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# Redescription of *Keronopsis helluo* Penard, 1922 from Antarctica and *Paraholosticha pannonica* Gellért and Tamás, 1959 from Alaska (Ciliophora, Hypotricha)

Kyung-Min Park[a,b], Namyi Chae[c], Jae-Ho Jung[d], Gi-Sik Min[b], Sanghee Kim[a,*], Helmut Berger[e,**]

[a]*Division of Life Sciences, Korea Polar Research Institute, Incheon 21990, South Korea*
[b]*Department of Biological Sciences, Inha University, Incheon 22212, South Korea*
[c]*Institute of Life Science & Natural Resources, Korea University, Seoul 02841, South Korea*
[d]*Department of Biology, Gangneung-Wonju National University, Gangneung 25457, South Korea*
[e]*Consulting Engineering Office for Ecology, Radetzkystrasse 10, 5020 Salzburg, Austria*

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## Abstract

The morphology of *Keronopsis helluo* Penard, 1922, type species of *Keronopsis*, and *Paraholosticha pannonica* Gellért and Tamás, 1959, two little-known members of the Keronopsidae Jankowski, 1979, was described using standard methods. In addition, we sequenced the SSU rRNA of both species. *Keronopsis helluo* was isolated from a mossy soil from Robert Island (Antarctica) while *P. pannonica* was found in terrestrial moss from Alaska. Our data correspond very well with the original descriptions. The frontal ciliature of *K. helluo* is identical with that of *Paraholosticha* spp., indicating that some *Keronopsis* species (*K. tasmaniensis*, *K. dieckmanni*) are misclassified in the keronopsids. The type species has distinctly more transverse cirri (8–13) than *K. wetzeli* (1–3), type species of *Parakeronopsis*, which is thus perhaps a valid genus or subgenus. The phylogenetic analyses confirm the position of the keronopsids outside the Dorsomarginalia. The species sequenced so far (*K. helluo*, *Paraholosticha muscicola*, *P. pannonica*) emerge from a soft polytomy, which also comprises *Bistichella*-like species and a large cluster composed of amphisiellids, trachelostylids, and gonostomatids, that is, the method failed to resolve the relationships within the keronopsids. The Keronopsidae and the two species studied are characterized based on previous studies and our data.
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***Keywords:*** Keronopsidae; Morphology; Moss; Robert Island; Soil ciliate; SSU rRNA gene

## Introduction

The Keronopsidae Jankowski, 1979 are a very small group (about 10 species) of hypotrichous ciliates with *Keronopsis* Penard, 1922 as name-bearing type genus. *Keronopsis helluo* Penard, 1922, type species by monotypy, is a little-known species discovered in terrestrial moss from Switzerland. It is

*Corresponding author. Fax: +82 32 760 5509.
**Corresponding author. Fax: +43 662 44 31 39.
*E-mail addresses:* sangheekim@kopri.re.kr (S. Kim), berger.helmut@protozoology.com (H. Berger).

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characterized, inter alia, by a frontal corona, two long frontoventral rows, and more than five (8–10) transverse cirri. In addition, Penard (1922) briefly mentioned that it divides in cysts. Kahl (1932), the first reviser, obviously ignored, or at least did not rate several features of *K. helluo* sufficiently highly, for example the specific kind of division. Thus, Kahl redefined *Keronopsis*, which he classified as subgenus of *Holosticha* Wrześniowski, 1877, from a rather conspicuous group of hypotrichs dividing in cysts to a large melting pot of hypotrichs (mainly urostyloids) with two central pseudorows (that is, a midventral complex composed of cirral pairs) which curve leftwards anteriorly ending in several, usually enlarged frontal cirri. This classification was basically accepted by Borror (1972) and Corliss (1979). Hemberger and Wilbert (1982) improved the situation in that they confined Penard's genus (with the invalid genus *Paraholosticha* Kahl, 1932 as synonym) to "true" *Keronopsis* species, which have, inter alia, a single frontal corona composed of a mixed row. Borror and Wicklow (1983) formally removed the urostyloids from *Keronopsis* and transferred them to *Pseudokeronopsis* Borror and Wicklow, 1983.

This brief summary demonstrates the rather tricky situation in the keronopsids, which is mainly due to the lack of a modern description of the type species *K. helluo*. In 2014, we found this obviously very rare species in a soil sample from Antarctica. In addition, we isolated a further, likewise little-known keronopsid from a moss sample collected in Alaska, namely *Paraholosticha pannonica* Gellért and Tamás, 1959. We describe their interphasic morphology with standard methods and estimate their phylogenetic position using their SSU rRNA gene sequences.

## Material and Methods

### Sample collection, cultivation, identification

*Keronopsis helluo* was isolated from a soil sample from the west coast of Robert Island (62°23′03.40″S, 59°41′36.84″W), Antarctica in January 2014. The sample, comprising the top soil (0–3 cm) and the moss cover (*Sanionia uncinata*), was transferred to the laboratory of the King Sejong Station on King George Island where it was air-dried for 14 days. It was stored at −20 °C during the four-month transport by ship and kept at −70 °C after arriving at the Korea Polar Research Institute (KOPRI), Incheon, South Korea.

*Paraholosticha pannonica* was found in terrestrial moss collected from near the Nome-Council Road (64°50′36.84″N, 163°42′38.04″W), about 5 km south of the village Council, Alaska in July 2014. The sample was kept in a freezer at −20 °C during the transport by the icebreaker Araon for about one month and kept at −70 °C after arriving at KOPRI.

In June 2015, the species were re-activated from the resting cysts using the non-flooded Petri dish method at 4 °C (Foissner et al. 2002). All morphological and molecular biological analyses are based on specimens from non-clonal raw cultures.

Living specimens were observed with a light microscope (Zeiss Axio Imager2, Carl Zeiss, Oberkochen, Germany) at magnifications of 50× to 1000×, and a stereo microscope (Leica M205C, Leica Microsystems, Wetzlar, Germany). Protargol preparations were made according to "Procedure A" described by Foissner (2014). The protargol was synthesized according to Pan et al. (2013). Drawings of live specimens are based on free-hand sketches and photos while those from protargol-prepared individuals were made with a drawing device. Counts and measurements on silvered specimens were performed at magnifications of 400× and 1000×, using an image analyzer (AxioVision SE64 Rel. 4.9.1; Zeiss Co.).

### Terminology

General terminology is according to Lynn (2008). For hypotrich-specifc terms (e.g., DE-value, dorsoventral flattening, true row, mixed row, pseudorow), see Berger (1999, 2006, 2008, 2011), Foissner and Al-Rasheid (2006), and Jung et al. (2015).

Terminology of the frontoventral ciliature in keronopsids is not uniform. In some works the cirral rows have been termed short and long ventral row or left or right frontal row and left or right ventral row (e.g., Berger and Foissner 1987; Kahl 1932), in other works the cirral rows have been numbered as ventral cirral row 1–4 starting with number 1 on the left side (e.g., Foissner and Al-Rasheid 2007; Jung et al. 2015). Tuffrau and Fryd-Versavel (1977) numbered all long cirral rows and dorsal kineties consecutively starting at the left frontoventral row (= row 1) and ending with the left marginal row (= row 7); the short frontal rows have been termed row b and c. Most *Paraholosticha* and *Keronopsis* specimens have two short frontal rows (rows 1 and 2 when using terminology of Foissner and Al-Rasheid 2007) and two long ventral rows (rows 3 and 4). However, *Keronopsis helluo* has usually three and sometimes four or five short rows (Fig. 1B, C, E, Table 1). Thus, when using the numbering system, the long rows would have a different designation (rows 4 and 5, 5 and 6, or 6 and 7) in *K. helluo*. We hypothesize that the long cirral rows are homologous in all keronopsid species. Consequently, we recommend designating these two long rows as left frontoventral row and right frontoventral row. The short rows should be designated as frontal rows 1, 2, 3, etc. (beginning from left) because left and right seems not usable due to the variable number. The frontal rows 1 and 2 in *Paraholosticha* and *Keronopsis* originate from anlagen II and III (Dieckmann 1988a, 1988b, 1989; Tuffrau and Fryd-Versavel 1977).

The frontal ciliature (corona) of the keronopsids differs distinctly from the corona of some urostyloids (e.g., *Pseudourostyla* Borror, 1972; *Pseudokeronopsis* Borror and Wicklow, 1983) or oxytrichids (e.g., *Neokeronopsis* Warren et al., 2002). The corona of the keronopsids is a mixed row, that is, the anlagen I, II, and III form more than one frontal cir-

**Table 1.** Morphometric data on Antarctic population of *Keronopsis helluo* (Kh) and Alaskan population of *Paraholosticha pannonica* (Pp).

| Characteristic[a] | Species | Mean | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|---|
| Body, length | Kh | 179.4 | 185.1 | 22.3 | 5.4 | 12.5 | 144.7 | 219.2 | 17 |
| | Pp | 93.5 | 93.5 | 5.3 | 1.2 | 5.6 | 85.4 | 107.3 | 19 |
| Body, width | Kh | 75.6 | 75.0 | 6.9 | 1.7 | 9.1 | 64.6 | 90.6 | 17 |
| | Pp | 43.4 | 43.4 | 3.8 | 0.9 | 8.8 | 36.2 | 49.6 | 19 |
| Body length:width, ratio | Kh | 2.4 | 2.3 | 0.4 | 0.1 | 16.3 | 1.7 | 3.2 | 17 |
| | Pp | 2.2 | 2.1 | 0.2 | 0.0 | 9.4 | 1.9 | 2.8 | 19 |
| Adoral zone of membranelles, length | Kh | 66.6 | 67.2 | 6.1 | 1.5 | 16.3 | 55.8 | 78.1 | 17 |
| | Pp | 33.3 | 33.2 | 1.7 | 0.4 | 5.2 | 30.9 | 37.1 | 19 |
| Body length:length of adoral zone, ratio | Kh | 2.7 | 2.6 | 0.2 | 0.0 | 7.7 | 2.5 | 3.3 | 17 |
| | Pp | 2.8 | 2.8 | 0.1 | 0.0 | 4.7 | 2.6 | 3.0 | 19 |
| Posterior end of cell to transverse cirri, distance | Kh | 10.4 | 9.7 | 3.7 | 0.9 | 35.8 | 5.2 | 18.6 | 17 |
| Anterior macronuclear nodule, length | Kh | 16.7 | 16.1 | 2.8 | 0.7 | 17.0 | 11.1 | 22.3 | 17 |
| | Pp | 15.7 | 15.7 | 2.8 | 0.6 | 17.6 | 9.9 | 19.7 | 19 |
| Anterior macronuclear nodule, width | Kh | 9.7 | 9.7 | 1.1 | 0.3 | 11.0 | 8.0 | 12.7 | 17 |
| | Pp | 8.1 | 8.4 | 1.0 | 0.2 | 12.7 | 5.6 | 9.9 | 19 |
| Macronuclear nodules, number | Kh | 4.2 | 4.0 | 0.4 | 0.1 | 9.4 | 4.0 | 5.0 | 17 |
| | Pp | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 2.0 | 2.0 | 19 |
| Micronuclei, length | Kh | 6.0 | 6.4 | 1.6 | 0.4 | 26.7 | 3.3 | 7.9 | 17 |
| | Pp | 5.1 | 5.0 | 0.5 | 0.1 | 9.2 | 4.4 | 5.8 | 19 |
| Micronuclei, width | Kh | 5.2 | 5.7 | 1.3 | 0.3 | 24.5 | 3.1 | 7.3 | 17 |
| | Pp | 4.4 | 4.3 | 0.5 | 0.1 | 10.4 | 3.8 | 5.2 | 19 |
| Micronuclei, number | Kh | 3.1 | 2.0 | 2.8 | 0.7 | 90.6 | 1.0 | 12.0 | 17 |
| | Pp | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 19 |
| Adoral membranelles, number | Kh | 63.9 | 63.0 | 6.3 | 1.5 | 9.8 | 53.0 | 76.0 | 17 |
| | Pp | 31.1 | 31.0 | 1.8 | 0.4 | 5.8 | 27.0 | 34.0 | 19 |
| Frontal corona, number of cirri | Kh | 26.1 | 25.0 | 2.8 | 0.7 | 10.9 | 22.0 | 31.0 | 17 |
| | Pp | 13.2 | 13.0 | 1.3 | 0.3 | 10.0 | 11.0 | 15.0 | 19 |
| Frontal and frontoventral rows, number | Kh | 5.4 | 5.0 | 0.6 | 0.1 | 11.4 | 5.0 | 7.0 | 17 |
| | Pp | 4.0 | 4.0 | 0.0 | 0.0 | 0.0 | 4.0 | 4.0 | 19 |
| Frontal row 1, number of cirri[b] | Kh | 4.8 | 5.0 | 1.0 | 0.2 | 19.7 | 3.0 | 6.0 | 17 |
| | Pp | 2.2 | 2.0 | 0.5 | 0.1 | 24.2 | 1.0 | 3.0 | 19 |
| Frontal row 2, number of cirri | Kh | 3.9 | 4.0 | 0.8 | 0.2 | 20.1 | 2.0 | 5.0 | 17 |
| | Pp | 2.1 | 2.0 | 0.4 | 0.1 | 19.7 | 1.0 | 3.0 | 19 |
| Frontal row 3, number of cirri | Kh | 6.4 | 7.0 | 1.6 | 0.4 | 24.9 | 3.0 | 9.0 | 17 |
| Frontal row 4, number of cirri | Kh | 6.8 | 7.0 | 2.3 | 0.9 | 33.9 | 3.0 | 10.0 | 6 |
| Frontal row 5, number of cirri | Kh | 7.0 | 7.0 | – | – | – | 7.0 | 7.0 | 1 |
| Left frontoventral row, number of cirri | Kh | 56.4 | 57.0 | 7.7 | 1.9 | 13.7 | 44.0 | 70.0 | 17 |
| | Pp | 23.0 | 23.0 | 1.9 | 0.4 | 8.3 | 19.0 | 26.0 | 19 |
| Right frontoventral row, number of cirri | Kh | 59.6 | 61.0 | 7.9 | 1.9 | 13.2 | 48.0 | 73.0 | 17 |
| | Pp | 29.4 | 29.0 | 3.2 | 0.7 | 10.9 | 23.0 | 35.0 | 19 |
| Right marginal row, number of cirri | Kh | 50.0 | 54.0 | 9.2 | 2.2 | 18.5 | 31.0 | 63.0 | 17 |
| | Pp | 26.9 | 27.0 | 2.7 | 0.6 | 10.2 | 20.0 | 31.0 | 19 |
| Left marginal row, number of cirri | Kh | 56.9 | 58.0 | 7.9 | 1.9 | 13.8 | 43.0 | 69.0 | 17 |
| | Pp | 24.0 | 24.0 | 2.5 | 0.6 | 10.5 | 18.0 | 28.0 | 19 |
| Buccal cirri, number | Kh | 4.1 | 4.0 | 1.3 | 0.3 | 32.0 | 3.0 | 8.0 | 17 |
| | Pp | 1.4 | 1.0 | 0.5 | 0.1 | 35.7 | 1.0 | 2.0 | 19 |
| Transverse cirri, number | Kh | 10.7 | 11.0 | 1.4 | 0.3 | 13.1 | 8.0 | 13.0 | 17 |
| | Pp | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19 |
| Dorsal kineties, number | Kh | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 17 |
| | Pp | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 19 |
| Dorsal kinety 1, number of bristles | Kh | 48.3 | 49.0 | 9.1 | 2.2 | 18.8 | 30.0 | 65.0 | 17 |
| | Pp | 16.6 | 17.0 | 1.7 | 0.4 | 10.3 | 14.0 | 20.0 | 19 |
| Dorsal kinety 2, number of bristles | Kh | 44.2 | 44.0 | 8.9 | 2.2 | 20.2 | 27.0 | 62.0 | 17 |
| | Pp | 18.9 | 19.0 | 1.9 | 0.4 | 10.3 | 16.0 | 22.0 | 19 |

Table 1 (*Continued*)

| Characteristic[a] | Species | Mean | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|---|
| Dorsal kinety 3, number of bristles | Kh | 47.1 | 47.0 | 7.3 | 1.8 | 15.6 | 37.0 | 62.0 | 17 |
| | Pp | 19.9 | 20.0 | 2.1 | 0.5 | 10.5 | 15.0 | 24.0 | 19 |

[a] Data based on protargol preparations. Measurements in μm. Abbreviations: CV, coefficient of variation (%); M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean; –, not applicable.

[b] This is the leftmost frontal row.

rus each. These three short true rows (each row is very likely homologous to the corresponding frontal cirrus of hypotrichs with three enlarged frontal cirri) form a bow (corona) along the distal portion of the adoral zone (for details, see Dieckmann 1988a, 1988b, 1989; Tuffrau and Fryd-Versavel 1977). By contrast, the corona of *Pseudourostyla*, *Pseudokeronopsis*, and *Neokeronopsis* is a pseudorow, that is, each frontal cirrus originates from a different anlage (e.g., Jerka-Dziadosz 1972; for revision, see Berger 2006). Usually the second cirri (from anterior) of these anlagen form the rear bow (corona) so that these taxa have a bicorona (Wirnsberger et al. 1987). Because of this difference, we recommend to add the vernacular names of the higher taxon, for example, keronopsid frontal corona.

Since this is a mainly taxonomic paper, "nomenclatural" references are also listed in the reference section.

## Voucher slides

Three voucher slides of protargol preparations of *Keronopsis helluo* (accession numbers ACNS000225–ACNS000227) and one voucher slide of *Paraholosticha pannonica* (ACNS000275) have been deposited in the Korea Polar Research Institute, South Korea. Relevant specimens are marked with circles on the bottom of the slides.

## ZooBank registration

ZooBank registration number of present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org:pub:41BC25BA-BAFB-44B6-8325-9C36ED362754.

## SSU rRNA gene sequencing

A single specimen of each species was washed several times with distilled water to remove any contaminants. It was used to extract genomic DNA using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA) following the manufacturer's instruction. The optimized condition for PCR was as follows: denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C

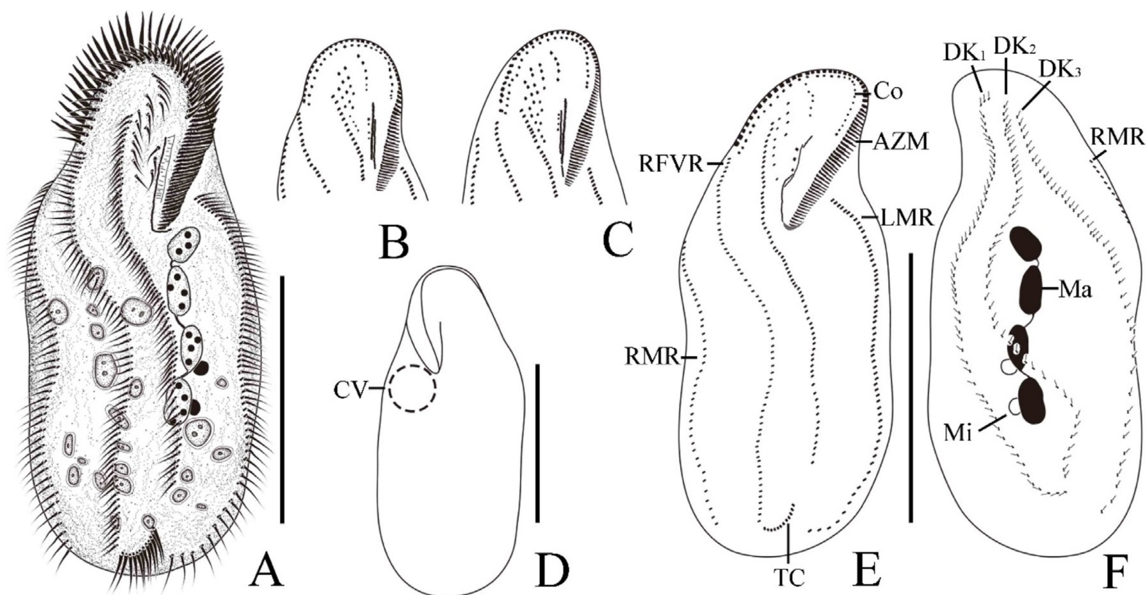


**Fig. 1. A–F** *Keronopsis helluo*, Antarctic population from life (A, D) and after protargol preparation (B, C, E, F). **A:** Ventral view of characteristic specimen with three frontal rows. **B, C:** Ventral views of oral region of a specimen with four (B) or five (C) frontal rows. **D:** Dorsal view showing contractile vacuole. **E, F:** Ciliature of ventral and dorsal side and nuclear apparatus of same specimen (see also Fig. 3A, B). AZM, adoral zone of membranelles; Co, frontal corona; CV, contractile vacuole; $DK_{1-3}$, dorsal kineties; LMR, left marginal row; Ma, macronuclear nodule; Mi, micronucleus; RFVR, right frontoventral row; RMR, right marginal row; TC, transverse cirri. Scale bars: 100 μm.

for 30 s, extension at 72 °C for 4 min, and a final extension step at 72 °C for 7 min. Nearly the complete small subunit ribosomal RNA (SSU rRNA) gene was amplified using two primers: slightly modified New EukA (5′-CTG GTT GAT YCT GCC AGT-3′) and LSU rev3 (5′-GCA TAG TTC ACC ATC TTT CG-3′) (Sonnenberg et al. 2007). The resulting PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany). Two internal primers were used for sequencing: 18S+810 (5′-GCC GGA ATA CAT TAG CAT GG-3′) and 18S−300 (5′-CAT GGT AGT CCA ATA CAC TAC-3′) (Jung et al. 2011). DNA sequencing was performed using an ABI 3700 sequencer (Applied Biosystems, CA, USA).

## Phylogenetic analyses

In order to analyze the phylogenetic positions of *Keronopsis helluo* and *Paraholosticha pannonica*, SSU rRNA gene sequences of 76 species were used. The oligotrichs *Strombidium styliferum* and *S. sulcatum* were selected as outgroup. GenBank accession numbers are mentioned after the species name in the phylogenetic tree (Fig. 7).

The sequences were aligned using Clustal X 1.81 (Jeanmougin et al. 1998) and manually trimmed at both ends in BioEdit 7.1.11 (Hall 1999). The alignment was then further checked by eye. A Best-fit substitution model for the phylogenetic analyses was chosen using the jModelTest 2.1.7 (Darriba et al. 2012). The model GTR + I (0.6540) + G (0.4900) under the Akaike information criterion (AIC) was selected. The Bayesian inference tree was built by MrBayes 3.2.5 (Ronquist et al. 2012) using the Markov chain Monte Carlo (MCMC) simulation for 1,000,000 generations with a burn-in of 300,000. PhyML version 20131022 (Guindon et al. 2010) in Bio-Linux 8 (Field et al. 2006) was used to infer Maximum Likelihood trees with 1000 bootstrap replicates.

For interpretation of bootstrap values we follow Vd'ačný and Rajter (2015), that is, we consider values ≥95 as high, from 71–94 as moderate, from 50–70 as low, and <50 as no support (Hillis and Bull 1993). Bayesian posterior probability values <0.95 are considered as low and values ≥0.95 as high (Alfaro et al. 2003).

## Results

### *Keronopsis helluo* Penard, 1922

1922 ***Keronopsis helluo* gen. nov. sp. n.** – Penard, Infusoires, p. 238, Fig. 228.1, 2 (original description; no type material available and no formal diagnosis provided). ZooBank registration number of *K. helluo*: urn:lsid:zoobank.org:act:CDA63454-B20E-4FFE-88DD-5CCCE246CFFD.

1932 ***Keronopsis helluo* Penard, 1922** – Kahl, Tierwelt Dtl., 25: 576, Fig. 8615 (revision of ciliates; combination with *Holosticha*, see nomenclature for correct name).

1972 ***Keronopsis helluo* Penard, 1922** – Borror, J. Protozool., 19: 11 (generic revision of hypotrichs).

**Nomenclature**: The species-group name *helluo* (Latin noun; squanderer, glutton; Menge and Pertsch 1988, p. 246) likely alludes to the fact that this species ingests rotifers and large ciliates (Penard 1922). Kahl (1932, p. 570, 571) classified *Keronopsis* as subgenus of *Holosticha*, that is, he transferred the species from the genus *Keronopsis* to the genus *Holosticha*. Thus, the correct name in his revision is *Holosticha (Keronopsis) helluo* (Penard, 1922) Kahl, 1932; a combination overlooked by Berger (2001, p. 44). *Keronopsis helluo* is type species of *Keronopsis* by monotypy and *Keronopsis* is the nominotypical genus of the family Keronopsidae.

No permanent preparations of the type population (Penard 1922) are available and no neotype has been fixed. A neotypification is desirable to define this species objectively. For example, from the original description it is not possible to recognize the frontal ciliature in detail, a very important taxonomic feature. However, since our population is from a sample collected very far away (Antarctica) from the original type locality (Switzerland), it is not possible to use our population as neotype because it has to come from as nearly as practicable from the original type locality (ICZN 1999, Article 75.3.6).

**Description of Antarctic population** (Figs 1A–F, 2A–I, 3A–E, Table 1): Body size in vivo about 160–235 × 65–85 µm (n = 3; 400×), on average 179 × 76 µm in protargol preparations; length:width ratio 2.4:1 on average (Table 1). Body-outline in ventral view broad to elongate elliptical in vivo, anterior portion distinctly narrowed, thus, more or less cephalized; rear body end usually broadly rounded. Body flexible, but not distinctly contractile, distinctly flattened dorsoventrally (Figs 1A, D, 2A–E, 3A–C). Nuclear apparatus in central body portion, usually somewhat left of midline, composed of four or five ellipsoidal (11–23 × 8–13 µm in protargol preparations) macronuclear nodules usually connected by thin thread; number of micronuclei very variable, namely 1–12 (median = 2), individual micronuclei spherical to slightly ellipsoidal, 3–8 × 3–7 µm in vivo (Figs 1A, F, 3A, B, E, Table 1). Contractile vacuole slightly anterior of mid-body near left margin of cell, approximately 23 µm in diameter when fully extended, no distinct collecting canals recognizable (Figs 1D, 2D). Cytoplasm grayish at low magnification, with about 5–15 µm-sized food vacuoles in middle and posterior portion with usually dark inclusions, mainly algae (e.g., chlorococcales). Cortical granules lacking. Locomotion without peculiarities, usually moderately fast creeping on bottom of Petri dish.

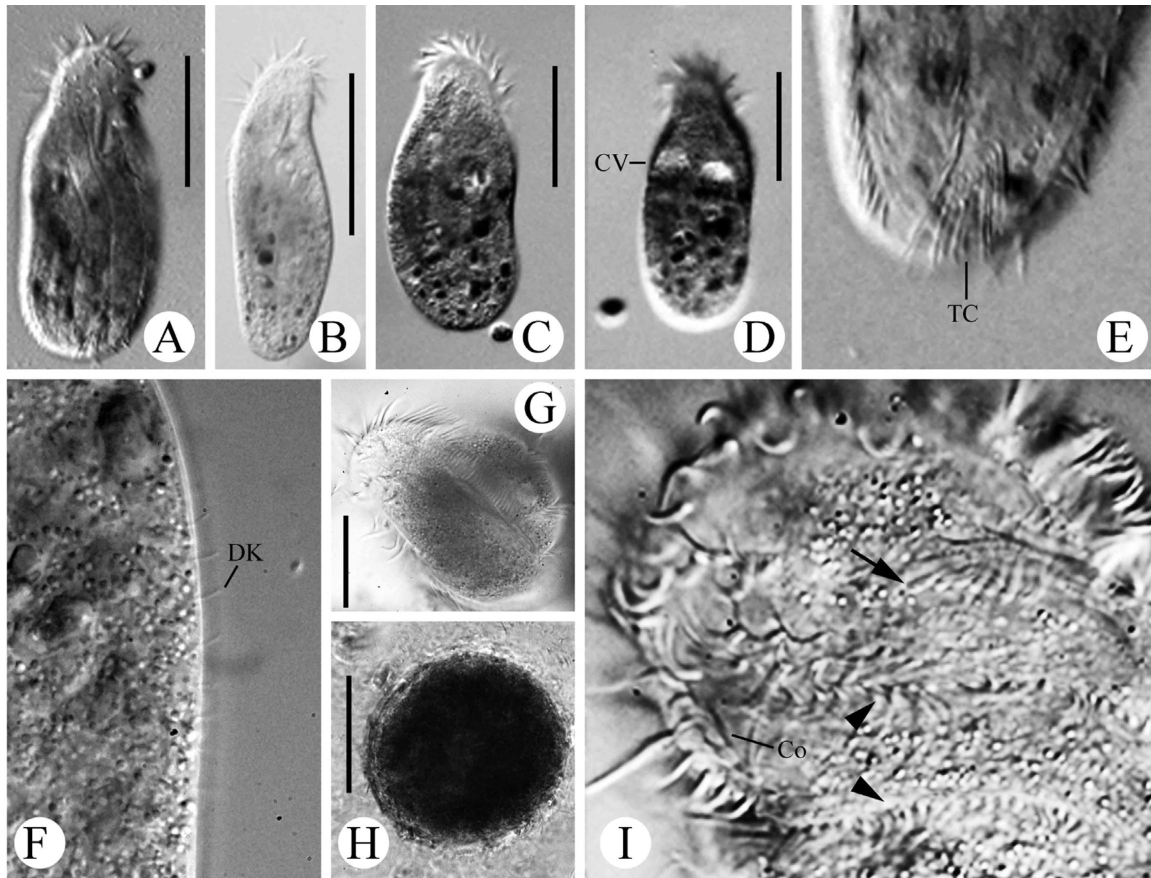


**Fig. 2.** **A–I** *Keronopsis helluo*, photomicrographs of Antarctic population from life (differential interference contrast). **A, B:** Ventral view of wide and slender specimen. **C, D:** Dorsal views. **E:** Ventral view of posterior body portion showing transverse cirri, right marginal row, right frontoventral row, and left marginal row. **F:** Dorsal view of right body margin showing dorsal bristles (4 μm long). **G, H:** Encysting specimen and resting cyst. **I:** Ventral view of frontal region showing, inter alia, buccal cirri (long arrow), adoral zone, frontal corona, and left and right frontoventral row (arrowheads). Co, frontal corona; CV, contractile vacuole; DK, dorsal bristles; TC, transverse cirri. Scale bars: A–D = 100 μm; G, H = 50 μm.

Adoral zone terminates at 37% of body length on average in protargol-prepared specimens; distal end extends far posteriorly, thus, DE-value about 0.50 (n = 6; Figs 1B, C, E, 3A–C); composed of 53–76, on average 64, membranelles; bases of largest membranelles 7.5 μm wide on average, cilia of membranelles up to 18 μm long. Paroral and endoral more or less straight and in parallel; endoral commences at level of anterior buccal cirrus (Figs 1A–C, E, 3A, C). Pharyngeal fibers not recognizable with preparation method used.

All cirri relatively fine, mostly 12–18 μm long in vivo (Figs 1A, 2E, I, 3A, C). Cirral pattern characteristic mainly due to keronopsid frontal corona, two long frontoventral rows, and dense row of transverse cirri. Frontal corona composed of 22–31, on average 26, cirri which form mixed row along anterior, curved portion of adoral zone (Figs 1A–C, E, 3A, C); cirri more or less of same size as other cirri, that is, not enlarged. 3–8, on average four, buccal cirri in longitudinal row right of paroral (Figs 1A–C, E, 3A, C). All specimens analyzed (n = 17) with two frontoventral rows (Figs 1A–C, E, 2A, 3A, C, Table 1). Most specimens (11 of 17 analyzed) with three frontal rows (Figs 1A, E, 3A, C), five with four frontal rows (Fig. 1B), and one with five frontal rows (Fig. 1C). Frontal rows usually distinctly separated from frontal corona (Figs 1A–C, 3C), sometimes still in contact, that is, not clearly separated during morphogenesis (Figs 1E, 3A); rows straight and arranged in parallel or slightly converging posteriorly. Left frontoventral row commences about at same level as rightmost frontal row, sigmoidally curved and thus extending posteriorly behind buccal vertex, ends distinctly subterminally, in specimen shown in Figs 1E, 3A about at 83% of body length, composed of 57 cirri on average. Right frontoventral row begins close to right end of adoral zone and frontal corona, respectively, extends in parallel to left row to near rightmost transverse cirrus, composed of 60 cirri on average (Figs 1A, E, 3A, C, Table 1). 8–13, on average 11, narrowly spaced transverse cirri form slightly to distinctly curved row (likely a pseudorow; see Discussion) somewhat subterminally so that only rearmost cirri protrude by about 50% of their length beyond rear body end; bases of transverse cirri of about same size as remaining cirri (Figs 1A, E, 2E, 3A, Table 1). Right marginal row commences dorsolaterally about at level of buccal cirri, extends on right cell

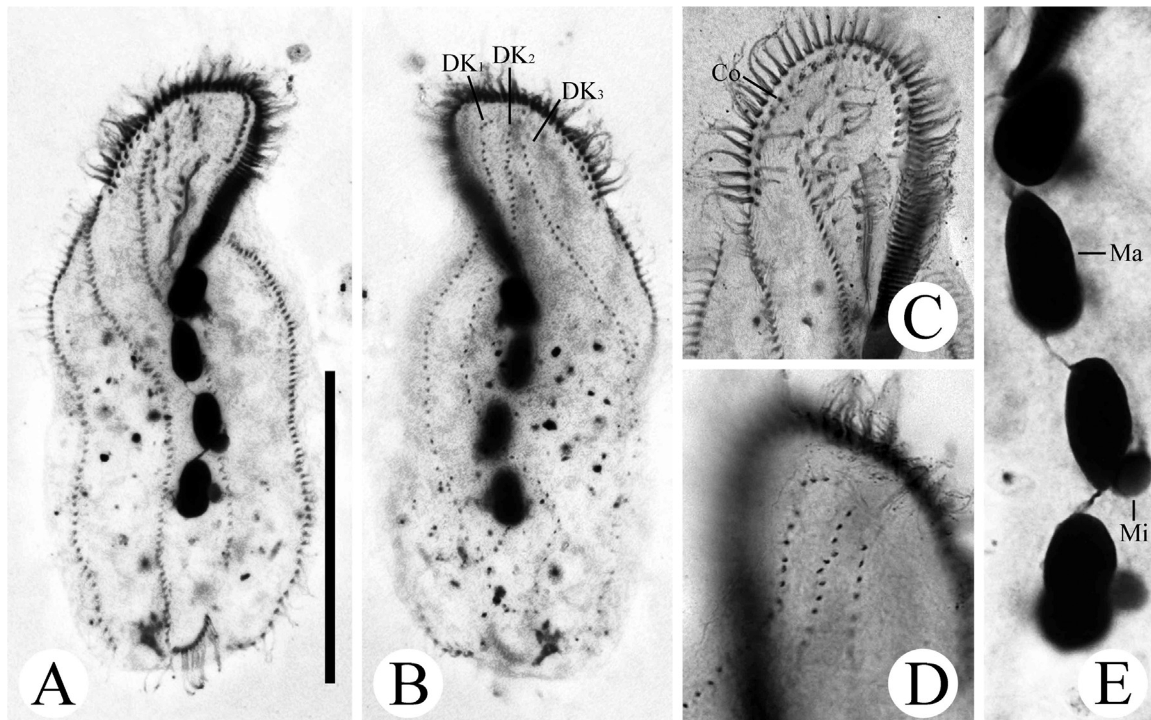


**Fig. 3.** **A–E** *Keronopsis helluo*, Antarctic population after protargol preparation. **A, B:** Ventral and dorsal view of same specimen. **C:** Ventral view of oral region showing, inter alia, frontal corona, three frontal rows, and straight and parallel undulating membranes. **D:** Anterior region in dorsal view showing dorsal kineties. **E:** Nuclear apparatus; usually composed of four macronuclear nodules and two micronuclei. $DK_{1-3}$, dorsal kineties; Ma, macronuclear nodule; Mi, micronucleus. Scale bar: 100 µm.

margin, and ends subterminally about at level of transverse cirri, composed of 31–63, on average 50, cirri. Left marginal row begins left of proximal portion of adoral zone, extends along left cell margin, and terminates about at same level as right row, that is, marginal rows widely separated posteriorly; composed of 43–69, on average 57, cirri (Figs 1A–C, E, 2E, 3A–C, Table 1).

Invariably three, more or less bipolar dorsal kineties, bristles about 4 µm long in vivo. Dorsomarginal kineties and caudal cirri absent (Figs 1F, 2F, 3B, D, Table 1).

**Resting cyst**: Few resting cysts observed; spherical, about 90 µm diameter in life (n = 5); cyst formation proceeds rapidly (Fig. 2G, H).

## *Paraholosticha pannonica* Gellért and Tamás, 1959

1959 ***Paraholosticha pannonica* n. sp.** – Gellért and Tamás, Annls Inst. biol. Tihany, 26: 225, Fig. 1 (original description; for type material see nomenclature). ZooBank registration number: urn:lsid:zoobank.org:act:10AC7C1A-5E4C-46A2-86B9-7C9B298725D7.

1975 ***Paraholosticha nana* Gellert, 1955** – Grolière, Protistologica, 11: 488, Fig. 12, 13 (description of French population; misidentification, see below).

**Nomenclature**: No derivation of the name is given in the original description or a later paper. The species-group name *pannonic·us*, *-a*, *-um* (Latin adjective [m, f, n]; living or occurring in Pannonia; Hentschel and Wagner 1996, p. 454) refers to the region (Pannonian Basin, Hungary) where the species was discovered. Gellért and Tamás (1959) mentioned neither Kahl (1932) nor Wenzel (1953) in the literature section. Thus, we determine that Gellért and Tamás (1959) have established their species in the valid genus *Paraholosticha* Wenzel, 1953 and not in the invalid genus *Paraholosticha* Kahl, 1932. Consequently, a new combination is not necessary.

Gellért and Tamás (1959) made opalblue preparations. Thus, it cannot be excluded that permanent preparations of *P. pannonica* still exist. We made no effort to locate them. A neotypification is desirable to define this species objectively. However, since our population was collected very far away (Alaska) from the original type locality (Lake Balaton, Hungary), it is not possible to use our population as neotype because it has to come from as nearly as practicable from the original type locality (ICZN 1999, Article 75.3.6).

**Description of Alaskan population** (Figs 4A–E, 5A–I, 6A–G, Table 1): Body size in vivo about 105–120 × 30–50 µm (n = 3; 400×); on average 94 × 43 µm, length:width ratio thus 2.2:1 in protargol preparations (Table 1). Body-outline in ventral view elongate elliptical with both ends broadly rounded in vivo; often inflated posteriorly in protargol preparations. Body flexible, but not

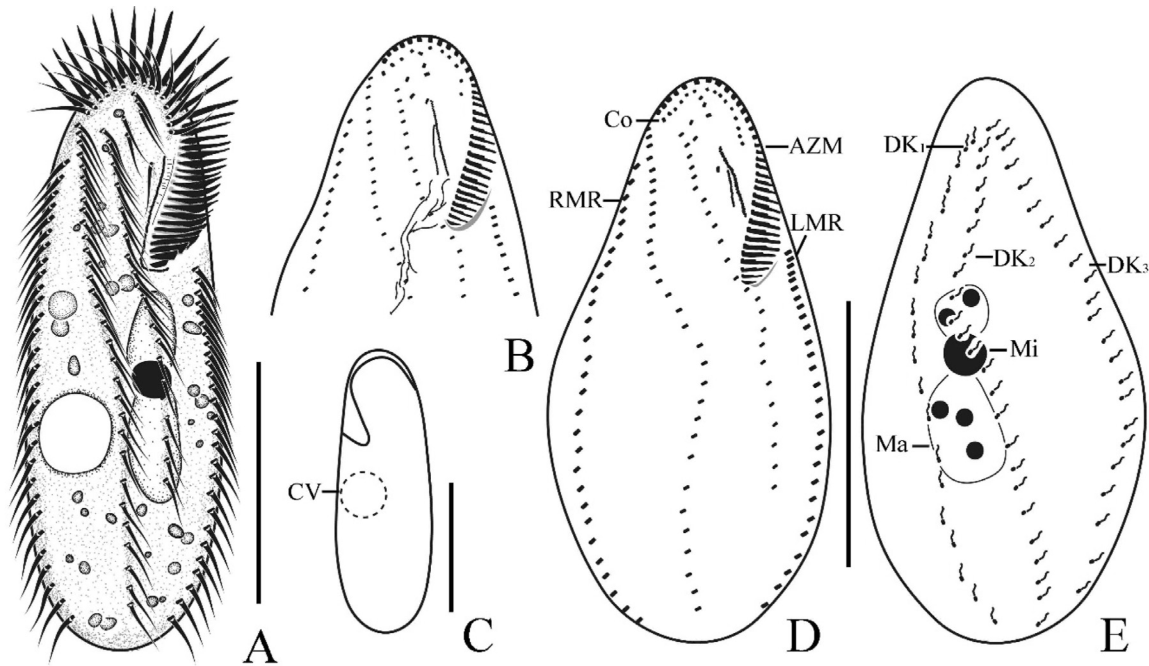


**Fig. 4. A–E** *Paraholosticha pannonica*, Alaskan population from life (A, C) and after protargol preparation (B, D, E). **A:** Ventral view showing, inter alia, nuclear apparatus with single micronucleus in between macronuclear nodules. The large vacuole in the right body portion is not the contractile vacuole. **B, D, E:** Ventral view of anterior portion and ventral and dorsal view of same specimen showing, inter alia, cirral pattern, arrangement of undulating membranes, dorsal kineties, and nuclear apparatus. **C:** Dorsal view showing position of contractile vacuole. AZM, adoral zone of membranelles; Co, frontal corona; CV, contractile vacuole; $DK_{1-3}$, dorsal kineties; LMR, left marginal row; Ma, macronuclear nodule; Mi, micronucleus; RMR, right marginal row. Scale bars: 50 µm.

distinctly contractile, distinctly flattened dorsoventrally (Figs 4A, C–E, 5A–D, H, 6A–C). Nuclear apparatus in central body portion slightly left of midline, invariably composed of two macronuclear nodules with a large, roughly spherical micronucleus in between; macronuclear nodules on average 16 × 8 µm in protargol preparations; micronucleus up to 8 µm across in vivo, about 5 × 4 µm in protargol preparations (Figs 4A, E, 5B, G, 6G, Table 1). Contractile vacuole about in mid-body near left margin of cell, approximately 20 µm in diameter when fully extended (Figs 4C, 5C). Cells grayish at low magnification. Cortical granules lacking. Movement without peculiarities, that is, glides moderately fast on bottom of Petri dish.

Adoral zone terminates at 36% of body length on average in protargol-prepared specimens; distal end extends only slightly posteriorly, DE-value thus only 0.15 on average (n = 3; Figs 4B, D, 6B); composed of 27–34, on average 31, membranelles; bases of largest membranelles approximately 6.5 µm wide on average, cilia of membranelles up to 15 µm long. Paroral and endoral straight, slightly diverging posteriorly. Pharyngeal fibers extend obliquely backwards (Figs 4A, B, D, 5A, 6B, Table 1).

All cirri relatively fine, frontal cirri 15 µm long, other cirri about 10 µm in vivo (Figs 4A, 5A, H). Cirral pattern characteristic mainly due to keronopsid frontal corona, two long frontoventral rows, and lack of transverse cirri. Frontal corona composed of 11–15, on average 13, cirri forming a roughly continuous, mixed row parallel to anterior, curved portion of adoral zone. One (11 of 19 specimens analyzed) or two buccal cirri right of anterior portion of undulating membranes. Invariably two frontal and two frontoventral rows. Each frontal row usually composed of two cirri only; left one arranged anterior of undulating membranes, right one left of anterior end of left frontoventral row. Left frontoventral row extends backwards via area of buccal vertex, terminates at 73% of body length in specimen shown in Fig. 4D; on average composed of 23 cirri. Right frontoventral row begins close to distal end of adoral zone, extends backwards about in midline of cell and terminates close to rear body end, consists of 29 cirri on average. Transverse cirri lacking. Right marginal row begins about at level of buccal cirrus, ends, like left row, slightly subterminally, marginal rows thus distinctly separated posteriorly; left row commences left of proximal portion of adoral zone, composed of 18–28 cirri, right row made of 20–31 (Figs 4A, B, D, 5H, 6A, B, F, Table 1).

Constantly three, more or less bipolar dorsal kineties, bristles about 3 µm long in vivo. Dorsomarginal kineties and caudal cirri absent (Figs 4E, 6C, E, Table 1).

**Resting cyst**: Few resting cysts observed; spherical, 47–56 × 45–48 µm in size in life (n = 3), surface more or less smooth; cytoplasm grayish (Fig. 5E, F).

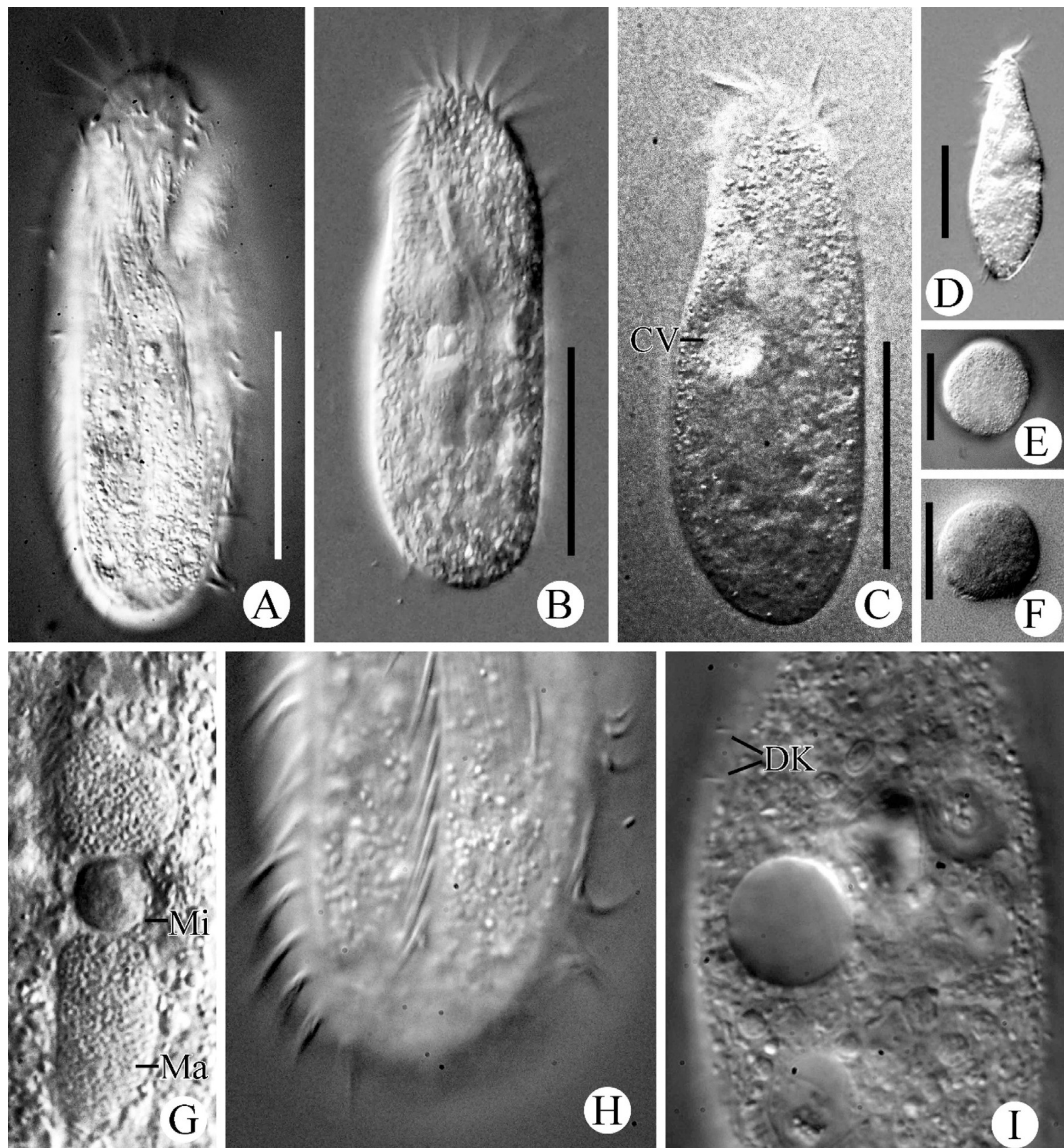


**Fig. 5.** **A–I** *Paraholosticha pannonica*, photomicrographs from life (differential interference contrast). **A, B:** Ventral and dorsal view showing body outline, parts of ciliature, and nuclear apparatus. **C:** Dorsal view showing contractile vacuole. **D:** Left lateral view showing dorsoventral flattening. **E, F:** Resting cysts. **G:** Nuclear apparatus. **H:** Ventral view of posterior body portion showing right marginal row, rear portion of right frontoventral row, rearmost cirrus of left frontoventral row, and left marginal row. **I:** Dorsal view showing some dorsal bristles of dorsal kinety 1 and contractile vacuole. CV, contractile vacuole; DK, dorsal bristles; Ma, posterior macronuclear nodule; Mi, micronucleus. Scale bars: 50 μm.

## Molecular analyses of *Keronopsis helluo* and *Paraholosticha pannonica*

The SSU rRNA gene sequences of *Keronopsis helluo* (KY492516) and *Paraholosticha pannonica* (KY492517) have a length of 1610 bp each and a GC-content of 44.5% and 44.7%, respectively. The phylogenetic trees obtained from BI and ML are similar and therefore the BI tree is represented (Fig. 7). The pairwise similarities between the three keronopsids, *Bistichella* spp., and *Orthoamphisiella breviseries* Foissner et al., 2002 range from 99.2% to 99.9% (Table 2).

*Keronopsis helluo* and *Paraholosticha* spp. form a soft polytomy with *Bistichella cystiformans* Fan et al., 2014,

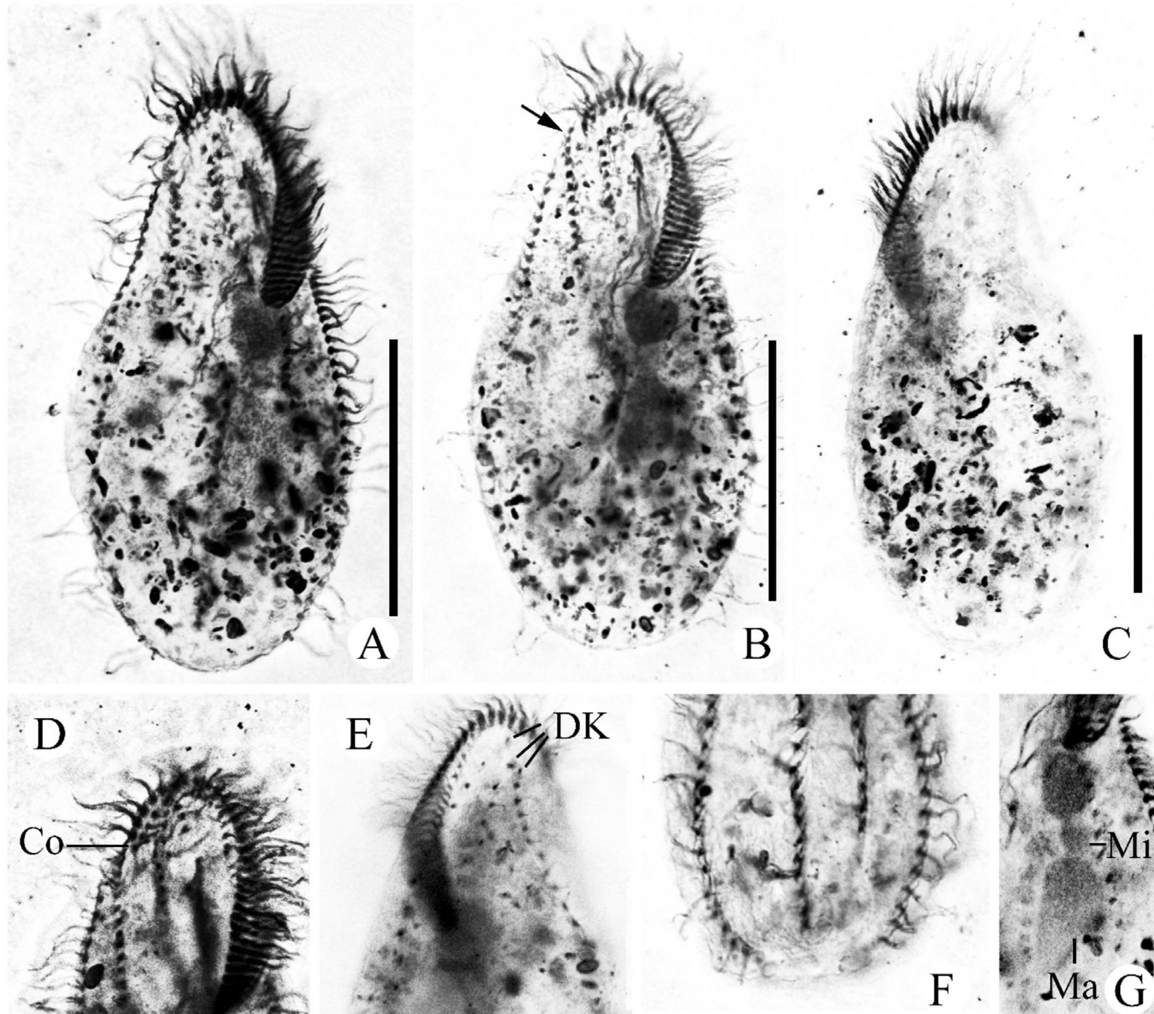


**Fig. 6.** **A–G** *Paraholosticha pannonica* after protargol preparation. **A–C:** Ventral views and dorsal view. Arrow in (B) marks anterior end of right frontoventral row. **D, E:** Ventral and dorsal view of anterior body portion showing frontal corona and dorsal kineties. **F.** Posterior body portion showing, inter alia, lack of transverse cirri. **G:** Nuclear apparatus in ventral view. Co, frontal corona; DK, dorsal kineties; Ma, posterior macronuclear nodule; Mi, micronucleus. Scale bars: 50 μm.

*B. variabilis* He and Xu, 2011, *Orthoamphisiella breviseries*, *Uroleptoides magnigranulosus* (Foissner, 1988) Berger, 2008 + *Parabistichella variabilis* Jiang et al., 2013, and a large cluster composed of amphisiellids, trachelostylids, and gonostomatids (Fig. 7). However, the support in the Bayesian inference analyses is low (posterior probability = 0.81) and there is no support (bootstrap value <50%) in the Maximum Likelihood analyses.

**Table 2.** Similarities (top right) and pairwise distances (bottom left) between SSU rRNA gene sequences of *Orthoamphisiella breviseries*, *Bistichella* spp., *Keronopsis helluo*, *Paraholosticha muscicola*, and *Paraholosticha pannonica*.

| Species | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 1 | *Orthoamphisiella breviseries* AY498654 | | 0.996 | 0.996 | 0.993 | 0.999 | 0.997 |
| 2 | *Bistichella cystiformans* KJ509196 | 0.004 | | 0.997 | 0.992 | 0.997 | 0.997 |
| 3 | *Bistichella variabilis* HQ699895 | 0.004 | 0.003 | | 0.993 | 0.997 | 0.997 |
| 4 | *Paraholosticha muscicola* KT003281 | 0.007 | 0.008 | 0.007 | | 0.993 | 0.993 |
| 5 | *Keronopsis helluo* KY492516 | 0.001 | 0.003 | 0.003 | 0.007 | | 0.999 |
| 6 | *Paraholosticha pannonica* KY492517 | 0.003 | 0.003 | 0.003 | 0.007 | 0.001 | |

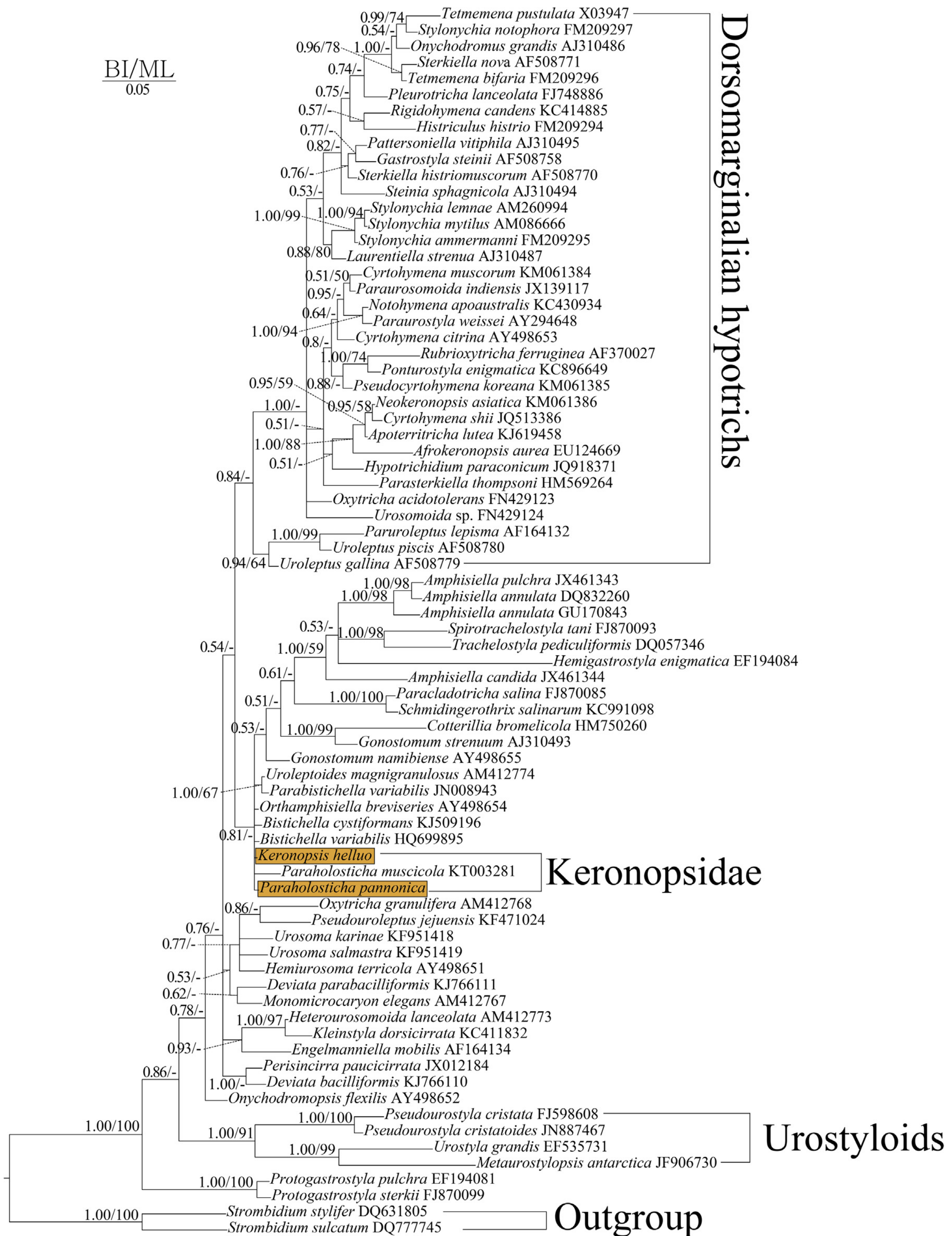


**Fig. 7.** Phylogenetic tree based on SSU rRNA gene sequences showing the position of *Keronopsis helluo* and *Paraholosticha pannonica* as determined by the Bayesian inference (BI) and Maximum Likelihood (ML) methods. GTR + I (0.6540) + G (0.4900) was selected as the best model from jModelTest. Posterior probability values for the BI and bootstrap values for the ML are shown on the interior branches (for interpretation of values, see Material and Methods). Dashes denote bootstrap values <50% or different topologies in BI and ML phylogenies.

## Discussion

### Identification of Antarctic population as *Keronopsis helluo* and comparison with similar species

*Keronopsis helluo*, type of the genus, is a poorly understood species because it is known only from the original description, which is rather long, but not very detailed, especially as concerns the frontal ciliature (Penard 1922). So far, it is reliably recorded only from terrestrial moss in Switzerland. Our isolate agrees with the type population in the large body size (160–235 μm long in vivo or 250–300 μm according to Penard 1922); the number of macronuclear nodules (four or five or 4–6); the presence of several, relatively large micronuclei (1–12, on average three or five in specimen illustrated by Penard 1922); the number of transverse cirri (8–13 or 8–10); the presence of a frontal corona; the presence of two long frontoventral rows; the far posteriorly extending distal end of the adoral zone; the presence of several buccal cirri; and the habitat (mossy soil). The dorsal ciliature of the type population is not known and details of the original description (e.g., cirral pattern in frontal region, oral apparatus) must not be over-interpreted because Penard (1922) did not have the advantage of silver preparation. In spite of that, we tentatively assume that our population is conspecific with *K. helluo*. However, it would not be a great surprise when more or less distinct morphological and/or molecular differences become evident when European populations are studied in detail because the populations are spatially (about 16.000 km; Google Maps) and chronologically widely separated (division of Pangea into Gondwana [including Antarctica] and Laurasia [including "Switzerland"] started about 160 my ago according to Cox and Moore 2010, p. 158).

Bovee (1979) provided an illustration of *K. helluo*, but no description. The outline of the depicted specimen and the posteriorly protruding transverse cirri agree very well with the specimen illustrated by Penard (1922). However, for the following reasons the identification cannot be accepted: (i) the single macronuclear nodule shown is rather large; this indicates that the specimen observed by Bovee had a maximum of only two nodules (vs. 4–6 relatively small nodules in type population and four or five in our population); (ii) the contractile vacuole is displaced posteriad while it is anterior of mid-body in the type population and the Antarctic specimens; and (iii) the depiction of the ciliature is too sketchy, although it cannot be excluded that Bovee observed a keronopsid, inasmuch as he found it in a *Sphagnum* bog (Buell Bog, Lake Itasca region, Minnesota, USA).

*Keronopsis polychaeta* (Borror, 1966) Jankowski, 1979 (original combination *Paraholosticha polychaeta*) has, like *K. helluo*, eight transverse cirri, which are, however, arranged at right angles (Borror 1966). It can be separated from the type species very easily by the lower number of macronuclear nodules (two vs. 4–6) and the habitat (tidal marsh pool vs. terrestrial moss). Jankowski (1979, p. 60) not only transferred this species to *Keronopsis* but also fixed it as type species of the subgenus *Paraholosticha (Estuaricola)*. However, further morphological, ontogenetic, and molecular studies on *K. polychaeta* are needed to support or disprove this hypothesis. The complicated nomenclature of *K. polychaeta* (e.g., simultaneously assigned to *P. (Estuaricola)* and *Keronopsis* by Jankowski 1979; three times combined with *Keronopsis*) is beyond the scope of the present paper. *Keronopsis* sp. in Dieckmann (1995, p. 377, his Fig. 9) is perhaps identical with *K. polychaeta*; however, the transverse cirri (eight or seven plus one pretransverse ventral cirrus) are not arranged perpendicularly, but in a slightly curved row.

*Keronopsis dieckmanni* Foissner, 1998 and *K. helluo* have a similar size (220 μm long in vivo or 160–300 μm) and nuclear apparatus (four or 4–6 macronuclear nodules). However, they differ, inter alia, in the number of transverse cirri (4–7, five on average vs. 8–13), the number of frontal cirri (five vs. 22–31), the oral apparatus (relative length in protargol preparations 25% of body length on average in type population vs. 37% in Antarctic population, Table 1; DE-value about 0.25 vs. about 0.50; undulating membranes distinctly curved vs. roughly straight), and the habitat (saline soil vs. mossy soil). Perhaps they also have a non-overlapping geographic distribution (Africa, Arabia vs. Europe, Antarctica; Foissner 1998; Foissner et al. 2002, 2008; Penard 1922; present study). *Keronopsis paradieckmanni* Ning et al., 2009 differs from *K. dieckmanni* only indistinctly in some morphometric features, indicating that it is a subspecies of, or even synonyms with, the latter.

*Keronopsis tasmaniensis* Blatterer and Foissner, 1988 has only two macronuclear nodules (vs. 4–6 in *K. helluo*) and five transverse cirri (vs. 8–13). So far, *K. tasmaniensis* is reliably recorded only from Tasmania (Blatterer and Foissner 1988), New Zealand (Foissner et al. 2012), and the Palaeotropis (Africa south of Sahara desert, Madagascar, India; Foissner 1998).

*Keronopsis dieckmanni* and *K. tasmaniensis* differ from the type species not only in the features mentioned above, but also in the formation of the frontal ciliature. *Keronopsis helluo* has, like *Paraholosticha* species, a characteristic keronopsid frontal corona which is a mixed row of normal-sized cirri (Figs 1A–C, E, 2I, 3A, C). In *Paraholosticha* and *K. wetzeli* these frontal cirri originate from the anlagen I to III (for details, see Dieckmann 1988a, 1988b, 1989; Tuffrau and Fryd-Versavel 1977). By contrast, the short corona of *K. dieckmanni* and *K. tasmaniensis* is, very probably, a pseudorow, that is, each frontal cirrus is more or less distinctly enlarged and originates from a different anlage. In addition, *K. dieckmanni* and *K. tasmaniensis* have distinctly curved undulating membranes while they are more or less straight in *K. helluo* (Blatterer and Foissner 1988; Foissner 1998; Foissner et al. 2008; Figs 1A, E, 3A, C). Of course, the supposed difference in the frontal ciliature has to be confirmed by ontogenetic data of *K. helluo*, *K. tasmaniensis*, and *K.*

*dieckmanni*; thus, we refrain from the transfer of the latter two species to a new genus.

According to our phylogenetic analyses based on the SSU rRNA (Fig. 7), the keronopsids sequenced so far (*K. helluo*, *P. pannonica*, *P. muscicola*; present paper; Jung et al. 2015) emerge from the node of a soft polytomy, which also comprises *Bistichella cystiformans*, *B. variabilis*, *Orthoamphisiella breviseries*, *Uroleptoides magnigranulosus* + *Parabistichella variabilis*, and a large cluster composed of amphisiellids, trachelostylids, and gonostomatids (for details on these taxa, see Berger 2008, 2011; Fan et al. 2014; Foissner 1988; Foissner et al. 2002, 2004; He and Xu 2011; Schmidt et al. 2007). All taxa are non-dorsomarginalian hypotrichs, but the non-keronopsid species can be easily separated from *Keronopsis* and *Paraholosticha* by the lack of the keronopsid frontal corona and, very likely, also by the mode of cell division, namely not in a cyst (for revision, see Berger 2008, 2011) against division in a cyst (e.g., Dieckmann 1988a, 1989; Garnjobst 1934, 1937; Penard 1922; Tuffrau and Fryd-Versavel 1977). Surprisingly, the molecular marker does not reflect these important morphological and ontogenetic differences, indicating that gene sequence data should not be over-interpreted.

## Identification of Alaskan population as *Paraholosticha pannonica* and comparison with congeners

Based on the ventral ciliature (keronopsid frontal corona; two short frontal rows; two long frontoventral rows; transverse cirri absent; two marginal rows) and the dorsal kinety pattern (three bipolar rows, that is, dorsomarginal kineties and kinety fragmentation lacking; caudal cirri absent) we assign the Alaskan population to *Paraholosticha*. Two species are known in this genus which have, like our population, a single micronucleus in-between the two macronuclear nodules, namely, *P. herbicola* (Kahl, 1932) Wenzel, 1953 (type locality: bog near Hamburg, Germany) and *P. pannonica* Gellért and Tamás, 1959 (Lake Balaton, Hungary) (Berger 2001). By contrast, *Paraholosticha nana* Gellért, 1956 has one micronucleus attached to each of the two macronuclear nodules (Gellért 1956). Thus, *P. nana* sensu Grolière (1975), isolated from a French *Sphagnum* pond, is a misidentification because it has the same nuclear apparatus as *P. herbicola* and *P. pannonica*. We synonymize the population studied by Grolière (1975) with *P. pannonica* because it agrees very well with the type population (i) in the body length (90–115 μm in protargol preparations; or 80 μm [according to text] to about 90 μm [from Fig. 1 in Gellért and Tamás 1959] in opal blue preparations); (ii) in the number of buccal cirri and cirri forming the frontal rows (three [from Fig. 12 in Grolière 1975; left frontal row not illustrated?] to five or six [from Fig. 13 in Grolière 1975, left frontal row and frontal corona almost not recognizable]; or six in *P. pannonica*); (iii) in the number of cirri forming the frontal corona (15 according to Fig. 12 in Grolière 1975; or 13 according to Fig. 1 in Gellért and Tamás 1959); and (iv) in the number of cirri in the left and the right frontoventral row (18 and 30, from Fig. 12 in Grolière 1975; or 20 and 26 from Fig. 1 in Gellért and Tamás 1959). The number of marginal cirri is somewhat higher in the population studied by Grolière (1975; 25–29 left [from text and Fig. 12 in Grolière 1975] and 24–27 right vs. 20 left and 22 right [from Fig. 1 in Gellért and Tamás 1959]). By contrast, the number of adoral membranelles is distinctly higher in the population studied by Grolière (1975) (28–34) than in the specimen illustrated by Gellért and Tamás (1959) (about 17). However, in the illustration of Gellért and Tamás (1959) the adoral zone ends much more anteriorly than the frontal corona indicating that the anterior end portion of the zone has been overlooked (not stained) so that the number of membranelles is very likely distinctly higher than 17. Since the specimens of the two populations have about the same body length and the same ratio of adoral zone length to body length we have to assume that the number of adoral membranelles is also very similar. The micronucleus is somewhat larger (diameter 7.8 μm according to text, but 5 μm in specimen illustrated) in the French population than in the type population (about 3 μm). By contrast, the distance between the macronuclear nodules is smaller in the French population. Considering that both descriptions and morphometric characterizations are not very detailed, the differences should not be over-interpreted (Gellért and Tamás 1959; Grolière 1975).

Our specimens from Alaska agree very well with the data above in size (body 105–120 μm long in vivo and 85–107 μm in protargol preparations; Table 1); total number (median = 5) of buccal cirri and cirri in frontal rows 1 and 2; number of cirri in frontoventral rows (19–26 left and 23–35 right; Table 1); number of coronal cirri (11–15); and number of adoral membranelles (27–34). These similarities suggest an identification of our population as *P. pannonica*. The dorsal bristles of our population are about 3 μm long while those of the French population have a length of 5–6 μm (Grolière 1975). For the type population no value is available (Gellért and Tamás 1959). Further, detailed morphological studies on other populations, supported by gene sequence data, will show whether or not *P. pannonica* is a somewhat variable species or a complex of two or more (sub)species.

*Paraholosticha herbicola*, the second species with a single micronucleus in-between the macronuclear nodules, differs from *P. pannonica* mainly in body size (150–190 μm long in vivo vs. up to 120 μm in Alaskan population of *P. pannonica*) and the number of cirri in frontal rows 1 (seven according to Fig. 88 in Kahl 1932) and 2 (nine, including buccal cirri which are not clearly separated), that is, the total number of these cirri is about three times as high (16 vs. usually five) as in *P. pannonica*. A detailed redescription of *P. herbicola* will certainly reveal further, relevant differences.

There exist two further, more or less similar species with a single micronucleus in-between the two macronuclear nodules. *Keronopsis wetzeli* Wenzel, 1953 has 1–3 transverse cirri (Berger and Foissner 1987; Grolière 1975; Wenzel 1953;

for brief explanation of problem with population studied by Wenzel 1953, see Foissner and Al-Rasheid 2007, p. 209). *Holosticha (Keronopsis) alpestris* Kahl, 1932, a little-known species, was transferred to the invalid genus *Paraholosticha* Kahl, 1932 by Borror (1972). Since it has a rather distinct midventral pattern and enlarged frontal cirri, Berger (2006) transferred it to the inhomogeneous melting-pot *Anteholosticha* Berger, 2003. It can be easily separated from *P. pannonica* by the presence of transverse cirri.

## *Parakeronopsis* Shi, 1999; a valid keronopsid genus?

Shi (1999, p. 256) established this genus for *Keronopsis wetzeli* Wenzel, 1953 (type species), a species with 1–3 transverse cirri (Berger and Foissner 1987; Grolière 1975; Wenzel 1953). In addition, he assigned – admittedly somewhat cryptically – *K. tasmaniensis* Blatterer and Foissner, 1988 to *Parakeronopsis* (see legend to Fig. 25A, B in Shi 1999; no formal combination made). From the brief discussion it can be concluded that Shi separated this new genus from *Keronopsis* by the presence of transverse cirri. Obviously, Shi (1999) overlooked that *Keronopsis helluo*, type and thus relevant species of *Keronopsis*, has very prominent transverse cirri (Penard 1922; Figs 1A, E, 2E, 3A). Consequently, *Parakeronopsis* is the junior subjective synonym of *Keronopsis* when considering the presence/absence of transverse cirri as diagnostic feature.

However, the situation is more complex because *Keronopsis helluo* and *K. wetzeli* differ significantly in the number of transverse cirri. The former has 8–13 while *K. wetzeli* has only 1–3 (Berger and Foissner 1987; Grolière 1975; Penard 1922; Wenzel 1953; Table 1).

Unfortunately, ontogenetic data are lacking for *K. helluo* so that we can only speculate about the origin of the transverse cirri. Most specimens of the Antarctic population of *K. helluo* have three frontal rows, and two frontoventral rows which are present in all specimens (Fig. 1E, Table 1); some specimens have four frontal rows (Fig. 1B) and only very rarely five frontal rows are present (Fig. 1C), that is, the number of frontoventral-transverse cirri anlagen very likely ranges from 5–7 without anlage I, which never forms a transverse cirrus (e.g., Berger 2008). Usually, a transverse cirral row is a pseudorow, that is, each cirrus originates from a different frontoventral-transverse cirri anlage (Berger 2008, p. 10). Consequently, the presence of 8–13 transverse cirri in *K. helluo* would require 8–13 anlagen (anlage I not included), a number which is distinctly higher than the number of frontal plus frontoventral rows present in interphasic specimens (e.g., the specimen shown in Fig. 1E has 12 transverse cirri, but only five rows, namely three frontal and two frontoventral rows). Several scenarios exist to explain this discrepancy: (i) the transverse cirral row is a pseudorow and the "surplus" transverse cirri (seven in present example) originate from anlagen which only form a transverse cirrus, but no frontal or frontoventral cirri. (ii) The transverse cirral row is a pseudorow and the "surplus" cirri originate from anlagen (nine in present case) which produce not only a transverse cirrus each, but also a short row of frontoventral cirri; these short rows (fragments) align longitudinally to form the two frontoventral rows (in that case the frontoventral rows would not be true rows, but mixed rows). (iii) The transverse cirral row is a mixed row, that is, some (all?) of the anlagen (five in present example) form more than one transverse cirrus. (iv) The transverse cirral row is a true row, that is, all transverse cirri are formed by the same anlage, perhaps from that which also forms the left frontoventral row, which is (for that reason?) distinctly shortened posteriorly in *K. helluo* (Fig. 1A, E). Only a detailed study of the cell division of *K. helluo* will reveal which of the four modes applies. Of course, we cannot exclude that the transverse cirri are formed in a way not described above, for example, de novo.

*Keronopsis wetzeli* is, like *K. schminkei* Foissner and Al-Rasheid, 2007, a species with a low number (1–3) of transverse cirri. The population studied by Dieckmann (1988b) had three transverse cirri, which have been formed by anlagen II and III (they also form the middle and right portion of the frontal corona) and the anlage for the left frontoventral row. The fact that the number of transverse cirri is lower than the number of anlagen is known from several other taxa, for example, most urostyloids, gonostomatids, and *Urosomoida* Hemberger in Foissner, 1982 (for revisions see Berger 1999, 2006, 2011).

The discussion demonstrates that there must be a distinct difference between *K. helluo* and *K. wetzeli* (type of *Parakeronopsis*) in the ontogenesis of the transverse cirri. Thus, it cannot be excluded that *Parakeronopsis* is a valid taxon. However, ontogenetic data on *K. helluo* and gene sequence analyses of *K. wetzeli* are needed to get an idea about the relationships within the keronopsids.

## Brief characterization of the Keronopsidae and *Keronopsis helluo* and *Paraholosticha pannonica*

Based on the data of the original descriptions, previous redescriptions, and the present study we provide brief characterizations of these little-known species. In addition, we update the characterization of the keronopsids, inter alia, based on the redescription of the type species of the name-bearing type genus. As demonstrated in the discussion above, the definitions of the genera and their relationships are not yet clear. Thus, we refrain from improved diagnoses.

Keronopsidae Jankowski, 1979: Non-dorsomarginalian hypotrichs (dorsomarginal kineties and kinety fragmentation lacking) with three bipolar dorsal kineties. Body flexible, but not distinctly contractile. Cortical granules lacking. Adoral zone continuous; undulating membranes more or less straight and arranged in parallel. Keronopsid frontal corona composed of three rows originating from anlagen I to III, that is, a mixed row; frontal cirri not or not distinctly enlarged. Two

or more (up to five in *K. helluo*) frontal rows. Two frontoventral rows. Transverse cirri present (*Keronopsis*, *Estuaricola* [valid genus?], *Parakeronopsis* [valid genus?]) or lacking (*Paraholosticha*). One left and one right marginal row. Caudal cirri lacking. Division in cyst. Name-bearing type genus: *Keronopsis* Penard, 1922. Genera assigned: *Keronopsis* Penard, 1922; *Paraholosticha* Wenzel, 1953. ZooBank registration number: urn:lsid:zoobank.org:act:6A9D5FC1-276A-40FF-ADB4-08A30226F15E.

*Keronopsis helluo* Penard, 1922 (based on data from original description and present study): Body size 160–300 × 65–105 μm in vivo. Body outline elongate-elliptical. 4–6 macronuclear nodules, usually 3–5 micronuclei. 53–76 adoral membranelles, DE-value about 0.50. Frontal corona composed of 22–31, on average 26, cirri. 3–8 buccal cirri. 3–5, usually three frontal rows composed of 3–6, 2–5, and 3–9 cirri. Left frontoventral row made of 44–70 cirri, right one of 48–73. 8–13 transverse cirri. Left marginal row composed of 43–69 cirri, right one of 31–63. Dorsal bristles about 4 μm long. So far recorded only from mossy soil.

*Paraholosticha pannonica* Gellért and Tamás, 1959 (based on data from original description, Grolière 1977, and present study): Body size about 80–120 × 30–50 μm in vivo. Body outline elongate-elliptical. Two macronuclear nodules with one micronucleus in between. 27–34 adoral membranelles, DE-value about 0.15. Frontal corona composed of 11–15, on average 13 cirri. One or two buccal cirri, usually one. Two frontal rows, each composed of 1–3 cirri. Left frontoventral row made of 18–26 cirri, right one of 23–35. Left marginal row composed of 18–28 cirri, right one of 20–31. Dorsal bristles 3–6 μm long. So far recorded from limnetic habitats and mossy soil.

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