves, see Table 1. Carapace (Fig. 2) orange colored (Fig. 1B); eye black; carapace soft, easily torn during dissection; outer surface smooth but with sparse tiny setae; widest point at about mid-length (Fig. 2A, B); anterior and posterior ends rounded, not beak-shaped in dorsal view (Fig. 2A); outer list absent (Fig. 2B). LV overlapping RV along entire margin, with slight ventral expansion (Fig. 2B, C); greatest

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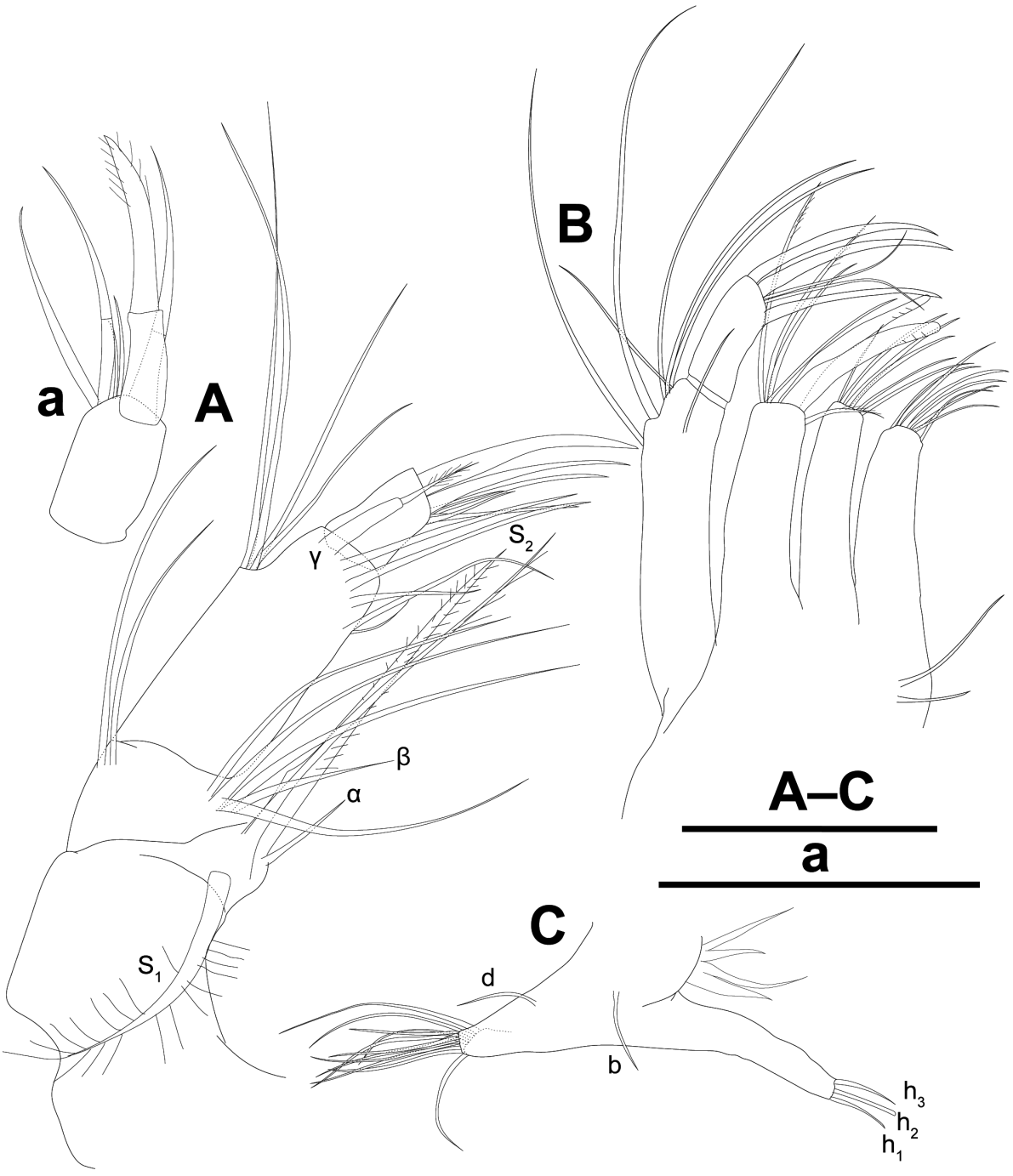


Fig. 4. *Thermocypris isabella* gen. et sp. nov., female: holotype (**A–C**, ICHUM-8925); paratype (**a**, ICHUM-8928). **(A)** Mandible, inner view, coxa and vibratory plate omitted. **(a)** Mandible, fourth palpal podomere. **(B)** Maxillula. **(C)** Fifth limb. Scale bars: 0.1 mm.

height of LV at mid-length; anterodorsal and posterodorsal margins smooth; posterior margin slightly more sharply rounded than anterior; ventral margin slightly concave; apex of anterior margin lower than mid-height of LV and higher than apex of posterior margin (Fig. 2D); in inner view, weak inner list present on anteroventral margin; inner list present on posterior margin; calcified inner lamella on anterior, posterior, and ventral margins narrow; without selvage (Fig. 2E). RV similar to LV in shape (Fig. 2C, F). Two oblong mandibular muscle scars and five oblong adductor muscle scars on LV and RV. Hinge adont.

An1 (Fig. 3A) with seven podomeres. Podomere length ratio from second to seventh podomeres 7: 12: 7: 6: 5: 4. First podomere with one dorsal and two ventrodistal setae; Wouters organ absent. Second podomere with dorsodistal seta reaching middle of third podomere; Rome organ present. Third podomere with dorsodistal seta reaching tip of fourth podomere and ventrodistal seta not reaching middle of fourth podomere. Fourth podomere with two long dorsodistal setae not reaching tip of long setae on sixth podo-

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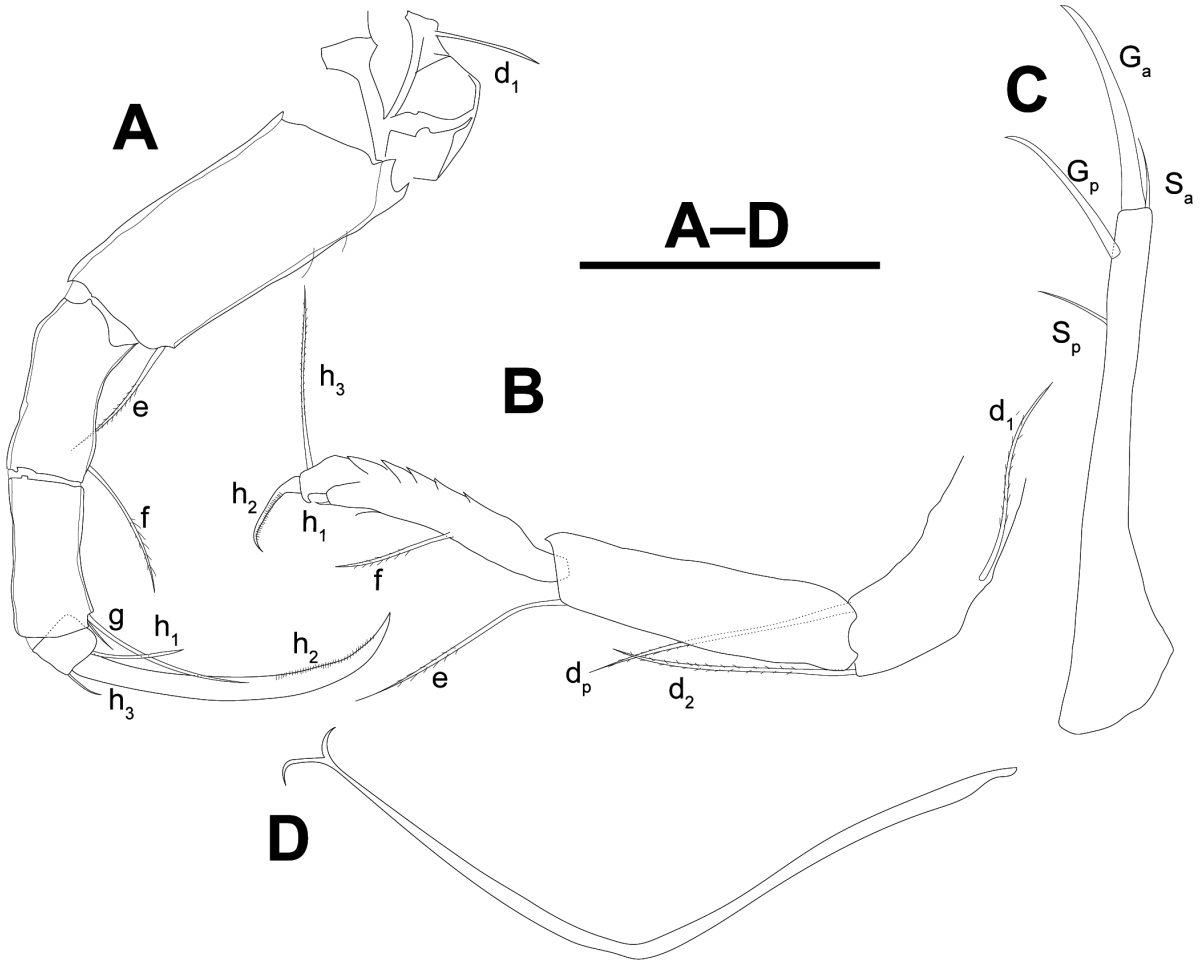


Fig. 5. *Thermocypris isabella* gen. et sp. nov., holotype, female (ICHUM-8925). **(A)** Sixth limb. **(B)** Seventh limb. **(C)** Caudal ramus. **(D)** Caudal ramus attachment. Scale bar: 0.1 mm.

mere, and two shorter ventrodistal setae (one reaching tip of fifth podomere; one not reaching tip of fifth podomere). Fifth podomere with two long dorsodistal setae reaching tip of long setae on seventh podomere, and two shorter ventrodistal setae reaching beyond tip of seventh podomere. Sixth podomere with four outer dorsodistal long setae as long as podomeres 2–7 and shorter inner dorsodistal seta. Seventh podomere with three long distal setae reaching tips of long setae on sixth podomere, and aesthetasc y_a (one-sixth as long as long setae).

An2 (Fig. 3B) with first podomere (coxa) having three ventral setae. Second podomere (basis) with ventro-subdistal seta reaching tip of third podomere. Third (first endopodal) podomere undivided, with six inner subdistal natatory setae (five long setae not reaching beyond tip of claws on fifth podomere and one short seta), ventrodistal plumed seta reaching tip of fourth podomere, and mid-ventral aesthetasc Y not reaching beyond tip of third podomere. Fourth podomere with two mid-dorsal setae, dorso-subdistal setae $z_{1–3}$ of unequal length, mid-ventral setae $t_{1–3}$ not reaching middle of claws G_1, G_3, and distal claws $G_{1–3}$, mid-ventral short aesthetasc y_1, and ventrodistal short aesthetasc y_2. Fifth podomere with plumed seta g reaching to about three-fourths length of claw G_M, bifurcate aesthetasc y_3 (longer than half-length of claw G_M), and G_m shorter than G_M; G_M reaching tip of claws $G_{1–3}$. Exopodite with one long and two short setae.

Md (Fig. 4A, a) with coxa, palp comprising four podomeres (one basal and three endopodal) and vibratory plate (not illustrated). Coxa with three sub-distal setae (not illustrated). First palpal podomere (basis) with one ventrodistal seta, ventrodistal setae S_1 and S_2, and ventrodistal short seta α (about one-third length of seta S2); setae S_1 and S_2 unequal in length, bearing rows of long setules. Vibratory plate (exopodite) with several rays. Second palpal (first endopodal) podomere with two dorsodistal setae of unequal length (longer seta reaching tip of third palpal podomere), four ventrodistal setae not extending beyond tip of claws on fourth palpal podomere, and ventral short seta β (about half length of ventrodistal setae). Third palpal podomere with four dorso-subdistal setae and two ventro-subdistal setae; inner region with distal plumed seta γ and three distal setae. Fourth palpal podomere with three distal setae (two short setae and one long seta) and three distal claws (Fig. 4a).

Mx (Fig. 4B) with palp comprising two podomeres, three endites, and vibratory plate (not illustrated). First palpal podomere with one short outer distal seta; on dorsodistal margin, one short and five long dorsodistal setae reaching

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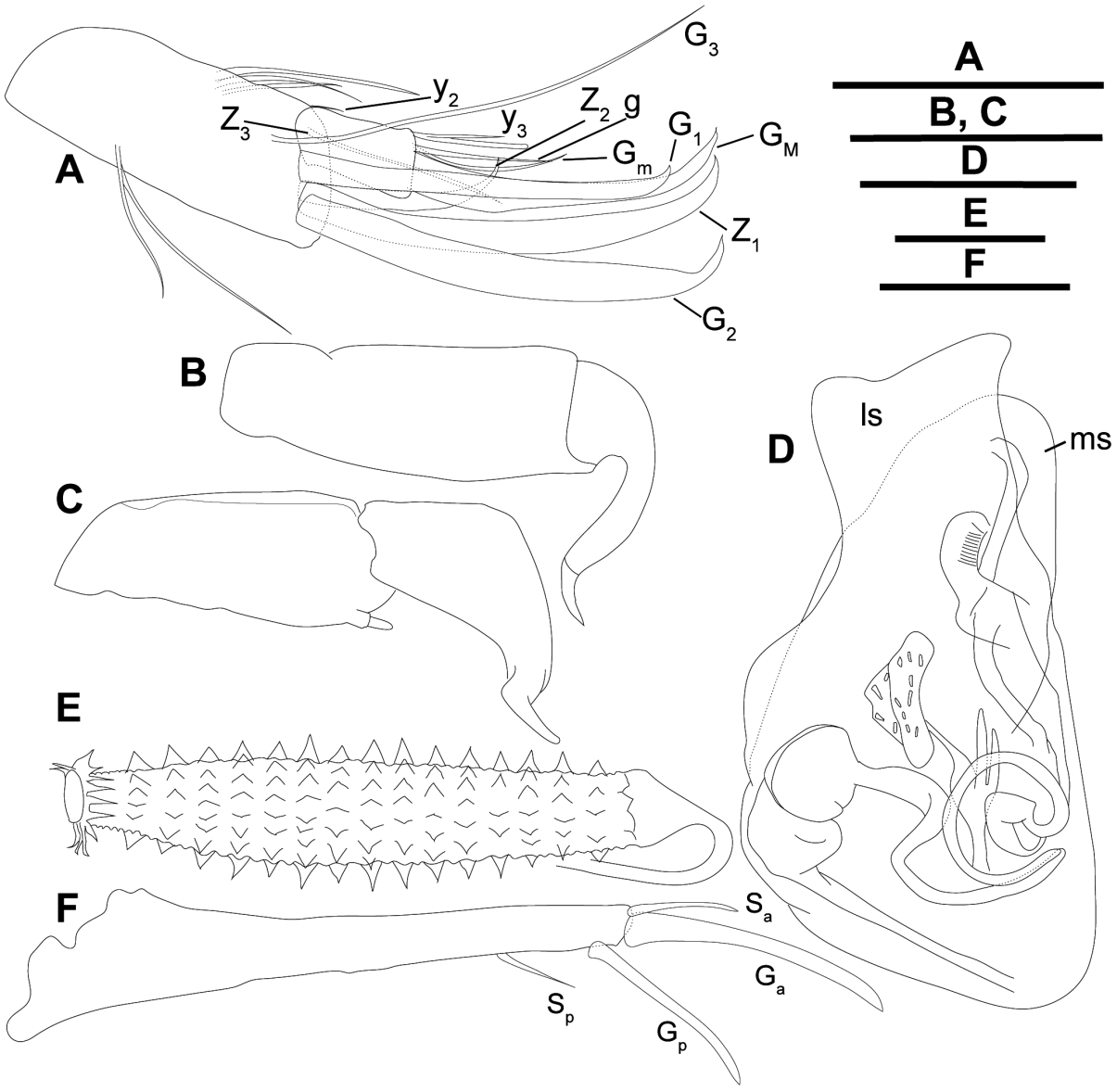


Fig. 6. *Thermocypris isabella* gen. et sp. nov., paratype, male (ICHUM-8930). **(A)** Fourth and fifth podomeres of antenna. **(B, C)** Left and right prehensile palps of fifth limb, respectively. **(D)** Hemipenis. **(E)** Zenker organ. **(F)** Caudal ramus. Scale bars: 0.05 mm.

Table 1. Measurements (in millimeters) of carapace and valves for *Thermocypris isabella* gen. et sp. nov. Abbreviations: Ca-W, width of the carapace; H, height; L, length; LV, left valve; RV, right valve. The asterisk indicates a holotype.

				LV			RV		
	sex	Ca-W	Ca-W/LV-L	H	L	H/L	H	L	H/L
ICHUM-8924	F	0.32	0.45	0.39	0.71	0.55	0.38	0.69	0.55
ICHUM-8925*	F	0.33	0.46	0.37	0.71	0.53	0.36	0.69	0.53
ICHUM-8926	–	0.29	0.43	0.36	0.69	0.53	0.30	0.58	0.52
ICHUM-8927	–	0.33	0.55	0.32	0.61	0.53	0.28	0.56	0.50
ICHUM-8928	F	0.32	0.47	0.34	0.67	0.51	0.33	0.65	0.50
ICHUM-8929	F	0.30	0.46	0.35	0.66	0.53	0.33	0.64	0.52
ICHUM-8930	M	0.32	0.46	0.35	0.68	0.52	0.33	0.67	0.49
Mean value		**0.32**	**0.47**	**0.36**	**0.68**	**0.53**	**0.33**	**0.64**	**0.52**

tip of claws on second palpal podomere. Second palpal podomere rectangular, L/W about 2.3, with two distal setae and three distal claws. First endite with two ventro-proximal and seven distal setae. Second endite with six distal setae. Third endite with two serrated tooth-bristles and five distal setae (three of them plumed).

L5 (Fig. 4C) with protopod, palp, and vibratory plate. Protopod with seta b, seta d, and approximately 11 distal setae of unequal length. Palp with distal setae $h_{1–3}$. Vibratory plate with several rays.

L6 (Fig. 5A) with five podomeres. Length ratio from second to fifth podomeres and terminal claw (h_2) 55: 31: 29: 8: 55. First podomere (protopod) with seta d_1. Second (first

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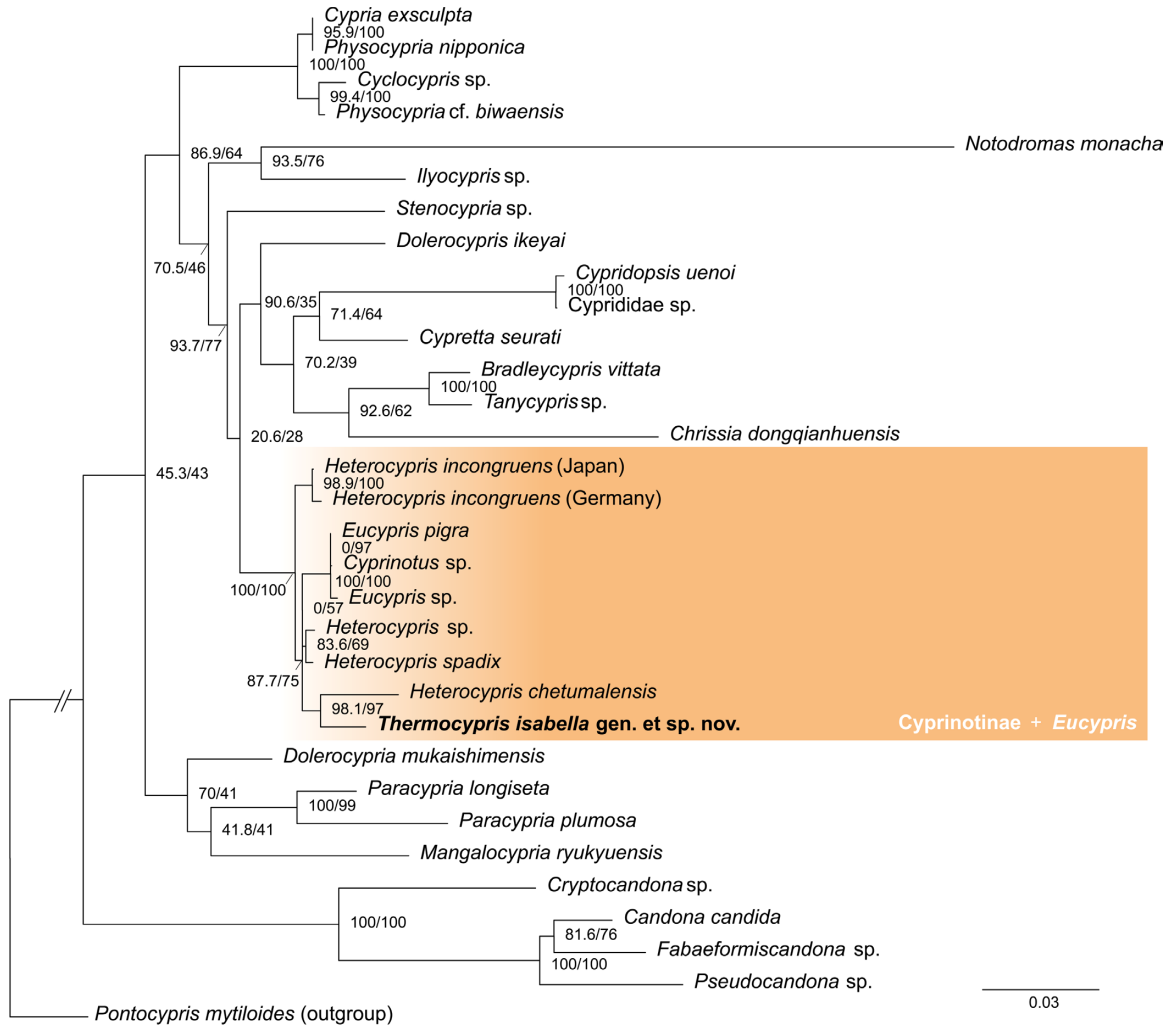


Fig. 7. Maximum-likelihood tree for cypridoidean ostracods based on 18S + 28S sequences (3556 positions). Numbers next to nodes indicate values from the Shimodaira–Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap support (uBS). The scale at bottom right indicates branch length in substitutions per site.

endopodal) podomere with ventrodistal plumed seta e reaching tip of third podomere. Third podomere not fused to fourth podomere, with ventrodistal plumed seta f not reaching tip of fifth podomere. Fourth podomere with ventrodistal seta g (half as long as h_2) and short seta. Fifth podomere with dorsodistal seta h_3, ventrodistal seta h_1, and distal curved claw h_2; h_1 twice as long as h_3.

L7 (Fig. 5B) with four podomeres, bearing pincer organ formed by third and fourth podomeres. First podomere (protopod) with plumed setae d_1, d_2, d_p. Second (first endopodal) podomere with ventrodistal plumed seta e not reaching beyond tip of fourth podomere. Third podomere with midventral plumed seta f not reaching tip of seta h_1. Fourth podomere fused to third podomere, with long plumed seta h_3, hook-like seta h_2, and tiny seta h_1.

CR (Fig. 5C) with CR-L/CR-W ratio about 17. L ratio of ramus, plumed seta S_a, claw G_a, claw G_p, and plumed seta S_p 50: 6: 20: 15: 7.

CR attachment (Fig. 5D) slender, longer than CR (ramus + G_a), with bifurcate proximal tiny end.

Description of male. For measurements of carapace and valves, see Table 1. Carapace, An1, Md, Mx, L6, L7, CR, and CR attachment as in female (not illustrated except for CR).

An2 (Fig. 6A) showing sexual dimorphism typical of this family in distal part (cf. Martens, 1987).

L5 (Fig. 6B, C) first podomere on left prehensile palp rectangular; second podomere curved, flagellate. First podomere on right prehensile palp rectangular, with ventrodistal seta; second podomere subtriangular, with distal flagellum.

Hemipenis (Fig. 6D) with two shields, ls and ms; ms unilobed, distal region bluntly pointed; ls tooth-shaped, narrower and longer than ms.

Zenker organ (Fig. 6E) about five times as long as wide, with about 15 spinous whorls.

CR (Fig. 6F) as in female.

Accompanying fauna. *Hyalella yashmara* Tomikawa, Kawasaki, Miranda, and Deza, 2023 (Crustacea: Amphipoda: Hyalellidae). No other ostracod species were collected.

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Molecular information and analysis

We determined a partial COI sequence (658 bp, encoding 219 amino acids; INSD accession number LC832419), a partial 28S sequence (3342 bp; LC832418), and the nearly complete 18S sequence (1762 bp; LC832419) for one *T. isabella* specimen (ICHUM-8931). The most similar known sequences to ours, as determined by BLAST searches, were COI from Podocopida sp. (HM432569.1; identity score 82.15%, query cover 98%; unpublished), 28S from *Stenocypria* sp. (AB674993; identity score 88.37%, query cover 100%; Hiruta et al., 2016), and 18S from *Eucypris pigra* (Fischer, 1851) (AB675004.1; identity score 98.30%, query cover 100%; Hiruta et al. 2016), respectively. No *Cardinium* infection was detected by PCR amplification with specific primers.

In the 18S + 28S based ML tree (Fig. 7), *T. isabella* appears as the sister taxon to *Heterocypris chetumalensis* (Yoo, Cohuo, Macario-Gonzalez, and Karanovic, 2017), with high branchial support (98.1/97%, SH-aLRT/uBS). A clade comprising Cyprinotinae (*Heterocypris*; *Cyprinotus*; *Thermocypris*) and Eucypridinae (*Eucypris* Vavra, 1891) is fully supported.

DISCUSSION

Taxonomy and phylogeny

Thermocypris gen. nov. differs from the other nine cyprinotine genera in having: (1) no dorsal hump on the carapace; (2) no tubercles on the RV or LV; (3) LV overlapping RV along all margins; (4) no selvage in the anterior part of the RV; (5) only d1 on the first podomere of L6; (6) only two lobes (medial and lateral shields) on the hemipenis; and (7) a unilobed medial shield and a tooth-shaped lateral shield. The following traits are also useful for identifying this genus: longest seta on exopodite of An2 short, not beyond one-third of first endopodal podomere in length; only three t-setae on An2; claw G_2 on An2 not reduced, almost same length as claw G_1; first endopodal podomere of Md with two dorsodistal setae; second palpal podomere of Mx rectangular; third endite on Mx with two serrated tooth-bristles.

In our 18S + 28S tree, *T. isabella* formed a highly supported clade with *Heterocypris chetumalensis*. The monophyly of *Heterocypris* has been questioned based on morphological evidence (see Halse and Martens, 2019), and this was also the case in previous molecular phylogenetic studies, though the corresponding branches were not well supported (Kong et al., 2014; Yoo et al., 2017). As *Thermocypris* cannot be assigned to *Heterocypris* morphologically (no tubercles on the RV or LV in the former), the high branchial support value for the *T. isabella* + *H. chetumalensis* clade is the first strong molecular evidence supporting non-monophyly for *Heterocypris. Heterocypris chetumalensis* was originally described as the type species of *Manuelcypris* Yoo, Cohuo, Macario-Gonzalez, and Karanovic, 2017 (Yoo et al., 2017), which is currently regarded as a junior synonym of *Heterocypris* (e.g., Halse and Martens, 2019; Meisch et al., 2024). Although the diagnoses largely overlap between *Manuelcypris* and *Heterocypris*, our result grouping *M. chetumalensis* in a clade separate from *H. incongruens* (Ramdohr, 1808) (the type species of *Heterocypris*) suggests that the synonymy of *Manuelcypris* with *Heterocypris* should be reconsidered through detailed morphological observations.

In previous studies (Kong et al., 2014; Yoo et al., 2017), Eucypridinae was nested in a Cyprinotinae clade. The validity of these two subfamilies should be extensively reexamined by means of integrative taxonomy based on topotypic specimens.

A new thermal-freshwater ostracod

The carapace of *T. isabella* was soft and easily broken during dissection. High water temperature is known to decrease carapace thickness (Glandon et al., 2018) and increase the Mg/Ca ratio (Chivas et al., 1986) in crustaceans, and the soft carapace is consistent with *T. isabella* inhabiting a high-temperature environment. The preliminary experiment to investigate survival temperature range for *T. isabella* using a bain-marie system found the following: when temperature was gradually increased, ostracods died at 61.8°C on average; when decreased, they exhibited immobility but remained alive at 25°C, and then died at 19°C on average (Nilton Deza, unpublished data). These results suggest that the distribution of this species may be restricted to warm water bodies around Baños del Inca hot spring.

Some cypridid species inhabit rice-fields where water temperature can become seasonally high (e.g., *Heterocypris incongruens*; *Cyprinotus uenoi* Brehm, 1936; Smith et al., 2018), suggesting that more ostracod species than we are currently aware of potentially have high-temperature tolerance.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

ND, YK, and AM collected specimens. MM and HT made morphological observations and dealt with taxonomy/classification. MM conducted molecular analyses, made figures, and wrote the first draft. All authors contributed to the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs240090)

Supplementary Table S1. List of species included in the molecular phylogenetic analysis, with GenBank accession numbers and source references.

Supplementary File S1. Aligned 18S + 28S sequences used for the maximum-likelihood analysis, trimmed in MEGA7 to the shortest length among the concatenated sequences.

Supplementary File S2. Aligned 18S + 28S sequences used for the maximum-likelihood analysis, reduced to 3556 positions by removing alignment-ambiguous sites with Gblocks ver. 0.91b in NGPhylogeny.fr under “relaxed” parameters.

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