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Morphology and molecular phylogeny of an Antarctic population of *Paraholosticha muscicola* (Kahl, 1932) Wenzel, 1953 (Ciliophora, Hypotricha)

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ABSTRACT

The morphology of an Antarctic soil population of *Paraholosticha muscicola*, type species of *Paraholosticha*, is described from life and after protargol preparation. The data agree rather well with that of relevant descriptions, but the total variability of several features is relatively high in this species. *Paraholosticha ovata* and *P. lichenicola* are very likely junior synonyms. In addition, we sequenced the SSU rRNA gene of *P. muscicola* and thus we can estimate for the first time the phylogenetic position of a member of the Keronopsidae, the sole hypotrichs that divide in cysts. The molecular data basically support the position derived from morphological concepts, that is, *P. muscicola* branches off outside the Dorsomarginalia because kinety fragmentation and dorsomarginal rows are lacking. However, as in many other molecular analyses, discrepancies with morphology-based hypothesis are present. The misclassification of *Paraholosticha* and its sister-group *Keronopsis* in the Keronidae, with *Kerona pediculus* as type species, is discussed.

1. Introduction

Unicellular eukaryotes, for example, ciliates, are considered as an important group in food web and nutrient cycling in Antarctic soil ecosystems because of the extreme environment for higher plants and animals (Bamforth et al., 2005; Petz, 1997). Ciliates from Antarctic terrestrial ecosystems are sparsely investigated and they are known to have a highly patchy distribution (Foissner, 1996; Smith, 1978; Sudzuki, 1979; Thompson, 1972).

Up to date, more than 70 ciliate species are reported from Antarctic soils (Foissner, 1998; Sudzuki, 1979), including some species of the *Paraholosticha*/*Keronopsis* group, namely, *Paraholosticha muscicola* (Kahl, 1932) Wenzel, 1953, *Paraholosticha herbicola* (Kahl, 1932) Wenzel, 1953, and *Keronopsis wetzeli* Wenzel, 1953 (Petz, 1997; Petz and Foissner, 1996, 1997; Sudzuki, 1979; present study). *Paraholosticha terricola* in Sudzuki (1979) is likely an unintended combination of a hypotrich having the species-group name *terricola* with the genus-group name *Paraholosticha*.

Paraholosticha Wenzel, 1953 (note that *Paraholosticha* Kahl, 1932 is invalid because no type species was fixed; ICZN, 1999, Article 67.4.1) and *Keronopsis* Penard, 1922 are, at the present state of knowledge, the sole hypotrichs which divide exclusively in cysts. Furthermore, they have a particular frontal ciliature, namely a corona which is formed by the anterior portion of the anlagen I, II, and III (e.g., Dieckmann, 1988; Penard, 1922).

In the Antarctic soil from King George Island, we found *P. muscicola*, the type species of *Paraholosticha*. We describe the morphology of this population and, more importantly, provide the first gene sequence-based phylogenetic analyses of a member of the Keronopsidae Jankowski, 1979. In addition, we briefly discuss why *Keronopsis* and *Paraholosticha* should not be classified in the Keronidae Dujardin, 1840 as proposed by several taxonomists (Hemberger and Wilbert, 1982; Jankowski, 2007; Lynn, 2008).

2. Materials and methods

2.1. Sample collection, identification, terminology

Paraholosticha muscicola was isolated from a moss-covered soil

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sample collected near the King Sejong station in the southwestern part (S62°13′49.1″ W58°42′37.3″) of King George Island, Antarctica, in December 2013. The sample was air-dried for 15 d and transported and analyzed in the Korea Polar Research Institute, Incheon, South Korea. The dried sample was kept in a refrigerator at −20 °C during the four-month transport and kept at −70 °C after arriving at the institute. After ten months from the sampling, the species was reactivated from the resting cysts using the non-flooded Petri dish method at 4 °C (Foissner et al., 2002). Attempts failed to set up clonal cultures in mineral water with wheat or rice grains to support bacterial growth. Thus, all data are based on specimens from a raw culture.

Live specimens were observed with a light microscope (Zeiss Axio Imager2, Carl Zeiss, Oberkochen, Germany) at magnifications ranging from 50× to 1000× using the bright field and the differential interference contrast method. Protargol preparation was performed to reveal the infraciliature (Foissner, 1991, procedure A). Drawing of live specimen is based on free-hand sketches while those from protargol-prepared individuals were made with a drawing device. Counts and measurements of silvered specimens were performed at a magnification of 1000×. Six voucher slides have been deposited in the collection of microscopic slides in the National Institute of Biological Resources (accession numbers NIBRPR0000105681, NIBRPR0000105682) and the Korea Polar Research Institute (accession numbers ACNS000007–ACNS00010).

General terminology is according to Lynn (2008), for terms specific for hypotrichs (e.g., DE-value, dorsoventral flattening, mixed row), see Berger (1999, 2006, 2008, 2011).

2.2. Polymerase chain reaction amplification and sequencing

Paraholosticha muscicola was washed several times with distilled water to isolate a single cell. Genomic DNA was extracted using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA), according to the manufacturer's instruction. The 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 for 30 s, extension at 68 °C for 4 min, and a final extension step at 72 °C for 7 min. Nearly complete SSU rRNA gene amplification was accomplished using two primers: slightly modified New EukA (5′-CTG GTT GAT YCT GCC AGT-3′) and LSUrev3 (5′-GCA TAG TTC ACC ATC TTT CG-3′) (Sonnenberg et al., 2007). The PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany). Two internal primers were used for sequencing: 18S+810 and 18S-300 (Jung et al., 2011). DNA sequencing was performed using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Phylogenetic analyses

In order to analyze the phylogenetic relationships of *P. muscicola*, SSU rRNA gene sequences of 72 species of hypotrichs were used. *Strombidium styliferum* and *Strombidium sulcatum* were selected as outgroup. GenBank accession numbers are provided after the species name in the phylogenetic tree (Fig. 4). 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 refined by checking using eye. Mega 5.2.2 was used to calculate the pairwise distances (Tamura et al., 2011). A substitution model for the phylogenetic analyses was chosen using the jModelTest 2.1.1 (Darriba et al., 2012). The model GTR + I (0.6560) + G (0.4960) under the Akaike information criterion (AIC) was selected as the best model. The Bayesian inference tree was built by MrBayes 3.2.5 (Ronquist et al., 2012) using the Markov chain Monte Carlo (MCMC) for 1,000,000 generations with a burn-in of 300,000. The maximum likelihood tree was obtained using the PhyML version 20131022 (Guindon et al., 2010) in Bio-Linux 8 (Field et al., 2006) with 1000 bootstrap replicates.

3. Results

3.1. Morphology of Antarctic population of Paraholosticha muscicola (Figs. 1–3; Table 1)

Body size in vivo 100–130 μm × 45–55 μm; 91 μm × 50 μm on average in protargol preparations. Body outline elongate-ellipsoidal, anterior end moderately wide, posterior end widely rounded (Figs. 1A and 2A, C). Body about 2:1 dorsoventrally flattened; flexible, but not contractile (Fig. 2B). Cells grayish at low magnification. Invariably two ellipsoidal macronuclear nodules arranged in slightly oblique, longitudinal row about in midline, nodules usually (18 out of 19 cells analyzed) connected by thin thread; individual nodules about 14 μm × 11 μm on average in protargol preparations (Table 1). Number of micronuclei highly variable, namely from 2 to 13; micronuclei scattered around macronuclear nodules and thin thread in between, spherical to ellipsoidal, on average about 3 μm across in protargol preparations (Figs. 1A, C–E and 3E, F; Table 1). Contractile vacuole slightly ahead of mid-body and near left margin of cell, approximately 11 μm in diameter when fully extended (Figs. 1A and 2C). Cortical granules lacking. Movement without peculiarities, that is, glides moderately fast on the bottom of Petri dish.

Adoral zone of membranelles terminates on average at 32% of body length in protargol preparations, DE-value of specimens documented about 0.42 (Fig. 3A, C); bases of largest membranelles approximately 6 μm long on average, cilia of membranelles up to 16 μm long. Paroral and endoral membrane arranged in parallel, paroral about 50% longer than endoral, terminate at same level; endoral commences at level of buccal cirrus (Figs. 1B and 3A, C; Table 1). Buccal cavity narrow. Pharyngeal fibers extend obliquely backwards.

All cirri relatively fine, mostly 10–16 μm long in vivo (Fig. 1A). Cirral pattern rather constant, number of cirri of usual variability (Table 1). Frontal corona composed of 12–20 cirri which form continuous bow arranged in parallel to distal portion of adoral zone, terminates about at same level on both sides (Figs. 1A, B, 2D, F and 3A, C, D). Buccal cirri, 1–3 in number, arranged in longitudinal row right of middle portion of paroral; never form continuous row with cirri of ventral row 1 (Figs. 1A, B and 3A, C, D). Ventral cirri arranged in four slightly oblique, longitudinal rows; row 1 (leftmost one) composed of one or two cirri; row 2 of 1–3; row 3 of 16–35; and row 4 of 21–37 cirri (Figs. 1A, B and 3A, C; Table 1). Rows 1 and 2 arranged in area ahead and right of anterior end of undulating membranes; rows 3 and 4 commence about at level of distal end of adoral zone, terminate distinctly (row 3) to slightly (row 4) subterminally. Transverse cirri lacking. Right marginal row commences about at level of buccal cirrus, terminates, like left row, about at 92% of body length in specimen illustrated, that is, marginal rows widely separated posteriorly; left row begins left of proximal end of adoral zone (Figs. 1B and 3A, C). Constantly irregularly shaped argyrophilic structures close to cirri, however, number, position, and size of structures rather variable (Fig. 3C, D). Invariably three roughly bipolar dorsal kineties; dorsal bristles 4 μm long in vivo. Caudal cirri lacking.

3.2. Division cysts (Fig. 2G, H)

Few division cysts were observed: slightly elongate-ellipsoidal (80 μm × 62 μm in Fig. 2G); covered with numerous bacteria-like structures, mostly 2–4 μm × 0.8 μm in vivo; content eight-

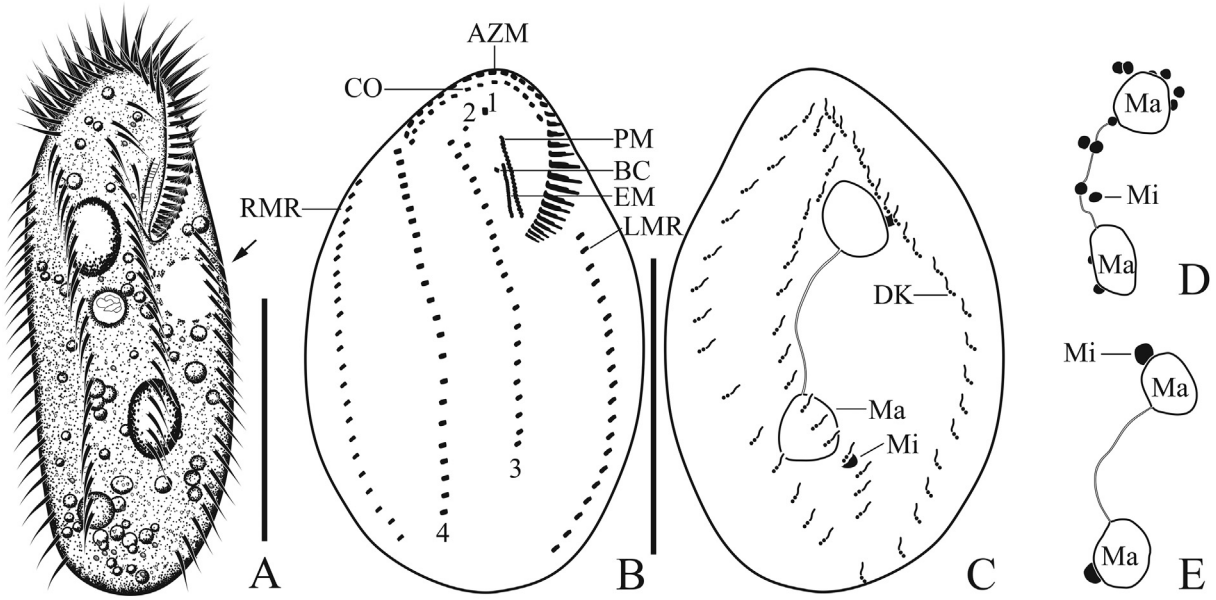

Fig. 1. A–E. *Paraholosticha muscicola* from life (A) and after protargol preparation (B–E). A. Ventral view of typical specimen; arrow denotes contractile vacuole. B, C. Infraciliature of ventral (B) and dorsal (C) side and nuclear apparatus of representative specimen. D, E. Nuclear apparatus with 13 and with two micronuclei, dorsal views (see also Fig. 3E, F). AZM, adoral zone of membranelles; BC, buccal cirrus; CO, coronal cirri; DK, dorsal kinety 3; EM, endoral membrane; LMR, left marginal row; Ma, macronuclear nodule; Mi, micronucleus; PM, paroral membrane; RMR, right marginal row; 1–4, ventral rows 1–4. Scale bars, 50 μm.

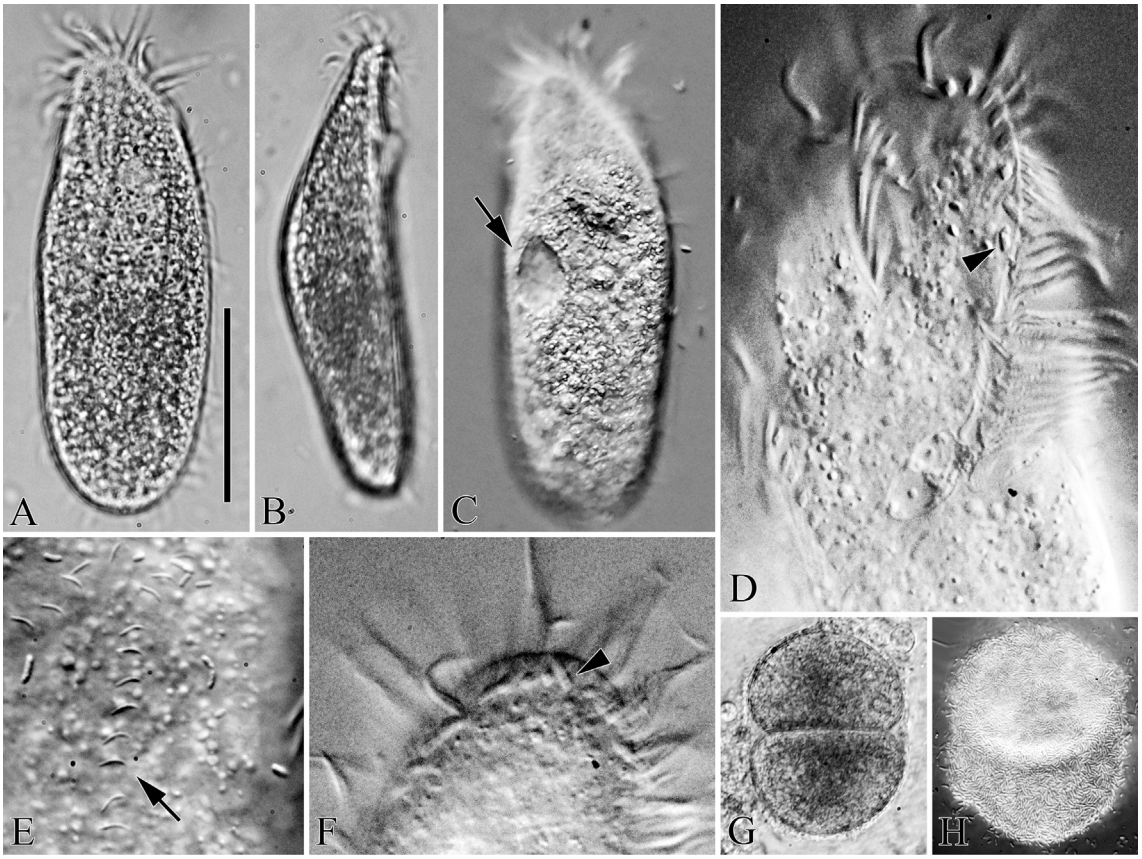

Fig. 2. A–H. *Paraholosticha muscicola* in vivo. A–C. Dorsal, right lateral, and dorsal view of a typical specimen; arrow marks contractile vacuole. D, F. Ventral view of anterior body portion; arrowhead marks left end (D) and middle portion (F) of frontal corona. E. Dorsal view showing the about 4 μm long dorsal cilia (arrow). G, H. Division-cyst showing two daughter cells (G) and dense layer of bacteria-like structures (H). Scale bars, 50 μm.

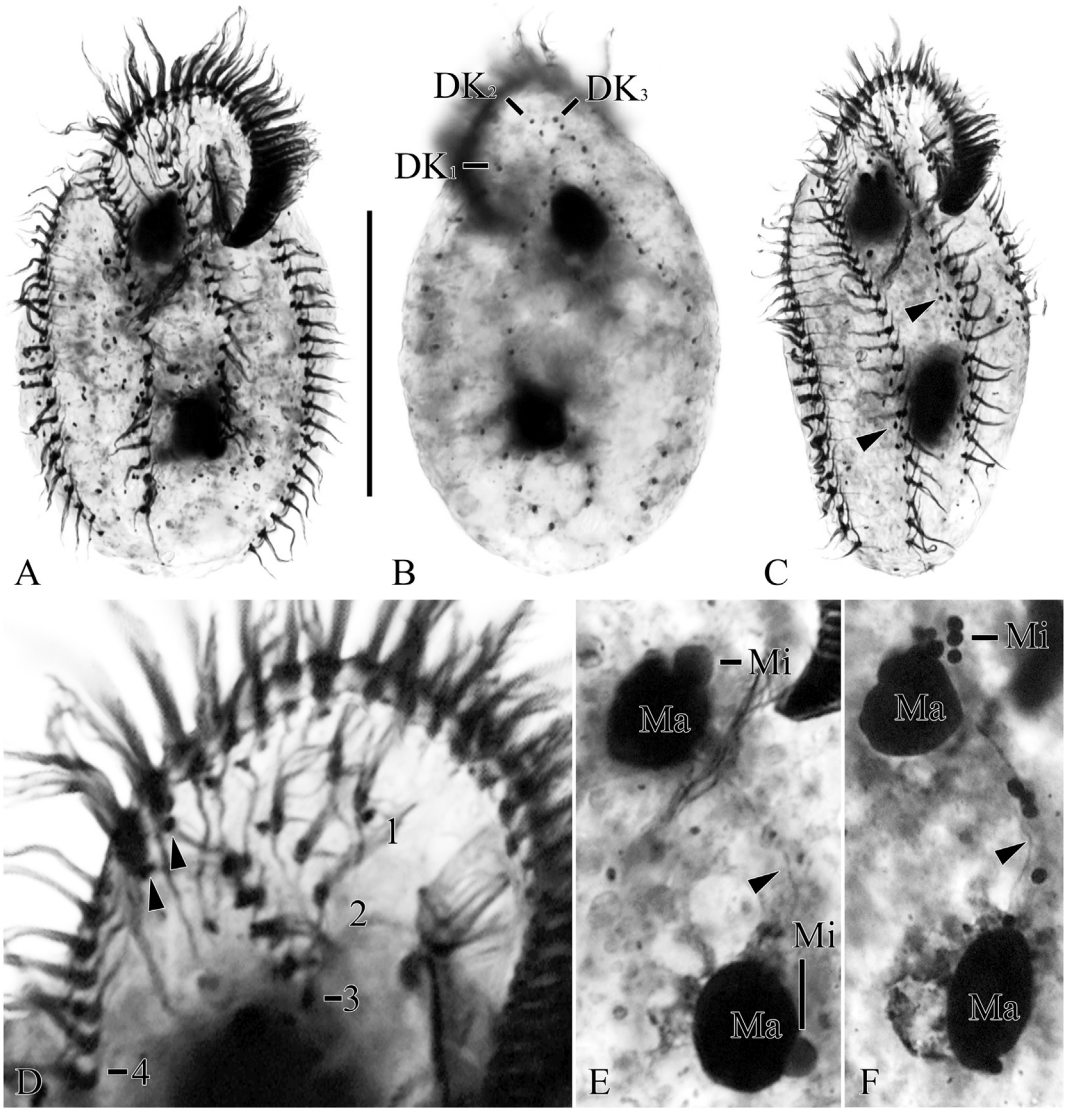

Fig. 3. A–F. *Paraholosticha muscicola* after protargol preparation. A, B. Ventral and dorsal view of representative specimen showing cirral and dorsal kinety pattern and macronuclear nodules. C. Ventral view of a specimen with three buccal cirri. Arrowheads mark argyrophilic structures near cirri. D. Arrangement of cirri in anterior body portion showing, inter alia, ventral rows 1 and 2 and argyrophilic structures (arrowheads) behind right end of frontal corona. E, F. Variation of nuclear apparatus, ventral views. DK1–3, dorsal kineties 1–3; Ma, macronuclear nodules; Mi, micronuclei; 1–4, ventral rows 1–4. Scale bar, 50 μm.

shaped because containing two daughter cells; thickness of cyst wall about 1.5 μm in vivo (Fig. 2G, H).

3.3. Molecular analyses (Fig. 4)

Paraholosticha muscicola is the first species of the Keronopsidae analyzed molecular biologically. The SSU rRNA gene sequence has a length of 1562 bp and a GC content of 45.8% (GenBank accession number KT003281). *Paraholosticha muscicola* nested within non-dorsomarginalian hypotrichs and the highest pairwise similarity with *P. muscicola* was 99.3% measured from *Bistichella variabilis* and *Orthoamphisiella breviseries* (Fig. 4). The non-dorsomarginalian hypotrichs are highly supported by a Bayesian posterior probability of 0.95, while a bootstrap value of maximum likelihood is less than 50%. As mentioned above, other congeneric and even confamilial species are not available from GenBank to infer their phylogenetic position in the Keronopsidae.

4. Discussion

4.1. Comparison of Antarctic population with other populations of Paraholosticha muscicola

So far, six populations of *P. muscicola* have been described, namely (i) by Kahl (1932) likely from mosses from North Germany (specific site not mentioned, that is, type locality sensu stricto not known); (ii) by Tuffrau and Fryd-Versavel (1977) from moss in the region Jura/Doubs in France; (iii) by Hemberger (1982) and Hemberger and Wilbert (1982) from forest soil (whether the soil is from Puerto Maldonado in Peru or from a site in Germany remains unclear; Hemberger, 1982, p. 2); (iv) by Foissner (1987) from a sample composed of lichens, moss, and soil from Denmark; (v) by Dieckmann (1989) likely from a sample from Germany; and (vi) by Shin (1994) from a moss-covered soil in Korea. Our data agree rather well with the descriptions (i) to (v) so that the identification of the Antarctic population as *P. muscicola* is beyond reasonable

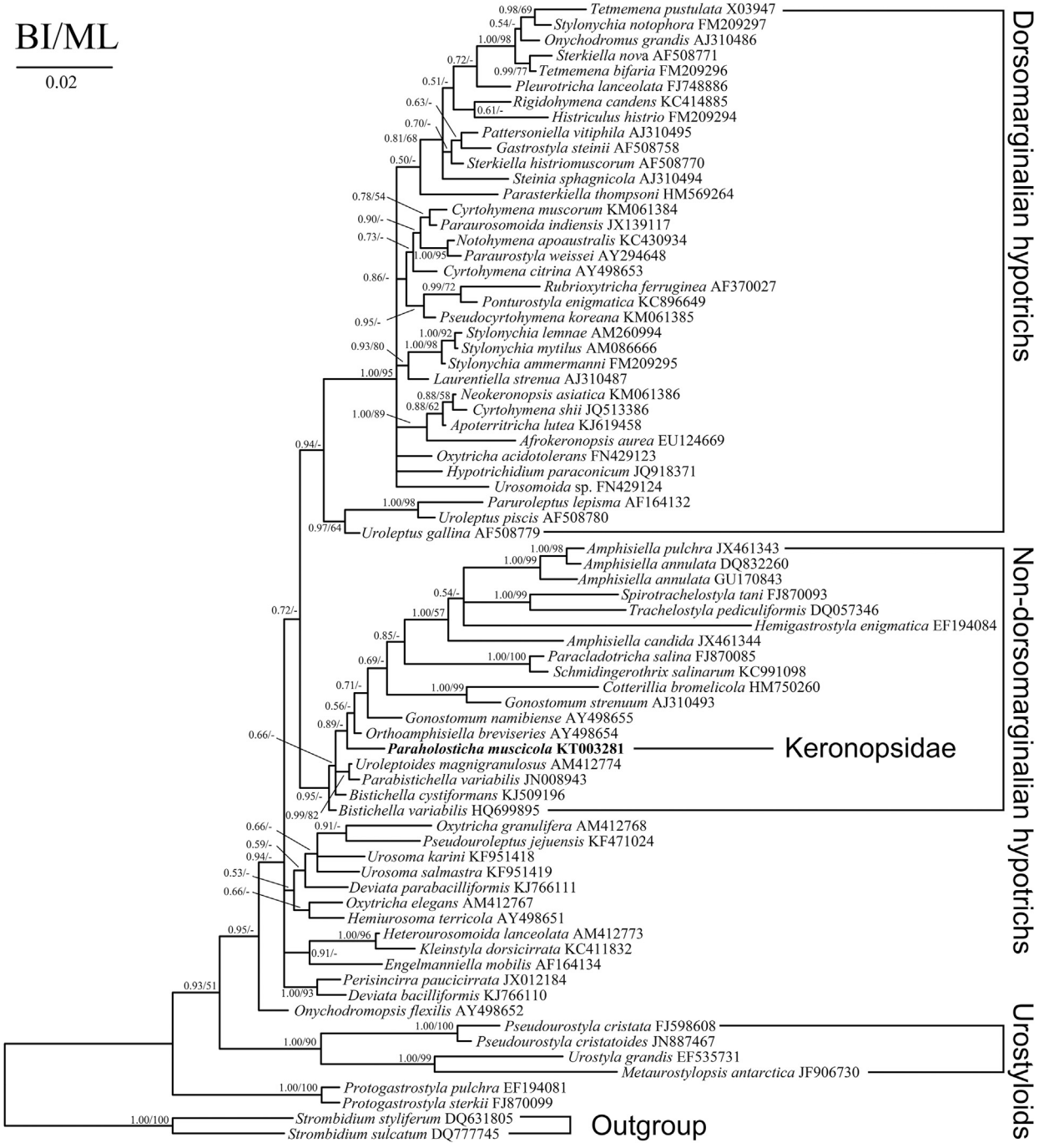

Fig. 4. Majority consensus tree of Bayesian inference using SSU rRNA gene sequences, indicating the position of *Paraholosticha muscicola* based on Bayesian inference (BI) and maximum likelihood (ML). Posterior probability values for BI and bootstrap values for ML are represented near the interior branches. A dash denotes a value of below 50% (ML).

doubt (Table 2). In addition, this is not the first record of *P. muscicola* from the Antarctic region; it was already listed by Sudzuki (1979), Petz (1997), and Petz and Foissner (1996, 1997). In *P. muscicola* sensu Shin (1994), the ventral rows 3 and 4 terminate slightly behind mid-body and the cirri of rows 1 and 2 are rather differently arranged so that it cannot be excluded that this population is not conspecific with *P. muscicola*.

The total variability of *P. muscicola* is considerable in some morphological features (Table 2). Our population has, like *P. muscicola* sensu Hemberger (1982) and Foissner (1987), a somewhat lower number of buccal cirri and cirri in the short ventral rows than the populations studied by Kahl (1932), Tuffrau and Fryd-Versavel (1977), and Dieckmann (1989). According to Hemberger (1982), *Paraholosticha lichenicola* Gellért, 1956 is a junior synonym of *P. muscicola*. We basically agree because there is no distinct difference to the populations described as *P. muscicola* (Table 2). For the same reason, we add *Paraholosticha ovata* Horváth, 1933 as junior supposed synonym. Borror (1972) classified this species incorrectly in *Uroleptopsis* Kahl, 1932, an urostyloid genus with a distinct, zigzagging midventral pattern (Berger, 2004, 2006; Huang et al., 2010).

Our population also resembles *Paraholosticha algivora* Gellért, 1942 and its supposed synonym *Paraholosticha nana* Gellért, 1956 as concerns the rather low number of cirri in the buccal row and

Table 1
Morphometric data on Antarctic population of *Paraholosticha muscicola*.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	91.1	86	10.1	2.3	11.1	77	111	19
Body, width	50.4	50	9.5	2.2	18.9	33	68	19
Anterior body end to end of adoral zone, distance	29.3	28	4.1	0.9	13.9	22	38	19
Macronuclear nodule, length[b]	14.3	13	3.0	0.7	20.8	10	21	19
Macronuclear nodule, width[b]	10.6	10	1.9	0.4	18.2	8	14	19
Macronuclear nodules, number	2.0	2	0.0	0.0	0.0	2	2	19
Macronuclear nodules, distance in between	21.5	21	4.2	1.0	19.3	14	31	19
Micronuclei, length[b]	3.0	3	1.0	0.2	33.3	2	4	19
Micronuclei, width[b]	2.9	2	1.0	0.2	34.3	2	4	19
Micronuclei, number[c]	5.0	4	3.4	0.8	68.0	2	13	19
Adoral membranelles, number	31.2	30	3.4	0.8	10.9	28	40	19
Longest adoral membranelles, length	5.9	6	0.9	0.2	15.9	4	7	19
Coronal cirri, number	15.7	15	2.3	0.5	14.3	12	20	19
Ventral cirral rows, number	4.0	4	0.0	0.0	0.0	4	4	19
Ventral cirral row 1, number of cirri	1.3	1	0.5	0.1	35.8	1	2	19
Ventral cirral row 2, number of cirri	2.1	2	0.5	0.1	25.5	1	3	19
Ventral cirral row 3, number of cirri	22.5	21	4.5	1.0	20.2	16	35	19
Ventral cirral row 4, number of cirri	26.1	25	4.1	0.9	15.8	21	37	19
Right marginal cirri, number	24.8	25	3.7	0.8	14.8	20	34	19
Left marginal cirri, number	22.5	22	3.7	0.9	16.5	18	30	19
Buccal cirri, number	1.7	2	0.7	0.2	39.8	1	3	19
Dorsal kineties, number	3.0	3	0.0	0.0	0.0	3	3	19
Dorsal kinety 1, number of bristles	22.4	22	4.4	1.0	19.8	12	32	19
Dorsal kinety 2, number of bristles	20.2	20	3.1	0.7	15.3	16	28	19
Dorsal kinety 3, number of bristles	17.1	17	3.1	0.7	18.2	12	23	19

[a] Data based on protargol preparations. Measurements in μm. CV, coefficient of variation in %; M, median; Max, maximum; mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean.
[b] A macro- or micronucleus was randomly chosen in each cell.
[c] Individual values: 2, 2, 2, 2, 2, 2, 2, 2, 3, 4, 6, 6, 6, 6, 7, 8, 10, 10, 13.

Table 2
Comparison of morphological characteristics between *Paraholosticha muscicola* populations and the supposed synonyms *P. ovata* and *P. lichenicola*.

Characteristic[a]	*Paraholosticha muscicola*							*P. ovata*	*P. lichenicola*
	Germany	France	Peru?	Denmark	Germany?	Antarctica	Total range		
Body length (μm)[c]	180–220	120	80–150	100–160	ca. 125 (protargol)	100–130	80–220	70–100	100
BC, No.	6[b]	3–5	1–4	1–2	4[b]	1–3	1–6	2	1
AM, No.	39[b]	ca. 55[b]	29–50	32–53	48[b]	28–40	28–55	~36	34
CO, No.	20[b]	15–21	11–20	15–22	20[b]	12–20	11–22	~14[b]	14–18
VR1, No. of cirri	3[b]	3–5	1–4	2–3	2[b]	1–2	1–5	NA	3
VR2, No. of cirri	6[b]	3–5	2–5	2–3	6[b]	1–3	1–6	NA	4
VR3, No. of cirri	16[b]	45[b]	15–38	22–42	45[b]	16–35	15–45	~36[b]	14[b]
VR4, No. of cirri	19[b]	47[b]	20–38	28–47	43[b]	21–37	19–47	~37[b]	20[b]
LMC, No.	18[b]	32[b]	17–30	24–35	41[b]	18–30	17–41	~29[b]	17[b]
RMC, No.	22[b]	42[b]	20–29	24–41	41[b]	20–34	20–42	~35[b]	23[b]
DK, No.	NA	3	3	3	3[b]	3	3	3	3
Ma, No.	2	2	2	2	2[b]	2	2	2	2
Mi, No.	4–6	2	2	2	3[b]	2–13	2–13	2	2
Habitat	Terrestrial	Terrestrial	Terrestrial	Terrestrial	Terrestrial?	Terrestrial	Terrestrial	Freshwater	Terrestrial
Data source	Kahl (1932)	Tuffrau and Fryd-Versavel (1977)	Hemberger (1982)	Foissner (1987)	Dieckmann (1989)	Original		Horváth (1933)	Gellért (1956)

[a] AM, adoral membranelles; BC, buccal cirri; CO, coronal cirri; DK, dorsal kineties; LMC, left marginal cirri; Ma, macronuclear nodules; Mi, micronuclei; NA, not available (or not clearly recognizable); No., number; RMC, right marginal cirri; VR1–4, ventral rows 1–4.
[b] Data from the illustrations.
[c] From life unless otherwise indicated.

the short ventral rows (likely invariably each one cirrus present in this species). However, this very little-known species is somewhat smaller (60–90 μm long vs. 100–130 μm) and has a lower number of adoral membranelles (22 vs. 28–40) and dorsal bristles per kinety (15 per kinety in specimen illustrated vs. on average 17–22, Table 1) indicating that our population is not conspecific with *P. algivora*.

This overview emphasizes the need for further investigations on the considerable range of variation in *P. muscicola* to show whether this is the natural variability or due to the inclusion of two or more cryptic (sub)species.

4.2. Estimation of phylogenetic position of Paraholosticha (Fig. 4)

Jankowski (2007), Lynn (2008), and some previous workers classified *Paraholosticha* (without transverse cirri; Dieckmann, 1988; Foissner and Al-Rasheid, 2007) and its supposed sister-group *Keronopsis* Penard, 1922 (with transverse cirri) in the Keronidae Dujardin, 1840 (type genus *Kerona* Müller, 1786), following the suggestion by Hemberger (1982) and Hemberger and Wilbert

(1982). Hemberger (1982) studied the cell division of *Kerona polyporum* Ehrenberg, 1835, the junior synonym of *Kerona pediculus* (Müller, 1773) Blochmann, 1886 (for revision and discussion of problems, see Berger, 1999). In this species the frontal corona is formed from anlage I; the anteriorly rightwards curved frontoventral rows formed by anlagen II and III are clearly separated from the cirri of anlage I and thus are not part of the corona. Hemberger (1982) and Hemberger and Wilbert (1982) supposed that in *Paraholosticha*, the junior synonym of *Keronopsis* according to these authors, the frontal corona is formed in the same way. They refer to Tuffrau and Fryd-Versavel (1977), but obviously misinterpret their data because the French workers clearly stated that the frontal corona of *P. muscicola* is formed from the anterior portion of anlagen I–III (see legend of their Fig. 1). In addition, Fig. 12 in Tuffrau and Fryd-Versavel (1977) shows, unfortunately not very clearly, that the frontal corona originates from more than one anlage, that is, the corona in *Paraholosticha* is a mixed row. Furthermore, they ignored the fact that *Paraholosticha* and *Keronopsis* divide in cysts while *K. pediculus* divides in the ordinary way (e.g., Penard, 1922; Garnjobst, 1937; Dieckmann, 1988, 1993).

The difference in the formation of the frontal corona is not the sole feature of the infraciliature showing that *K. pediculus* and *Paraholosticha* and *Keronopsis* species do not belong to the same higher taxon. Berger (1999, p. 827) – who classified *K. pediculus* in the oxytrichids, a subgroup of the Dorsomarginalia Berger, 2006 – already stated that important differences in the dorsal ciliature (kinety fragmentation and dorsomarginal rows present in *Kerona* vs. both features lacking in *Paraholosticha* and *Keronopsis*) indicate that *Keronopsis* and *Paraholosticha* branch off outside the oxytrichids. In addition, in *K. pediculus* the frontoventral ciliature is formed from the ordinary six anlagen (I–VI), while in *Keronopsis* and *Paraholosticha* only five anlagen are present (Hemberger, 1982; Dieckmann, 1988, 1989). The lack of fragmentation and dorsomarginal rows demonstrates that *Keronopsis* and *Paraholosticha* are non-dorsomarginalian hypotrichs (Berger, 2008, p. 46), like, for example, the urostyloids, the amphisiellids, and the gonostomatids (Berger, 2006, 2008, 2011). Consequently, *Keronopsis* and *Paraholosticha* should not be classified in the Keronidae, but in the Keronopsidae Jankowski, 1979, as already proposed by Shi (1999) and Foissner and Al-Rasheid (2007).

Our molecular analyses basically support the morphology-based classification of *Paraholosticha* outside the core-Dorsomarginalia with high support in the Bayesian inference tree (Fig. 4). In addition, the dorsomarginalian hypotrichs were not nested within the non-dorsomarginalian hypotrichs. However, as in almost all other published trees, morphology-based and gene-based analyses show some serious discrepancies. Not all species (e.g., *Hemiurosoma terricola*, *Heterourosomoida lanceolata*, *Oxytricha granulifera*, *Onychodromopsis flexilis*, *Pseudouroleptus jejuensis*, *Urosoma* spp., *Kleinstyla dorsicirrata*) with dorsal kinety fragmentation and/or dorsomarginal rows branch off within the major part of the dorsomarginalians. Instead, they are rather irregularly distributed within the remaining tree. The reasons for these mismatches (The concepts of dorsomarginalians and/or oxytrichids are incorrect? Some populations morphologically misidentified are sequenced? The molecular methods are inadequate? Number of species analyzed are too low?) remain obscure. In addition, the bootstrap values of maximum likelihood were not strong enough to support their relationships inferred from the Bayesian phylogenetic analysis. Consequently, we need multiple-gene based approaches with more taxon-sampling, especially on soil ciliates.

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