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Taxonomy and natural history of *Cavernocypris hokkaiensis* sp. nov., the first ostracod reported from alpine streams in Japan

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Abstract

We describe the cypridoidean ostracod *Cavernocypris hokkaiensis* sp. nov. from riverbed sediments in an alpine stream at an elevation of ca. 1850 m in the Taisetsu Mountains, Hokkaido, Japan. This species differs from congeners in having (1) the outer surface of the carapace smooth, with sparse, tiny setae, but without shallow pits; (2) the carapace elongate rather than triangular in lateral view; (3) the antennula consisting of seven podomeres; (4) first palpal podomere of maxillula with five dorsodistal and one ventro-subdistal setae; (5) the fifth limb lacking setae b and d; and (6) the fifth limb lacking a vibratory plate. We provided the key to the *Cavernocypris* species. We determined partial sequences for the cytochrome *c* oxidase subunit I (COI; *cox1*) and 18S rRNA (18S) genes in *C. hokkaiensis*. Our sample contained only females, and we obtained a partial 16S rRNA sequence for the endosymbiotic bacterium *Cardinium* from *C. hokkaiensis*, indicating the possibility that this ostracod species reproduces parthenogenetically. Our field survey and observations of captive individuals suggested that *C. hokkaiensis* may be endemic to the Taisetsu Mountains, with a low population density, narrow distributional range, and slow maturation to sexual maturity.

Key Words

Crustacea, cryophilic, Cypridoidea, ecology, lotic, Ostracoda

Introduction

The genus *Cavernocypris* Hartmann, 1964, one of 20 genera in the subfamily Cypridopsinae Kaufmann, 1900 (Savatenalinton 2018, 2020; Meisch et al. 2019), is distinguished from the other 19 genera by the following combination of features (cf. Smith et al. 2017): (1) carapace elongate to triangular in lateral view, (2) left valve overlapping right valve along ventral margin, (3) surface of valves smooth or with small pits, (4) swimming setae of antennae very short, (5) distal segment of maxillular palp elongate, (6) terminal segment of seventh limb not separated, and (7) uropodal ramus flagellum-like and present only in females.

To date, six *Cavernocypris* species have been described from the Palearctic and Nearctic regions

(Meisch et al. 2019; Külköylüoğlu 2020); in Japan, one species, *C. cavernosa* Smith, 2011, has been reported from Shiga and Shizuoka prefectures (Smith 2011; Tanaka et al. 2015). *Cavernocypris* species inhabit the interstitial environment of riverbed sediments, the littoral zone of mountain lakes, springs, and caves (Marmonier et al. 1989; Smith et al. 2017). Although there is little information on their ecology, Forester (1991) found *C. wardi* Marmonier, Meisch & Danielopol, 1989 only in cold-water habitats at ca. 0–14 °C and suggested that the species may be cryophilic.

Streams in the alpine vegetation zone between the treeline and the permanent snow line are cold and nutrient-poor (Niedrist and Füreder 2017). They arise from glacial melt, snowmelt, rain runoff, and groundwater springs and are highly environmentally heterogeneous (Hotaling et

al. 2017). Many organisms inhabiting alpine streams are endemics (e.g. Muhlfield et al. 2011), uniquely adapted to harsh conditions (Lencioni et al. 2009). Ostracods are often detected in ecological research in alpine streams (e.g., Suren 1993; Zbinden et al. 2008), but their taxonomy, particularly outside Europe, has not been well studied.

The Taisetsu Mountains in Daisetsuzan National Park are located in the center of Hokkaido, Japan, and consist of several gently sloping volcanic peaks in the 2000 m class. Above the treeline at ca. 1400–1500 m elevation (Amagai et al. 2018), there are several aquatic features, including alpine streams, but except for insects (e.g. Konno 2003; Konno et al. 2003), the aquatic invertebrates have not yet been investigated.

Here we describe a new species of *Cavernocypris* from an alpine stream in the Taisetsu Mountains, the first record of an ostracod from cold alpine waters in Japan. We present nucleotide sequences for this species for parts of the mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 18S rRNA (18S) genes and provide preliminary comments on its phylogenetic position based on 18S data. We also present information on its natural history based on a field survey and preliminary rearing results. Finally, we demonstrate with molecular data (part of the mitochondrial 16S rRNA gene, 16S) the infection of this species by *Cardinium*, a group of “reproduction-manipulating” endosymbiotic bacteria (Ma and Schwander 2017).

Materials and methods

Sampling was conducted at seven sites, including four streams (Stns 1–3, 7) fed by springs, a hot spring, and/or snowmelt, two ponds (Stns 5, 6), and one waterfall basin (Stn. 4) (Table 1, Fig. 1). Bottom sediment and water were placed in a bucket and stirred, and all but the sediment was filtered through a plankton net (mesh size 63 µm). This process was repeated several times at each site. Specimens were picked from the samples using a stereomicroscope (Olympus SZX9, Japan). Geographical coordinates and the elevation were obtained from GSI Maps (Geospatial Information Authority of Japan 2022). Water temperature was measured by using an O-274 thermometer (DRETEC, Japan).

Ostracods were fixed in 80% 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). All material studied has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, under catalog numbers ICHUM-8247 to 8252.

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, sixth, and seventh limbs, respectively; UR, uropodal ramus. 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 UR. The following measurements were made from digital images by using ImageJ (Rasband 2022): L and H of the LV and RV (LV-L, LV-H, RV-L, and RV-H); W of the carapace (Ca-W). Measurements in the text are in millimeters, followed by the mean value and sample size in parentheses.

An attempt was made to extract total DNA from the soft parts of three individuals by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany) following the manufacturer’s protocol, but only one of the three extracts allowed successful PCR amplification. Primers used for the PCR amplification and sequencing of ostracod COI, ostracod 18S, and *Cardinium* 16S are listed in Munakata et al. (2021), except that CLO-f2 (GGTGC GTGGGCGGCTTATT) and CLO-r2 (AAAGGGTTTCGCTCGTTATAG) (Gotoh et al. 2007) were used instead of CLO-f1 and CLO-r1. PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan), for 18S with KOD FX Neo (Toyobo Life Science, Japan), and for 16S with TaKaRa Ex Taq were as described by Munakata et al. (2021). All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated using MEGA7 (Kumar et al. 2016). BLAST (Altschul et al. 1990) was used to search the International Nucleotide Sequence Database (INSD; International Nucleotide Sequence Database Collaboration 2022) for nucleotide sequences most similar to our sequences.

Table 1. List of sampling sites in Daisetsuzan National Park.

Station number and name	Environment	Coordinates	Elevation	Mean water temperature	Sampling date
1. Hokkai-sawa Stream	Stream fed by spring	43°41'08"N, 142°55'28"E	1853 m	3.5 (2.2–4.8) °C	25.viii.2020, 26.vii.2021
2. Hokkai-sawa Stream	Stream fed by spring and snowmelt	43°41'17"N, 142°54'33"E	1837 m	4.1 (2.4–5.7) °C	25.viii.2020, 26.vii.2021
3. Akaishi Stream	Stream fed by hot spring with high-H ₂ S concentration and snowmelt	43°41'23"N, 142°54'35"E	1829 m	13 °C	26.vii.2021
4. Momizi Fall	Fall basin in Akaishi Stream	43°43'72"N, 142°57'41"E	813 m	13 °C	27.vii.2021
5. Sugatami Pond	Pond	43°39'41"N, 142°49'58"E	1665 m	No data	26.viii.2020
6. Unnamed Pond	Pond	43°39'43"N, 142°49'32"E	1597 m	No data	26.viii.2020
7. Daisetsu Asahidake Spring	Stream fed by spring	43°37'59"N, 142°41'31"E	445 m	7.0 °C	26.viii.2020

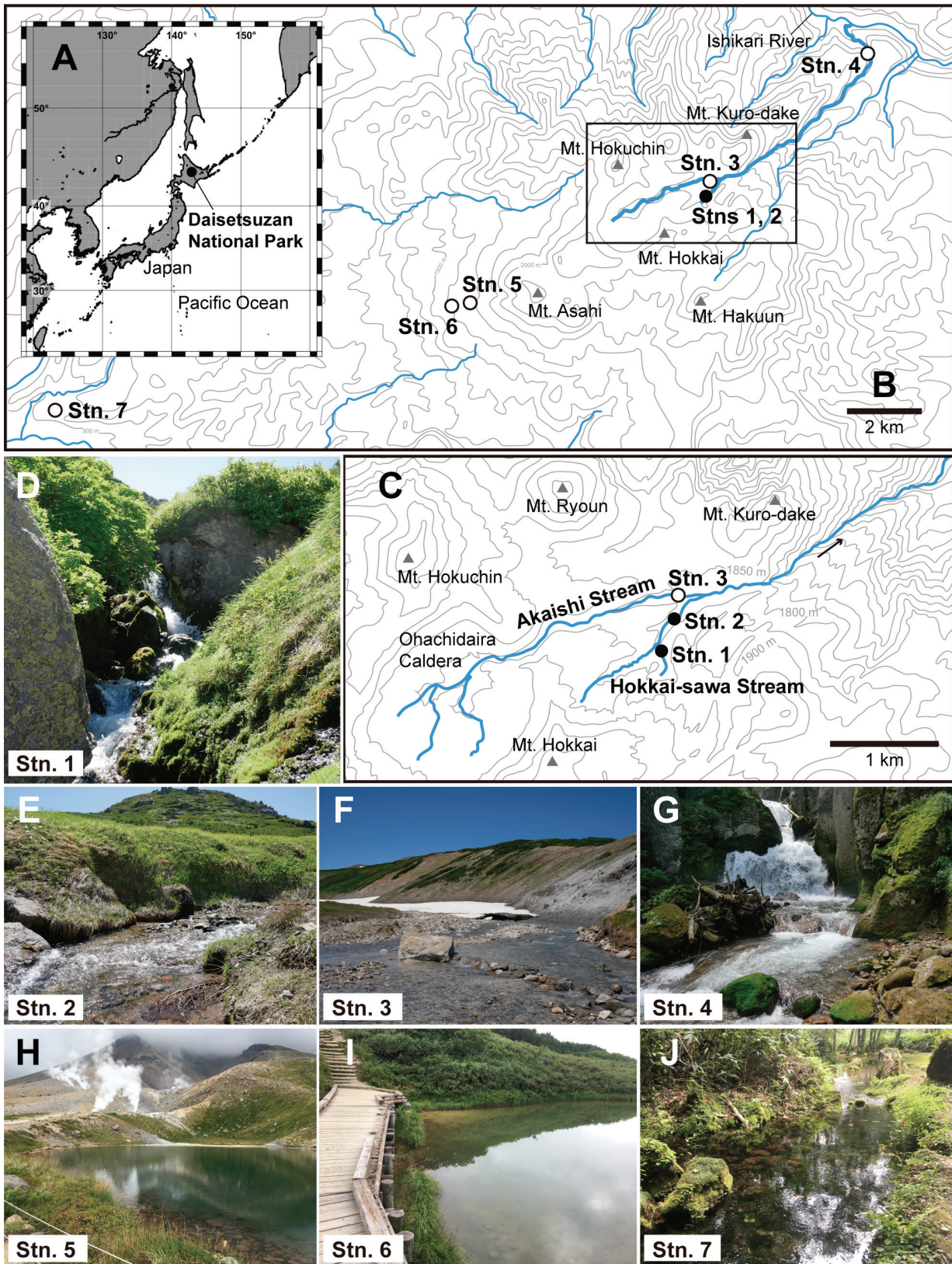


Figure 1. Sampling sites for *Cavernocypris hokkaiensis* sp. nov. **A.** Map showing location of Daisetsuzan National Park in Japan; **B.** Map showing the sampling sites (Stns 1–7) in Daisetsuzan National Park; **C.** Enlarged map of the area corresponding to the square in Fig. 1B; arrow indicates the stream flow direction; **D–J.** Photographs of the sampling sites; **D.** Hokkai-sawa Stream (Stn. 1); **E.** Hokkai-sawa Stream (Stn. 2); **F.** Akaishigawa Stream (Stn. 3); **G.** Momizi Fall (Stn. 4); **H.** Sugatami Pond (Stn. 5); **I.** Unnamed pond (Stn. 6); **J.** Daisetsu Asahidake Spring (Stn. 7). Maps were generated by using GMT6 (A Wessel et al. 2019) or were based on GSI Maps (B, C Geospatial Information Authority of Japan 2022). Filled and open circles indicate sites where *Cavernocypris* individuals were collected or not found, respectively.

To explore the phylogenetic position of this species, a maximum likelihood (ML) phylogenetic tree was constructed based on the 18S dataset comprised of 66 ostracod sequences (one our sequence, and 64 cypridoidean and one pontocypridoidean (outgroup) sequences from INSD; 1547 positions in the aligned dataset; see Suppl. material 1–3: Table S1, Alignments S1, S2). The detailed method and result are shown in Suppl. material 4: File S1.

To obtain information on the life cycle, three non-adult individuals collected on 26 July 2021 were maintained singly in wells of a tissue culture plate filled with water collected from sampling site Stn. 1 and placed in a refrigerator at a temperature of 7 °C. Detritus collected from sampling site Stn. 1 was added to each well as a food source. Observations were made twice or more per month.

Results

Field survey and observation of captive individuals

Among seven sampling sites, *Cavernocypris* ostracods were collected from only two sites in the Hokkai-sawa Stream (Stns 1 and 2) (Fig. 1B, C). Ostracod density at both sites was low, with fewer than 10 individuals per 500 ml of filtered residue. No male individuals were detected.

Three captive non-adult individuals have remained alive and active for more than five months. The body length (LV-L) of each individual was 0.47, 0.48, 0.39 mm. No molts have been observed to date (the latest observation was on 7 January 2022).

Taxonomy

Family Cyprididae Baird, 1845

Genus *Cavernocypris* Hartmann, 1964

Cavernocypris hokkaiensis sp. nov.

<http://zoobank.org/3DBCDFE2-957F-485F-A91E-036E71EC75C0>

Figs 2, 3

Etymology. The epithet *hokkaiensis* is an adjective referring to the type locality, Hokkai-sawa Stream in Japan.

New Japanese name. *Shibare-doukutsu-kaimijinko*, referring to the habitat having low water temperature. *Shibare* is derived from the Japanese verb *shibare-ru* (freeze), in a Hokkaido dialect; *Doukutsu-kaimijinko* is the Japanese name for *Cavernocypris* (Tanaka et al. 2015).

Type locality. Hokkai-sawa Stream, Daisetsuzan National Park, Hokkaido, Japan (Stn. 1: 43°41'08"N, 142°55'28"E).

Holotype: female, ICHUM-8247, one slide and one SEM stub, Stn.1, Hokkai-sawa Stream, riverbed sediment, 26 July 2021. **Paratypes** (five females): ICHUM-8248, 8249, one SEM stub and one slide for

each; ICHUM-8250, 8251, one SEM stub; ICHUM-8252, one slide, voucher specimen for LC666823 (COI) and LC666824 (18S). Collection data for ICHUM-8249, 8252 are same as holotype; ICHUM-8248, 8251 were collected from Stn. 2 (43°41'17"N, 142°54'33"E) on 25 August 2020; ICHUM-8250 was collected from Stn. 1 on 25 August 2020. All individuals were collected by Mizuho Munakata.

Description of females. Measurements (in millimeters, except for ratios) of carapace and valves: LV-L, 0.59–0.61 (0.60, N=3); LV-H, 0.30–0.31 (0.31, N= 3); LV-H/LV-L, 0.50–0.51 (0.51, N=3); RV-L, 0.58–0.61 (0.60, N=3); RV-H, 0.29–0.31 (0.30, N=3); RV-H/RV-L, 0.50–0.51 (0.51, N=3); Ca-W, 0.25–0.26 (0.26, N=2); Ca-W/LV-L, 0.41–0.42 (0.42, N=2).

Carapace (Fig. 2) translucent white, with black eye; outer surface smooth, with sparse, tiny setae but without shallow pits; widest point at about mid-length (Fig. 2A, B); LV overlapping RV at ventral margin (Fig. 2A); no dorsal hump on LV (Fig. 2B–D); outer list present in ventral margin of both valves (Fig. 2A).

LV (Figs 2C, E, G, 3A) with greatest height at about mid-length; anterior and posterior margins evenly rounded; apex of anterior margin lower than mid-height of LV and slightly higher than apex of posterior margin; in inner view, calcified inner lamella on anterior and posterior margins well developed (Figs 2E, 3A); inner list present in ventral region (Fig. 2G); mid-ventral margin with inner triangular projection (Fig. 2A, G). RV (Figs 2D, F, H, 3B) similar to LV in shape; inner list in ventral region absent (Fig. 2H). Two oblong mandibular muscle scars and four oblong adductor muscle scars on LV and RV (Figs 2E, F, 3A, B). Hinge adont.

An1 (Fig. 3C) with seven podomeres. First podomere with one dorsal and two ventrodiscal plumed setae and Wouters organ. Second podomere with dorsodistal seta reaching distal edge of third podomere and Rome organ. Third podomere with dorsodistal seta reaching distal edge of fourth podomere and ventrodiscal seta extending beyond middle of fourth podomere. Fourth podomere with two dorsodistal setae of unequal length (longer one extending to distal edge of seventh podomere) and two ventrodiscal setae reaching distal edge of sixth podomere. Fifth podomere with two dorsodistal setae of unequal length (longer one as long as podomeres 2–7) and two shorter ventrodiscal setae. Sixth podomere with four outer distal setae as long as podomeres 1–7 and shorter inner distal seta. Seventh podomere with three distal setae of unequal length and aesthetasc y_a (ca. 60% length of longest seta).

An2 (Fig. 3D) with five podomeres. First podomere (coxa) with two ventral setae. Second podomere (basis) with ventrosudistal seta reaching distal edge of third podomere. Exopodite with one long and two unequal short setae. Third (first endopodal) podomere with six inner sudistal short natatory setae extending slightly beyond distal edge of third podomere, ventrodiscal plumed seta reaching distal edge of fifth podomere, and mid-ventral

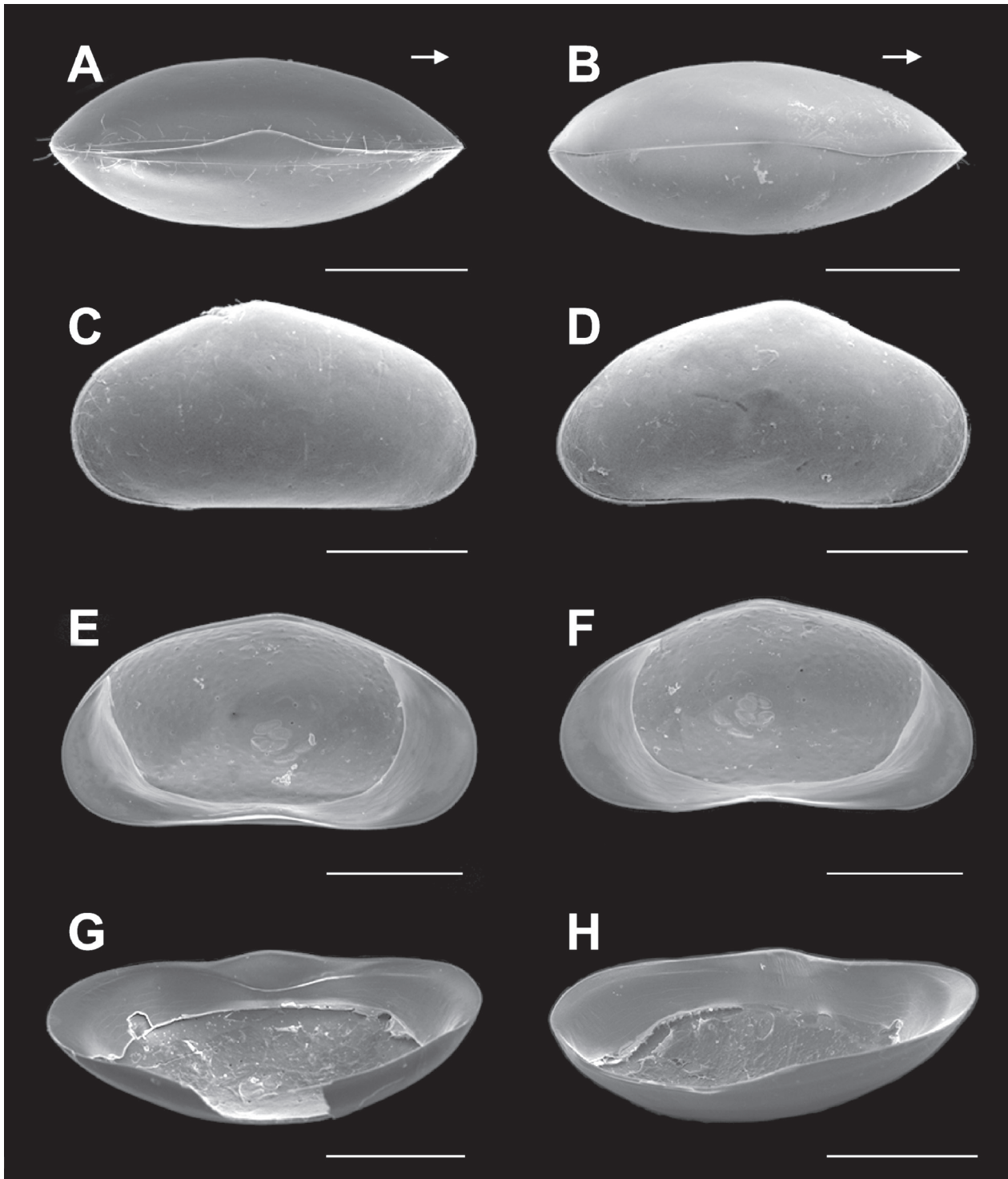


Figure 2. SEM images of carapaces and valves of female *Cavernocypris hokkaiensis* sp. nov. **A.** Paratype (ICHUM-8250); **B.** Paratype (ICHUM-8251); **C, D.** Paratype (ICHUM-8248); **E, F.** Holotype (ICHUM-8247); **G, H.** Paratype (ICHUM-8249); **A, B.** Ventral and dorsal views of whole carapace; **C, D.** Outer views of left and right valves; **E, F.** Inner views of left and right valves; **G, H.** Inner dorsal views of left and right valves; dorsal portion of left valve broken. Arrows indicate anterior direction. Scale bars: 0.2 mm.

aesthetasc Y. Fourth podomere undivided, with two mid-dorsal setae, dorsosubdistal setae z_{1-3} extending beyond middle of claws $G_{2,3}$, mid-ventral plumed setae t_{1-4} of unequal length, and distal claws G_{1-3} of nearly equal length. Fifth podomere with bifurcate aesthetasc y_3 (longer part half the length of claw G_M) and claws $G_{m,M}$; G_m ca. 70% length of G_M ; G_M reaching tip of claws G_{1-3} .

Md (Fig. 3E, F) with coxa, palp comprising four (one basal and three endopodal) podomeres, and vibratory plate. Coxa with several distal teeth and one subdistal plumed seta. First podomere (basis) with one ventrodorsal seta, ventrodorsal setae $S_{1,2}$, and ventrodorsal short seta α ; setae $S_{1,2}$ subequal in length, bearing row of long setules. Vibratory plate (exopodite) with four rays. Second (first

endopodal) podomere with three dorsodistal setae of unequal length (longest reaching tip of claws on fourth podomere), four mid-ventral long plumed setae (not extending beyond tip of claws on fourth podomere), and mid-ventral plumed short seta β (shorter than half the length of mid-ventral plumed setae). Third podomere with four dorsosubdistal setae and two ventrosubdistal setae; inner distal region with plumed seta γ and two plumed setae. Fourth podomere with two distal setae and four distal claws.

Mx (Fig. 3G) with palp comprising two podomeres, three endites, and vibratory plate (not illustrated). First palpal podomere with five dorsodistal setae of unequal length and one ventro-subdistal seta. Second palpal podomere not spatula-like, but rectangular, with two distal setae and three distal claws. First endite with two ventroproximal setae and ca. nine distal setae. Second endite with ca. eight distal setae. Third endite with two distal serrated spines and six distal setae.

L5 (Fig. 3H) with protopod and palp; vibratory plate absent. Protopod with two setae a and at least nine distal plumed setae of unequal length; setae b, c, and d absent. Palp with distal plumed setae h_{1-3} .

L6 (Fig. 3I) with six podomeres. First and second podomeres (protopod) with seta d_2 . Third (first endopodal) podomere with ventrodistal plumed seta e reaching middle of fifth podomere. Fourth podomere with ventrodistal plumed seta f reaching beyond distal edge of fifth podomere. Fifth podomere with ventrodistal plumed seta g. Sixth podomere with dorsodistal seta h_3 , ventrodistal plumed seta h_1 , and distal curved claw h_2 .

L7 (Fig. 3J) with four podomeres; third and fourth podomeres fused to form pincer organ. First podomere (protopod) with plumed setae $d_{1,2,p}$. Second (first endopodal) podomere with ventrodistal plumed seta e not reaching middle of fused podomeres 3 and 4. Fused third and fourth podomeres with mid-ventral plumed seta f not reaching tip of L7, subdistal long plumed seta h_3 , distal hook-like seta h_2 , and subdistal tiny seta h_1 .

UR (Fig. 3K) strongly reduced. Proximal part longer than wide, with one seta. Distal part flagellar in shape.

Rake organ (Fig. 3L) with stout rod and ca. eight blunt distal teeth.

Genital hooks on female copulatory organ present (not illustrated).

Genetic information and phylogenetic analysis

The partial COI sequence (658 bp, encoding 219 amino acids; LC666823), the nearly complete 18S sequence (2053 bp; LC666824), and a *Cardinium* 16S sequence (907 bp; LC666825) were determined from paratype individual ICHUM-8252.

The sequences in the INSD most similar to our sequences, determined by BLAST searches, were from the ostracod *Bennelongia scanloni* Martens et al., 2013 (KF724989.1; identity score 81.28%, query cover 99%; Martens et al. 2013) for COI, from the ostracod

Cyprretta seurati Gauthier, 1929 (AB674999.1; identity score 87.60%, query cover 89%; Hiruta et al. 2016) for 18S, and from “*Cardinium* endosymbiont of *Heterocypris spadix*” (LC589665.1; identity score 98.90%, query cover 100%; Munakata et al. 2021) for *Cardinium* 16S. In our ML tree based on 18S sequences (Suppl. material 4: File S1), *C. hokkaiensis* appears as the sister taxon to a strongly supported clade (97% ultrafast bootstrap support) comprising all other cypridoideans. Cyprididae and Cypridopsinae, in which *Cavernocypris* is classified, do not appear as monophyletic.

Discussion

Morphological comparison

Cavernocypris hokkaiensis sp. nov. resembles *C. cavernosa* and *C. danielopoli* Smith & Kamiya, 2017 in lacking setae b and d on L5, but differs from them in that (1) the outer surface of the carapace is smooth, with sparse, tiny setae, but without shallow pits (pits present in *C. cavernosa* and *C. danielopoli*); (2) the carapace is elongate rather than triangular in lateral view (triangular in *C. danielopoli*); (3) first palpal podomere of Mx has five dorsodistal and one ventro-subdistal setae (only five dorsodistal setae present in *C. danielopoli*; not described in *C. cavernosa*); and (4) L5 lacks the vibratory plate (vibratory plate present in *C. cavernosa* and *C. danielopoli*). Character states in all congeners are summarized in Table 2.

Reproductive mode

Our sample comprised only females, indicating that *C. hokkaiensis* may be parthenogenetic. The endosymbiotic bacterium *Cardinium* has previously been detected (e.g., by means of 16S sequences) in non-marine ostracods engaged in parthenogenetic or mixed reproduction, and infection with *Cardinium* might be a causative factor in the parthenogenetic reproductive mode (Schön and Martens 2019). Our study is the first to detect *Cardinium* in a species of *Cavernocypris*, implying that *C. hokkaiensis* may be parthenogenetic. It should be noted that male individuals have likewise not been reported among the congeners *C. cavernosa*, *C. danielopoli*, *C. wardi*, and some populations of *C. subterranea* (Wolf, 1920) (Marmonier et al. 1989; Külköylüoğlu and Vinyard 1998; Smith 2011; Smith et al. 2017).

Ecology, distribution, and life cycle

The results of our field survey suggest that *C. hokkaiensis* is distributed in an extremely narrow area, only in Hokkai-sawa Stream. It was not found at three sites distant from Hokkai-sawa Stream (Stns 5, 6, and 7). Its absence at two sites in Akaishi Stream (Stns 3 and 4), which Hokkai-sawa

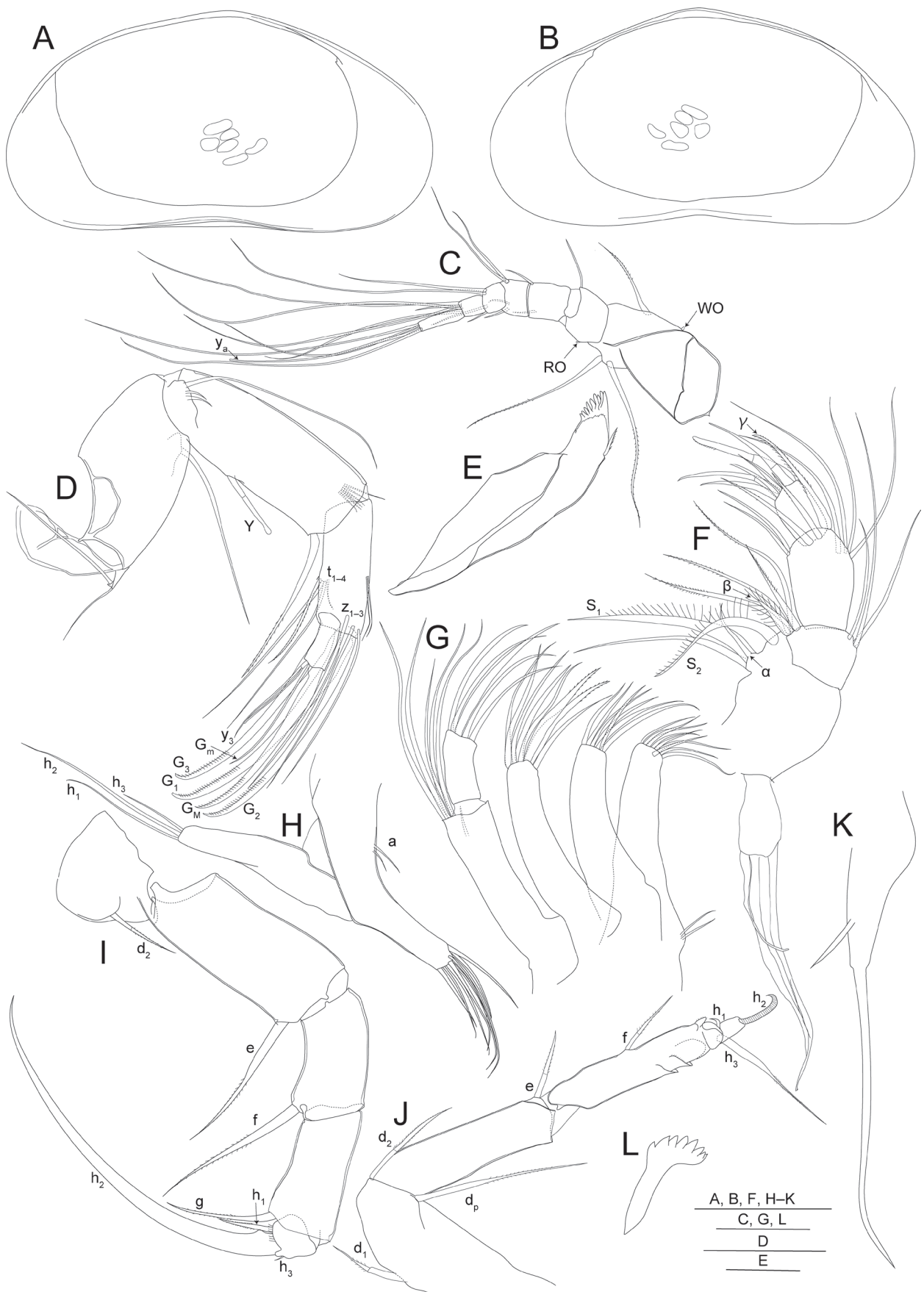


Figure 3. *Cavernocypris hokkaiensis* sp. nov., female. **A–G, I–L.** Holotype (ICHUM-8247); **H.** Paratype (ICHUM-8249); **A, B.** Inner views of left and right valves; **C.** Antennula; **D.** Antenna, outer view; **E.** Coxa of mandible; **F.** Mandible, inner view; **G.** Maxillula (vibratory plate omitted); **H–J.** Limbs 5–7; setules of distal setae on protopod and palp of limb 5 omitted; **K.** Uropodal ramus; **L.** Rake organ. Abbreviations: RO, Rome organ; WO, Wouters organ. Scale bars: 0.2 mm (**A, B**); 0.5 mm (**C–K**); 0.25 mm (**L**).

Table 2. Comparison of morphological characteristics between species of *Cavernocypris*.

	<i>C. cavernosa</i>	<i>C. coreana</i>	<i>C. danielopoli</i>	<i>C. reddelli</i>	<i>C. subterranea</i>	<i>C. wardi</i>	<i>C. hokkaiensis</i> <i>sp. nov.</i>
Shape of valves	elongate	elongate	triangular	elongate	elongate	elongate	elongate
Surface of valves	with numerous shallow pits, most distinct towards anterior and posterior margins, less distinct in central area	with very finely pitted	with elongate shallow pits in central area, and with much smaller rounded pits in posterior region	with small shallow pits	with small shallow pits in a central transverse band; pits may be reduced or even absent	smooth	smooth
Number of An1 podomeres	7	6	7	7	7	7	7
Number of setae on 1 st podomere of Mx-palp	not described	5 distal and 1 subdistal	5 distal	5 distal	5 distal and 1 subdistal	4 distal and 1 subdistal	5 distal and 1 subdistal
Seta b on L5	absent	present	absent	absent	present	present	absent
Seta d on L5	absent	absent	absent	present	present	present	absent
Number of rays comprising vibratory plate on L5	2	4	1	4	2	3	0
Reference	Smith (2011)	McKenzie (1972); Marmonier et al. (1989)	Smith et al. (2017)	Külköylüoğlu (2020)	Wolf (1920); Marmonier et al. (1989)	Marmonier et al. (1989)	this study

Stream joins, may be related to environmental differences between two streams. Hokkai-sawa Stream is fed by spring water and snowmelt, and is thus cold (1.4–6.8 °C measured in the summer season; Table 1; Konno et al. 2003) and slightly acidic (pH 6.2; Konno et al. 2003). In contrast, Akaishi Stream is fed by water from the Yudokunosen hot spring in the Ohachidaira Caldera, which has high water temperature (48 °C) and high H₂S content (245 mg/l) (Uzumasa et al. 1959), and measurements taken at Stn. 3 indicate that Akaishi Stream is warmer (13 °C) and more acidic (pH 2.8–3.3) than Hokkai-sawa Stream (Table 1; Konno et al. 2003). No pH data were available for our Stn. 4, but the water temperature was similar to that at Stn. 3 (13 °C; Table 1). Based on samples collected from sites almost identical to our Stns 1 and 3 in Hokkai-sawa and Akaishi Streams, Konno et al. (2003) found no lotic aquatic insects in common between the two streams. The warmer, more acidic condition of Akaishi Stream may be an unsuitable habitat for *C. hokkaiensis*.

Our rearing experiment, though we could prepare only three live individuals, provided preliminary data about the life history of *C. hokkaiensis*. We observed no molting by captive *C. hokkaiensis* individuals for more than five months at 7 °C. Ostracod life cycles typically comprise eight non-adult and

one adult instars, i.e., ostracods molt eight times before becoming sexually mature adults. Instars are not uniform in duration, but tend to become longer with successive instars (e.g., Heip 1976; Liberto et al. 2014). Although we could not determine the true instar for three captive individuals, their estimated instar was A-1 (0.47- and 0.48-mm individuals) or A-2 (0.39-mm individual) if this species follows Brooks's rule (Brooks 1886; Watabe and Kaesler 2004). Our result could be an artefact from the artificial conditions during culturing, but it may also be a natural phenomenon, and we could speculate that this species may require more than a year to become sexually mature.

Our field survey and observation of captive individuals may indicate that *C. hokkaiensis* is an endemic species adapted to the harsh alpine environment of the Taisetsu Mountains, with a low population density, narrow distributional range, and potentially slow maturation. If this is the case, then habitat loss and fragmentation due to anthropogenic activities, or a decrease in snowfall and snowfields due to climate change, could lead to a rapid population decline of this species. Additional ecological and biological information is necessary to confirm whether *C. hokkaiensis* is a narrow endemic, and to design an informed conservation strategy.

Key to the *Cavernocypris* species (modified after Külköylüoğlu (2020))

- 1 An1 with 7 podomeres 2
- An1 with 6 podomeres *C. coreana* (McKenzie, 1972)
- 2 6th swimming seta of An2 longer than the other 5 setae, second podomere of Md-palp with β+4 setae 3
- 6th swimming seta of An2 shorter than others, second podomere of Md-palp with β+3 setae
..... *C. reddelli* Külköylüoğlu, 2020
- 3 Seta b present on L5 4
- Seta b absent from L5 5
- 4 First palpal podomere of Mx with 5 distal and 1 subdistal setae, vibratory plate of L5 with 2 rays
..... *C. subterranea* (Wolf, 1920)
- First palpal podomere of Mx with 4 distal and 1 subdistal setae, vibratory plate of L5 with 3 rays
..... *C. wardi* Marmonier, Meisch & Danielopol, 1989

- 5 Carapace triangular in lateral view, with distinctive hump on LV *C. danielopoli* Smith & Kamiya, 2017
 – Carapace elongate in lateral view, without hump on LV 6
 6 Surface of valves covered with numerous shallow pits, vibratory plate of L5 with 2 rays *C. cavernosa* Smith, 2011
 – Surface of valves smooth, L5 without vibratory plate *C. hokkaiensis* sp. nov.

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Supplementary material 1

Table S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: table (Excel format)

Explanation note: List of species included in the molecular phylogenetic analysis and respective GenBank accession numbers.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl1>

Supplementary material 2

Alignment S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: molecular dataset (fasta format)

Explanation note: Aligned 18S sequences used for the maximum-likelihood analysis, trimmed in MEGA7 to the shortest length among the sequences.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl2>

Supplementary material 3

Alignment S2

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: molecular dataset (fasta format)

Explanation note: Aligned 18S sequences used for the maximum-likelihood analysis, reduced to 1547 positions by removing alignment-ambiguous sites with Gblocks ver. 0.91b in NGPhylogeny.fr under “relaxed” parameters.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl3>

Supplementary material 4

File S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: text with one figure (docx format)

Explanation note: Phylogenetic analysis of cypridoidean ostracods based on 18S rRNA sequences.

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