



Title	Taxonomy and natural history of <i>Cavernocypris hokkaiensis</i> sp. nov., the first ostracod reported from alpine streams in Japan
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Zoosystematics and Evolution: Supplementary file 4

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

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## Supplementary File 4: File S1.

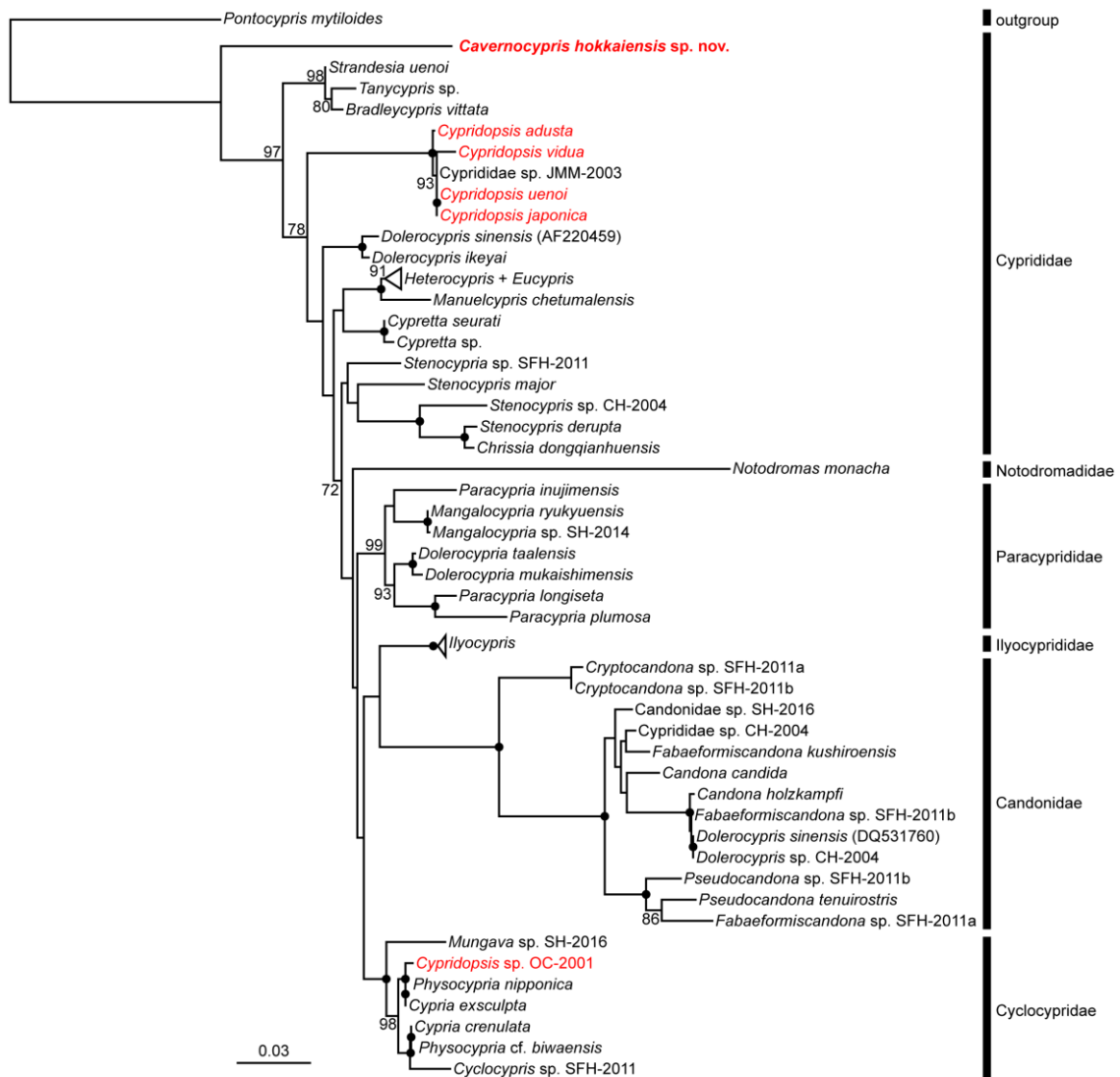
### Phylogenetic analysis of cypridoidean ostracods based on 18S rRNA sequences

#### Methods

The 18S dataset for a phylogenetic analysis included one sequence we determined and 65 sequences greater than 1600 bp long from 64 cypridoidean species and one outgroup taxon (*Pontocypris mytiloides*, Pontocypridoidea) taken from the INSD (see supplementary file 1: Table S1). Five cypridoidean sequences (AY191451–191453, AY191457, AY457057), greater than 1600 bp long and available in the INSD were not included in our dataset because a preliminary alignment showed that they contained long indels not present in the other species. Sequences were aligned by eye according to the secondary structure of the honeybee 18S sequence (Gillespie et al. 2006) and the structure predicted with the RNAfold WebServer (Gruber et al. 2008; Lorenz et al. 2011); the aligned sequences were trimmed in MEGA7 (Kumar et al. 2016) to the shortest length among them (see supplementary file 2: Alignment S1). Alignment-ambiguous sites were then 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) (1547 positions in the alignment dataset; see supplementary file 3: Alignment S2). The optimal substitution model (GTR + G + I) was determined under the corrected AIC (Akaike information criterion) option in PartitionFinder 2.1.1 (Lanfear et al. 2017), using the greedy algorithm (Lanfear et al. 2012). A maximum likelihood (ML) analysis was conducted in IQ-TREE 2.1.2 (Minh et al. 2020), with nodal support values obtained from an ultrafast bootstrap analysis of 1000 pseudoreplicates under the “bnni” option (Hoang et al. 2018). The ML tree was drawn with FigTree v1.4.4 (Rambaut 2021).

#### Results

In our ML tree based on 18S sequences (the figure below), *C. hokkaiensis* sp. nov. appears as the sister taxon to a strongly supported clade (97% ultrafast bootstrap support [uBS]) comprising all other cypridoideans. Cyprididae and Cypridopsinae, in which *Cavernocypris* is classified, do not show as monophyletic. Four other families for which two or more species each were included in our analysis (Paracyprididae, Ilyocyprididae, Candonidae, and Cyclocypridae) comprise strongly supported clades (99–100% uBS). Cyclocypridae contains one sequence and Candonidae three sequences determined from specimens possibly misidentified as cypridids (*Dolerocypris sinensis* [DQ531760], *Dolerocypris* sp. CH-2004 [AY622194], Cyprididae sp. CH-2004 [AY622196], and *Cypridopsis* sp. OC-2001 [AF363307]).



**Figure.** Maximum-likelihood (ML) tree based on 18S sequences (1547 positions). Numbers near nodes are ultrafast bootstrap support (uBS) greater than 60%. Filled circles indicate 100% uBS. Taxa in Cyridopsinae are shown in red letters. Two clades each comprising *Ilyocypris* and *Heterocypris*+*Eucypris* sequences were collapsed (terminal triangle). The taxon names labeling sidebars are family names.

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