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Monographic treatment of *Paraholosticha muscicola* (Ciliophora, Keronopsidae), including morphological and molecular biological characterization of a brackish water population from Korea

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Abstract

Paraholosticha muscicola, type species of *Paraholosticha* Wenzel, inhabits mainly terrestrial habitats, but also freshwater. A brackish water population from Korea is described, the first record from such a habitat. Principal component analysis shows that this population is more similar to a terrestrial population from Denmark than to a population from Antarctic soil. Keronopsids have two strong morphological/ontogenetic apomorphies (frontal corona formed from anlagen I–III; division in cysts). However, the SSU rRNA sequence of the Korean population does not cluster with that of the Antarctic population in the phylogenetic tree, but both branch off consecutively and immediately before a mixture of other non-dorsomarginalian hypotrichs, including two further keronopsids. Furthermore, the keronopsids cluster in the phylogenetic network, providing phylogenetic conflicts, which cannot be exemplified in the conventional gene tree. To complete the picture of *P. muscicola*, we provide a detailed overview about nomenclature, history, taxonomy, and its geographic distribution. From the four synonyms proposed so far, we tentatively accept only *P. lichenicola* and *P. ovata*. *Paraholosticha algivora* is likewise very similar. Thus we propose to include these three taxa as members of the *P. muscicola* complex. *Stylonethes sterkii* and *P. algivora* are transferred to *Paraholosticha* Wenzel. A key to the *Paraholosticha* species is provided.

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Introduction

The Keronopsidae Jankowski, 1979 is a small (about 10 species), unique group of hypotrichous ciliates. This taxon is characterized by two apomorphies, namely (i) a frontal corona, that is, a mixed, curved row made of the anterior portion of the frontal cirral anlagen I–III (thus the corona is homologous to the three frontal cirri of many hypotrichs) and (ii) a division in cysts. In addition, they lack both dorsal kinety fragmentation and dorsomarginal rows (e.g., Dieckmann 1989; Park et al. 2017; Penard 1922). At present, the keronopsids comprise the genera *Keronopsis* Penard, 1922 (transverse cirri present) and *Paraholosticha* Wenzel, 1953 (transverse cirri lacking) (Dieckmann 1989; Park et al. 2017).

Paraholosticha muscicola (Kahl, 1932) Wenzel, 1953 is the type species of *Paraholosticha*, a genus initially established by Kahl (1932) with two new species (*P. muscicola*, *P. herbicola*), however, without fixing one as type species as requested by the International Code of Zoological Nomenclature (Code) from 1927 (Heikertinger 1930, p. 8; Article 25c (3)). Wenzel (1953) established the genus validly in that he

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fixed *P. muscicola* Kahl, 1932 as type species. This species is well known morphologically (e.g., Foissner 1987a), ontogenetically (e.g., Dieckmann 1989), and molecular biologically (Jung et al. 2015). Most populations have been found in terrestrial habitats (moss, soil), few in freshwaters.

Recently, we isolated a population from brackish water in Korea, which is the first record from such a biotope substantiated by morphological data. In the present work, this population is characterized morphologically and molecular biologically, but we also provide a detailed overview about the nomenclature, taxonomy, and biogeography of *P. muscicola* and its synonyms.

Material and Methods

Sample collection, cultivation, identification

Paraholosticha muscicola was isolated from the River Gyeongpocheon (37°47′23.633″N 128°54′32.886″E), Unjeong-dong, Gangneung-si, Gangwon-do, northeastern coast of South Korea. The river is only about 11 km long and drains the farmland south of Lake Gyeongpoho. At the sample site the water is brackish (10.3–16.2 psu) and the water temperature was about 10 °C during sampling in February 2017.

Approximately 200 ml of stirred water from the riverbank (water depth about 30 cm) containing mud and leaves was collected in a sterile flask and transferred to a Petri dish (15 cm in diameter) within 1 h. The raw culture containing *P. muscicola* was maintained at room temperature. Rice grains were added to enhance growth of bacteria and other preys as a food source for the ciliates. Establishment of a clonal culture failed and therefore we cannot be sure that the specimens used for the morphological studies and the gene sequence analyses belong to the same species. However, we found only the *P. muscicola* morphotype in the protargol preparations and thus the probability is very high that the morphological and molecular studies deal with the same species.

Live specimens were observed with a stereomicroscope (Olympus SZ11, Japan) and an compound microscope (Olympus BX53, Japan) at low (×40–200) and high (×400–1000) magnifications using bright field and differential interference contrast. Micrographs were made with a digital camera (Olympus DP74, Japan). Protargol preparation (‘procedure A’) using an acetone developer was performed to reveal the ciliature and nuclear apparatus (Foissner 2014; Kim and Jung 2017). An inverted microscope with time-lapse option (Eclipse T*i*-U, Nikon, Japan) was used to document cell division in a cyst in vivo.

Terminology

General terminology is according to Lynn (2008). For terms specific for hypotrichs (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). For terms specific for keronopsids, see Park et al. (2017). Since this is a mainly taxonomic paper, nomenclatural references are also listed in the reference section. To complete the picture of *P. muscicola*, we also considered dissertations, inasmuch as they are not explicitly excluded by the Code, especially when they have a certain edition (e.g., Article 9 of ICZN 1964, the relevant Code for Hemberger 1982).

Multivariate analyses

To study the morphological variability of *P. muscicola*, two data sets were set up for principal component analysis using vegan package in R (Oksanen et al. 2018). One set comprised three populations of *P. muscicola*: (i) 19 individuals of Antarctic population (Jung et al. 2015); (ii) six individuals of Danish population (Foissner 1987a); and (iii) 17 individuals of Korean population (this study). A total of 20 quantitative features were examined for this data set (for details, see legend to Fig. 7B).

The other set comprised nine populations shown in Table 2. A total of 11 features were measured. Usually, the median or the arithmetic mean was used, in some cases values from the drawings have been applied.

Voucher material

Two voucher slides with protargol-prepared specimens (NIBRPR0000107902, NIBRPR0000107903) were deposited in the National Institute of Biological Resources (NIBR), Incheon, South Korea. One slide (GUC000790) was deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seochun-gun, South Korea.

ZooBank registration

ZooBank registration number of present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org:pub:FE19712-3744-4FEC-8491-2F6376AFCF99. For ZooBank registration numbers of species, see list of synonyms.

DNA extraction, PCR amplification, sequencing

Two specimens of *Paraholosticha muscicola* were isolated from the raw culture and transferred to 0.22 μm-filtered culture water using a micropipette. Genomic DNA of each cell was extracted with a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA), using 10% of the volume of the manufacturer’s instructions. The conditions for PCR were as follows: denaturation at 94 °C for 1 min 30 s; followed by 40 cycles of denaturation at 98 °C for 10 s, annealing at 58.5 °C for 30 s, and extension at 72 °C for 3 min; and a final extension step at 72 °C for 7 min. Two primers (New

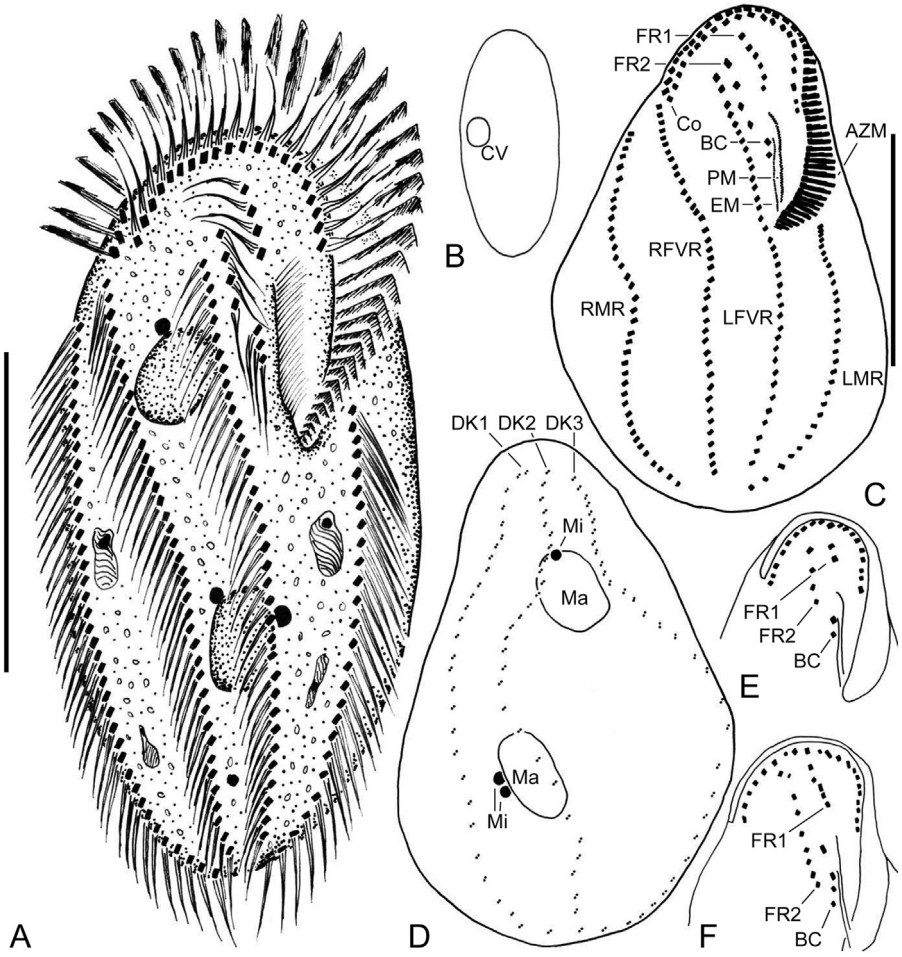

Fig. 1. A–F *Paraholosticha muscicola*, Korean population from life (A, B) and after protargol preparation (C–F). **(A)** Ventral view of a representative specimen. **(B)** Dorsal view showing position of contractile vacuole. **(C, D)** Ciliature of ventral and dorsal side and nuclear apparatus of same specimen. **(E, F)** Frontal region to show variability of ciliature. AZM, adoral zone of membranelles; BC, buccal cirri; Co, frontal corona; CV, contractile vacuole; DK1–3, dorsal kineties 1–3; EM, endoral membrane; FR1, 2, frontal row 1 and 2; LFVR, left frontoventral row; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; RFVR, right frontoventral row; RMR, right marginal row. Scale bars: 50 μm.

Euk A and LSU rev4), slightly modified from Sonnenberg et al. (2007), were used to amplify nearly the complete nuclear small subunit ribosomal RNA (SSU rRNA) gene. After the amplification, the PCR products were purified using a MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON, Korea). DNA sequencing was performed using two internal primers (18SF790v2:5′-AAA TTA KAG TGT TYM ARG CAG-3′ and 18SR300:5′-CAT GGT AGT CCA ATA CAC TAC-3′) and an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses

To infer the phylogenetic position of the present population of *Paraholosticha muscicola*, SSU rRNA gene sequences of 83 ciliates were retrieved from NCBI database, including 81 hypotrichs. Two oligotrichs, *Novistrombidium orientale* (FJ422988) and *Strombidium stylifer* (DQ631805), were used as outgroup. These sequences were aligned using ClustalW (Thompson et al. 1994) in Geneious 9.1.6 (Kearse et al. 2012), and both ends of this alignment were manually trimmed using Geneious. jModelTest 2.1.10 selected GTR + I (0.6310) + G (0.4690), based on the Akaike information criterion (AIC), as the best-fit model of substitution for phylogenetic analysis (Darriba et al. 2012). IQ-TREE 1.5.3 was used to infer maximum likelihood (ML) trees, with 1000 bootstrap replicates (Nguyen et al. 2015). The Bayesian inference (BI) tree was inferred using MrBayes 3.2.6 (Ronquist et al. 2012), with Markov chain Monte Carlo (MCMC) for 1,000,000 generations. The first 300,000 generations were discarded as a burn-in. A consensus ML tree was annotated using ggtree in R (Yu et al. 2017). Uncorrected pairwise distances were calculated using Geneious (Kearse et al. 2012).

For computing the phylogenetic network, the software SplitsTree 4 was used (Huson and Bryant 2006). A neighbor-

net analysis was conducted using the same sequence alignment as in the ML/BI analyses, with uncorrected pairwise distance and 1000 bootstrap replicates (Vd'ačný 2017).

Note that several sequences branch off at positions which do not agree with the position of the corresponding species in systems which are largely based on non-molecular features. This problem, which does not only apply to hypotrichs, is beyond the scope of the present paper.

Results and Discussion

Paraholosticha muscicola (Kahl, 1932) Wenzel, 1953 (Figs. 1 A–F, 2 A–G, 3 A–C, 4 A–D; Tables 1 and 2)

1932 ***Paraholosticha muscicola*** **spec. n.** – Kahl, Tierwelt Dtl., 25: 545, Fig. 89 (original description; no type material available; ZooBank registration number: urn:lsid:zoobank.org:act:2D6D3CAD-3B35-4EFB-B8C7-AA952ED5DBF2).

1933 ***Paraholosticha ovata*** **sp. nov.** – Horváth, Arch. Protistenkd., 80: 282, Figs. 1–3 (original description of junior synonym; no type material available; ZooBank registration number: urn:lsid:zoobank.org:act:BA4C32AF-CA3A-4913-B80D-AB051C16D7BA).

1953 ***Paraholosticha muscicola*** **(Kahl, 1932)** – Wenzel, Arch. Protistenkd., 99: 104 (fixation as type species of *Paraholosticha* Wenzel, 1953; combination with *Paraholosticha* Wenzel, 1953).

1956 ***Paraholosticha lichenicola*** **n. sp.** – Gellért, Acta biol. hung., 6: 92, Abb. 8 (original description of synonym; likely no type material available; ZooBank registration number: urn:lsid:zoobank.org:act:738B3FF0-B4C6-4F3D-8AB4-2E7A39C5FFB2).

1972 ***Paraholosticha muscicola*** **Kahl, 1932** – Borror, J. Protozool., 19: 11 (generic revision of hypotrichs).

1972 ***Uroleptopsis ovata*** **(Horváth, 1933)** n. comb. – Borror, J. Protozool., 19: 11 (combination of junior synonym with *Uroleptopsis* Kahl, 1932).

1974 ***Uroleptopsis ovata*** **(Horváth, 1933)** comb. n. – Stiller, Annls hist.-nat. Mus. natn. hung., 66: 132 (combination of synonym with *Uroleptopsis* Kahl, 1932).

1977 ***Paraholosticha muscicola*** **Kahl, 1935** – Tuffrau and Fryd-Versavel, Protistologica, 13: 321, Figs. 1–15 (detailed redescription based on protargol preparations and cell division; incorrect year).

1979 ***Paraholosticha (Paraholosticha) muscicola*** **Kahl** – Jankowski, Trudy zool. Inst., Leningr., 86: 60 (classification in invalid subgenus *Paraholosticha (Paraholosticha)* Kahl, 1932; see nomenclature).

1982 ***Keronopsis muscicola*** **(Kahl, 1932)** n. comb. – Hemberger, Dissertation, p. 71, Abb. 11 (redescription; combination with *Keronopsis* and synonymization with four species; revision of hypotrichs).

1982 ***Keronopsis ovata*** **(Horváth, 1933)** n. comb. – Hemberger, Dissertation, p. 72 (combination of junior synonym with *Keronopsis*; revision of hypotrichs).

1982 ***Keronopsis muscicola*** **(Kahl)** – Hemberger and Wilbert, Arch. Protistenkd., 125: 269, Abb. 2 (illustrated record based on protargol preparations).

1983 ***Pseudokeronopsis ovata*** **(Horváth, 1933) nov. comb.** – Borror and Wicklow, Acta Protozool., 22: 116, 124 (combination of junior synonym with *Pseudokeronopsis* Borror and Wicklow, 1983).

1986 ***Keronopsis muscicola*** **(K.) Hem.** – Schmitz, Dissertation, p. 88, Abb. 35a, b (description of limnetic population from River Rhine).

1987 ***Keronopsis muscicola*** **(Kahl, 1932) Hemberger & Wilbert, 1982** – Foissner, Zool. Beitr. (N. F.), 31: 204, Abb. 7a–e, Tabelle 4 (description of Danish population from life and after protargol preparation; two slides have been deposited in the Upper Austrian Museum in Linz [LI] according to Aescht 2003, p. 391 and Aescht 2008, p. 167: accession numbers 1988/145, 1988/146; see nomenclature).

1989 ***Paraholosticha muscicola*** **Kahl, 1932** – Dieckmann, Arch. Protistenkd., 137: 143, Abb. 1a–c, 2–27 (detailed description of cell division).

2001 ***Paraholosticha muscicola*** **(Kahl, 1932) Wenzel, 1953** – Berger, Catalogue of ciliate names 1. Hypotrichs, p. 68 (nomenclator containing all original combinations, combinations, and higher taxa of hypotrichs; first correct spelling of full name).

2001 ***Paraholosticha ovata*** **(Horváth, 1933)** comb. nov. – Berger, Catalogue of ciliate names 1. Hypotrichs, p. 68 (combination of junior synonym with *Paraholosticha* Wenzel, 1953).

2007 ***Paraholosticha muscicola*** – Jankowski, Phylum Ciliophora, p. 470 (generic revision of ciliates).

2015 ***Paraholosticha muscicola*** **(Kahl, 1932) Wenzel, 1953** – Jung, Park, Min, Berger, and Kim, Polar Sci., 9: 374, 375, Fig. 1A–E, 2A–H, 3A–F, Table 1 (description of Antarctic population and analysis of SSU rRNA sequence KT003281).

2016 ***Paraholosticha muscicola*** **(Kahl, 1932) Wenzel, 1953** – Xu, Shao, Fan, Warren, Al-Rasheid, Song, and Wilbert, Polar Biol., 39: 1447, Fig. 5A, B, 6A–G, Table 5 (description of Antarctic population and synonymization with *Stylonethes sterkii*).

Nomenclature

No derivations of the names are given in the original descriptions or later papers. The-species group name *muscicola* is a composite of the Latin noun *musc·us* (moss; Hentschel and Wagner 1996, p. 410), the thematic vowel *·i-*, and the Latin noun *-cola* (dweller, inhabitant, tiller; Brown 1954, p. 478). It refers to the habitat (moss) where the species was discovered by Kahl (1932). The species-group name *ovat·us*, *-a*, *-um* (Latin adjective [m, f, n]; egg-shaped; www.en.wiktionary.org/wiki/ovatus) obviously refers to the oval body outline. The species-group name *lichenicola* is a composite of the Greek noun *lichen* (Brown 1954, p. 475), the thematic vowel *·i-*, and the Latin noun *cola* (see at *muscicola*). It refers to the habitat (lichen) where this species was discovered by Gellért (1956a).

Paraholosticha muscicola Kahl, 1932 is the type species of *Paraholosticha* Wenzel, 1953 by original designation.

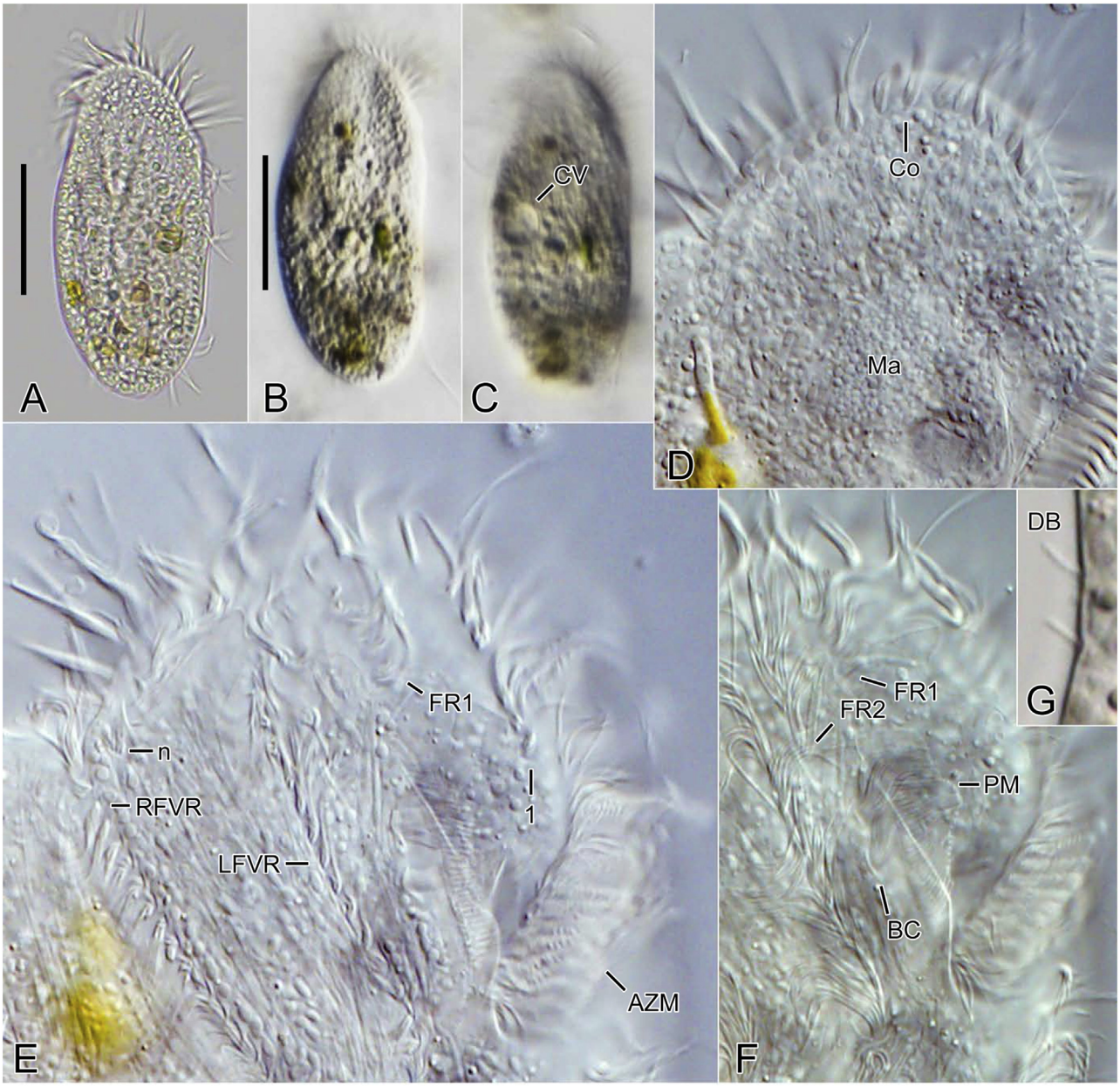

Fig. 2. A–G *Paraholosticha muscicola*, Korean population from life (A, bright field; B, C, differential contrast, inverted microscope; D–G, differential interference contrast). **(A–C)** Dorsal views showing position of contractile vacuole, cell inclusions, and variability of body shape. **(D–F)** Ventral views showing buccal field with adoral membranelles, undulating membranes, and cirri. **(G)** Dorsal bristles, about 3 μm long. AZM, adoral zone of membranelles; BC, buccal cirri; Co, frontal corona; CV, contractile vacuole; DB, dorsal bristles; FR1, 2, frontal rows 1 and 2; LFVR, left frontoventral row; Ma, macronuclear nodule; n, right end of frontal corona; PM, paroral membrane; RFVR, right frontoventral row; 1, left end of frontal corona. Scale bars: 50 μm.

Borror (1972, p. 11) incorrectly fixed *P. herbicola* Kahl, 1932 as type species of the invalid genus *Paraholosticha* Kahl, 1932 by subsequent designation, obviously ignoring Article 69 of the ICZN (1964) which says that a subsequent designation is only possible for nominal genera that were established before 1931. The incorrect type species was accepted by later workers (Aescht 2001, p. 116; Jankowski 1979, p. 60; 2007, p. 470).

Wenzel (1953) did not formally combine *P. muscicola* Kahl, 1932 with *Paraholosticha* Wenzel, 1953, but he put Kahl in brackets as requested by Article 23 of the ICZN (Heikertinger 1930, p. 7). In addition, due to the type fixation the combination is likely made automatically.

Borror (1972) transferred *P. ovata* to *Uroleptopsis* Kahl, 1932 (see list of synonyms). Obviously, Stiller (1974) did not consult Borror's revision because she made the same

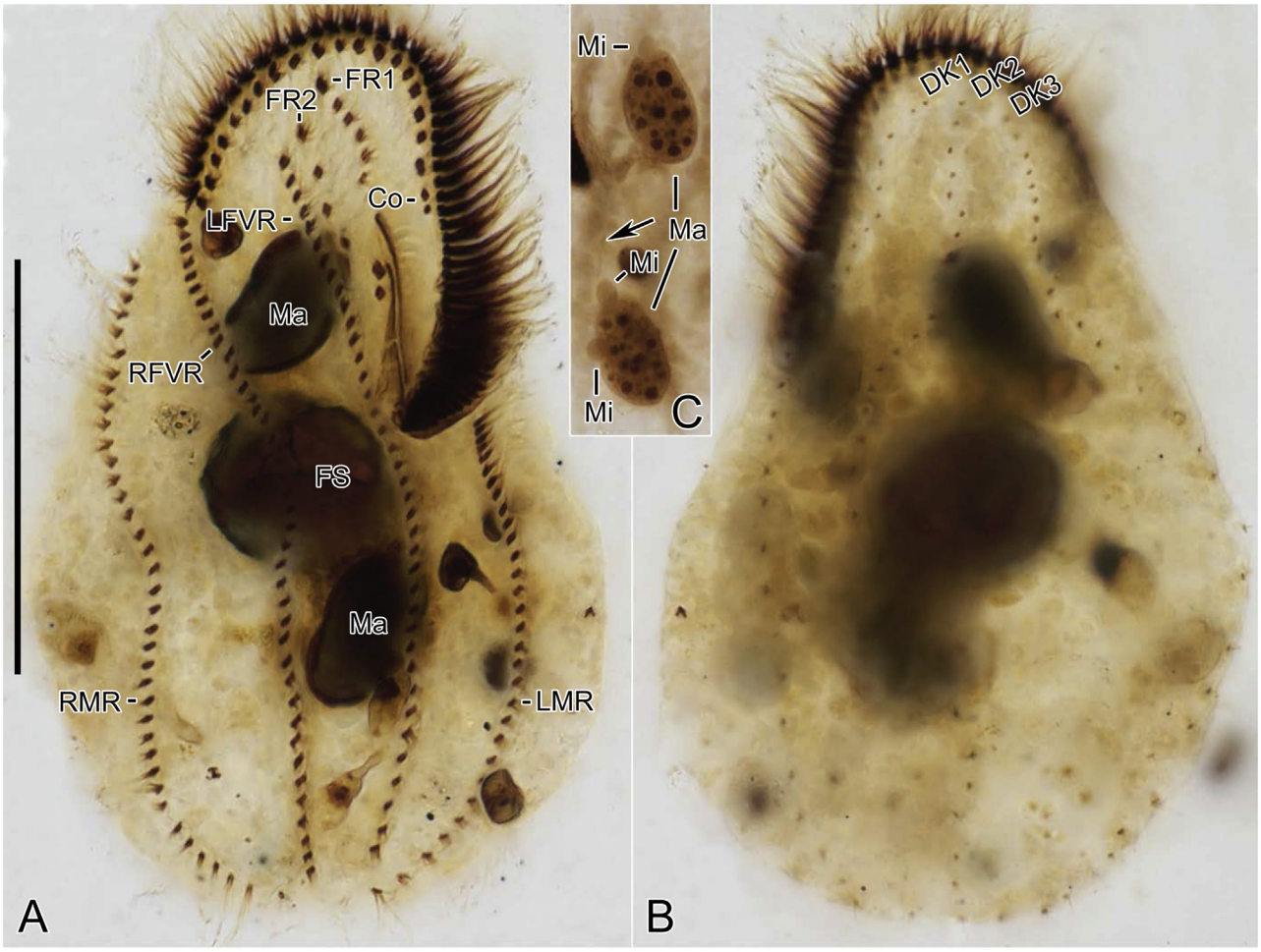

Fig. 3. A–C *Paraholosticha muscicola*, Korean population after protargol preparation. **(A, B)** Ventral and dorsal view of representative specimen. **(C)** Nuclear apparatus in dorsal view; arrow denotes thin thread connecting macronuclear nodules. Co, frontal corona; DK1–3, dorsal kineties; FR1, 2, frontal rows 1 and 2; FS, fungal spore; LFVR, left frontoventral row; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; RFVR, right frontoventral row; RMR, right marginal row. Scale bar: 50 μm.

combination. Jankowski (1979) classified *Paraholosticha muscicola* and *P. lichenicola* in the genus *Paraholosticha* Kahl, 1932 and the subgenus *Paraholosticha (Paraholosticha)* Kahl, 1932 (as *P. lichenicola* and *P. muscicola*); however, both the genus established by Kahl (1932) and the corresponding nominotypical subgenus are invalid due to the lack of type fixation (see introduction).

Foissner (1987a, p. 190) wrote that for most other species (the "other species" are those which are not new in Foissner 1987a) at least one slide is deposited in the Upper Austrian Museum in Linz; he did not mention a neotypification. According to Aescht (2008, p. 167), the slides have been labelled as neotypes. However, since the qualifying conditions of the ICZN (1985, Article 75d) have not been published by Foissner (1987a), a neotype was not validly designated. For several reasons a neotype should be fixed for *P. muscicola* to define it objectively: (i) Kahl (1932) made no permanent preparations; (ii) its taxonomy is rather complicated, as indicated, inter alia, by the description of several very similar (synonymous?) species; and (iii) the type locality is not known in detail ("mosses from northern Germany"; Kahl 1932). Thus, the neotype population should come from a terrestrial habitat of this area and should comprise a very detailed description and morphometry and a molecular characterization.

Paraholosticha ovata Horváth, 1933 was established in the invalid genus *Paraholosticha* Kahl, 1932. Thus, Berger (2001) transferred it to the valid genus *Paraholosticha* Wenzel, 1953. Whether *P. lichenicola* Gellért, 1956a was established in *Paraholosticha* Kahl, 1932 or in *Paraholosticha* Wenzel, 1953 remains obscure. Since Gellért (1956a) listed only Kahl (1932) in the reference section, he probably classified his new species in Kahl's genus. In that case it has to be transferred to *Paraholosticha* Wenzel when relevant studies show that it is a valid species and not a synonym of *P. muscicola*.

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

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	101.0	101	11.4	2.5	11.2	88.0	134.0	20
Body, width	59.0	60	10.6	2.4	17.9	43.0	80.0	20
Body length:width, ratio	1.8	1.7	0.2	0.1	13.4	1.5	2.2	20
Adoral zone of membranelles, length	46.5	45	4.9	1.1	10.5	40.0	59.0	20
Body length:length of adoral zone, ratio	2.2	2.2	0.1	0.0	4.6	1.9	2.5	20
DE-value	0.4	0.4	0.1	0.0	12.6	0.3	0.5	20
Anterior macronuclear nodule, length	17.3	16.0	2.6	0.6	15.0	12.0	22.0	20
Anterior macronuclear nodule, width	12.2	12.0	1.3	0.3	10.8	10.0	15.0	20
Macronuclear nodules, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	20
Macronuclear nodules, distance in between	20.6	20.0	5.4	1.2	26.4	12.0	34.0	20
Micronuclei, length	4.1	4.0	0.6	0.1	15.6	3.0	5.0	20
Micronuclei, width	3.7	4.0	0.6	0.1	16.1	3.0	5.0	20
Micronuclei, number	2.8	3.0	1.0	0.2	35.7	1.0	6.0	19
Adoral membranelles, number	45.9	46.0	4.2	0.9	9.0	38.0	55.0	21
Largest adoral membranelle, width[b]	7.2	7.0	0.7	0.2	9.9	6.0	8.0	19
Frontal corona, number of cirri	20.1	21.0	1.7	0.4	8.6	17.0	24.0	21
Buccal cirri, number	2.6	3.0	0.7	0.1	25.5	2.0	4.0	21
Frontal and frontoventral rows, number	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
Frontal row 1, number of cirri[c]	3.7	4.0	0.9	0.2	24.9	2.0	6.0	21
Frontal row 2, number of cirri	4.3	4.0	1.2	0.3	28.7	3.0	9.0	21
Left frontoventral row, number of cirri	36.3	36.0	3.6	0.8	10.0	28.0	45.0	21
Right frontoventral row, number of cirri	37.4	37.0	3.3	0.7	8.8	33.0	46.0	21
Left marginal row, number of cirri	25.9	25.0	4.5	1.0	17.3	18.0	41.0	21
Right marginal row, number of cirri	34.8	34.0	3.1	0.7	9.0	31.0	44.0	21
Dorsal kineties, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Dorsal kinety 1, number of bristles	23.5	23.0	3.0	0.8	12.9	20.0	29.0	13
Dorsal kinety 2, number of bristles	26.2	25.0	4.3	1.2	16.3	21.0	38.0	13
Dorsal kinety 3, number of bristles	29.1	28.0	4.3	1.2	14.6	24.0	40.0	12
Dorsal bristles, total number	78.8	75.0	10.6	3.0	13.4	67.0	106.0	12

[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.
[b] See Fig. 1a in Berger (2011).
[c] This is the left frontal row.

According to Foissner and Al-Rasheid (2007, p. 204), Brunberg-Nielsen (1968) and/or Chardez (1967) have recorded the present species in Belgium and/or Denmark. Obviously, they confused "*Keronopsis muscorum*" (original combination *Holosticha (Keronopsis) muscorum* Kahl, 1932, p. 576; for revision of this urostyloid, see Berger 2006, p. 317) in Brunberg-Nielsen (1968, p. 85) with the present species, which was classified in *Keronopsis* by Hemberger (1982) for the first time (see list of synonyms).

History and taxonomy

In this section the most important taxonomic events listed in the synonymy above are discussed. The original description of *P. muscicola* is based on live observations only, but in spite of that the characterization is rather detailed (Kahl 1932). The specimens are somewhat larger than that described by later workers (Table 2).

Tuffrau and Fryd-Versavel (1977) studied the morphology of a French population. Furthermore, they described the division in a cyst as new among the hypotrichs. Obviously, they overlooked the papers by Penard (1922) and Garnjobst (1934, 1937) who already mentioned and described this type of cell division for *Keronopsis helluo* and *Stylonethes sterkii*, two other keronopsids.

Hemberger (1982) put *Paraholosticha* Kahl into the synonymy of *Keronopsis*. He synonymized several species with *Keronopsis muscicola*, namely *Stylonethes sterkii* Garnjobst, 1934, *Paraholosticha vitrea* Vörösváry, 1950, *P. lichenicola* Gellért, 1956a, and *P. pannonica* Gellért and Tamás, 1959 because he considered the differences in the number of macronuclear nodules and cirri as insufficient for species separation. Hemberger (1982) provided an illustration of the ventral ciliature and the nuclear apparatus and a brief morphometric characterization (Table 2; the same illustration was published by Hemberger and Wilbert 1982). The data agree basically with that of the original description (Kahl 1932), except for the number of cirri forming the frontal rows (two in each vs. six in each). However, the low number of cirri in the frontal rows agrees with the redescriptions by Schmitz (1986) and Foissner (1987a). A population also having only two cirri per frontal row was described by Gellért (1956b, p. 345).

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

Characteristic[a]	*Paraholosticha muscicola* population										*P. ovata*	*P. lichenicola*
	Germany	France	Peru?	Germany	Denmark	Germany?	Antarctica	Antarctica	Korea	Total range		
Body length in vivo (μm)	180–220	120	80–150[c]	80–100[c]	100–160	ca. 125 (protargol)	100–130	110–155 (protargol)	100–180	100–220[e]	70–100	100
BC, number	6[b]	3–5	1–4 (mostly 2)	1 or 2	1 or 2 (2)	3–6 (4[g])	1–3 (1.7)	0–5 (2.3)	2–4 (2.6)	0–6	1	1
AM, number	39[b]	ca. 55[b]	29–50 (35)	35–47	32–53 (42.9)	39–48[b]	28–40 (31.2)	30–42 (33.7)	38–55 (45.9)	28–55	~36	34
Co, NoC	20[b]	15–21	11–20 (14)	15–18 (17)	15–22 (17.7)	20–22[g]	12–20 (15.7)	14–24 (19.1)	17–24 (20.1)	11–24	14[b]	14–18
FR1, NoC	3[b]	3–5	1–4 (2)	2–4	2 or 3 (2.8)	2[g]	1 or 2 (1.3)	1–6 (4.1)[d]	2–6 (3.7)	1–6	NA	3
FR2, NoC	6[b]	3–5	2–5 (3)	3 or 4	2 or 3 (2.7)	5–7[g]	1–3 (2.1)	–	3–9 (4.3)	1–9	NA	4
LFVR, NoC	16[b]	45[b]	15–38 (20)	30–35 (33)	22–42 (34.8)	45[g]	16–35 (22.5)	14–47 (31.7)	28–45 (36.3)	14–47	36[b]	14[b]
RFVR, NoC	19[b]	47[b]	20–38 (25)	33–38 (35)	28–47 (40.2)	44[g]	21–37 (26.1)	20–56 (34.5)	33–46 (37.4)	19–56	37[b]	20[b]
RMR, NoC	22[b]	42[b]	20–29 (26)	30–36	24–41 (35.9)	41[g]	20–34 (24.8)	26–46 (31)	31–44 (34.8)	20–46	29[b]	17[b]
LMR, NoC	18[b]	32[b]	17–30 (21)	24–30	24–35 (31.0)	41[g]	18–30 (22.5)	22–40 (28.6)	18–41 (25.9)	17–41	35[b]	23[b]
Ma, number	2	2	2	2	2	2[g]	2	1–4 (2.2)	2	1–4[f]	2	2
Mi, number	4–6	2	2	2	2	3[g]	2–13 (5.0)	1–5 (2.5)	1–6 (2.8)	1–13	2	2
Habitat	Terrestrial	Terrestrial	Terrestrial	Freshwater	Terrestrial	Terrestrial?	Terrestrial	Glacier	Brackish	T, F, B	Freshwater	Terrestrial
Data source	Kahl (1932)	Tuffrau and Fryd-Versavel (1977)	Hemberger (1982)[h]	Schmitz (1986)	Foissner (1987a)	Dieckmann (1989)	Jung et al. (2015)	Xu et al. (2016)	Original	–	Horváth (1933)	Gellért (1956a)

[a] Values are the minimum and maximum and the arithmetic mean in parentheses. Abbreviations: AM, adoral membranelles; B, brackish water; BC, buccal cirri; Co, frontal corona; F, freshwater (including melt-water, slush, and algae from glacier); FR1, frontal row 1; FR2, frontal row 2; LFVR, left frontoventral row; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; NoC, number of cirri; RFVR, right frontoventral row; RMR, right marginal row; T, terrestrial. All populations have three dorsal kineties (value not known for population studied by Kahl 1932).

[b] Data from the corresponding illustrations.

[c] Method (in vivo or protargol preparation) not indicated.

[d] Sum of cirri in frontal row 1 and frontal row 2.

[e] In vivo; when the uncertain value of Hemberger (1982) is considered, then the range is 80–220 μm.

[f] Most populations have constantly two macronuclear nodules.

[g] From Fig. 1A, 18, 19 in Dieckmann (1989).

[h] For note on locality (Peru or Germany), see chapter Occurrence and ecology.

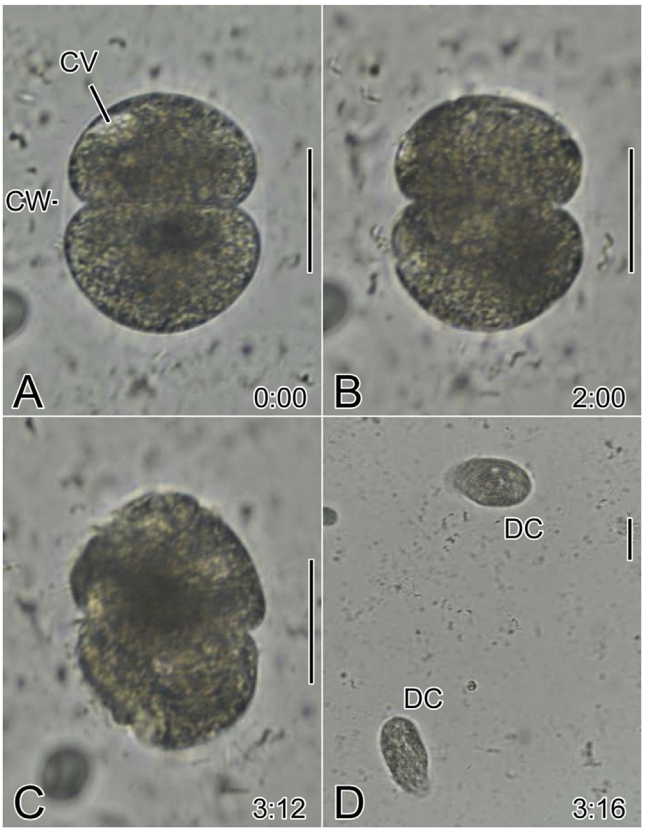

Fig. 4. A–D Chronological sequence of division in cyst of *Paraholosticha muscicola* from life (for details, see text). CV, contractile vacuole; CW, cyst wall; DC, daughter cells. Scale bars: 50 μm.

Gellért found specimens which differ from the "stem form" described by Kahl (1932; Table 2) in the body length (only 90 μm), the number of cirri forming the frontal corona (only four cirri), and the number of cirri forming the frontal rows (only two cirri each). This population is obviously not identical with *P. muscicola* or any other *Paraholosticha* species because of the low number of cirri forming the corona.

Of the synonyms proposed by Hemberger (1982; see above), we accept only *Paraholosticha lichenicola* (Table 2). Recently, we classified *P. ovata* as further (supposed) junior synonym of *P. muscicola* (Berger 2006, p. 985; Jung et al. 2015). This species was transferred, obviously by mistake, to the urostylid genus *Uroleptopsis* Kahl, 1932 by Borror (1972) and Stiller (1974). Very likely, they referred to the redrawing of *P. ovata* provided by Kahl (1935, his Fig. 155 $_{14}$), who obviously misinterpreted the frontal corona and the cirri of the frontal rows as bicorona, a characteristic of the pseudokeronopsids. Later, Borror and Wicklow (1983) transferred it to *Pseudokeronopsis* because they incorrectly put the older genus *Uroleptopsis* Kahl, 1932 into the synonymy of the newly established genus *Pseudokeronopsis* Borror and Wicklow, 1983. *Uroleptopsis* and *Pseudokeronopsis* are pseudokeronopsids and therefore the classifications proposed by these workers are certainly incorrect (for revision of *Pseudokeronopsis* and *Uroleptopsis*, see Berger 2006, p. 886, 980). Hemberger (1982) transferred *P. ovata* to *Keronopsis*. He stated that this species is not yet unambiguously described, but the assignment to *Keronopsis* is definite. Dieckmann (1995, p. 373) published an improved protargol preparation method. In the material and methods section he mentioned *P. muscicola* and *P. ovata*, indicating that he did not synonymize these two species. Unfortunately, no distinguishing features were described by Dieckmann (1995). Further studies are necessary to describe *P. ovata* based on standard methods to convince its synonymization

Paraholosticha sterkii (Garnjobst, 1934) comb. nov.[1] has usually four macronuclear nodules (Dieckmann 1988a; Garnjobst 1934, 1937). Xu et al. (2016), who described an Antarctic population of *P. muscicola*, found that six out of 22 specimens had "three or four" macronuclear nodules and three specimens had "four or five" (average 2.2). According to their Tables 5 and 6, at least one individual had only one macronucleus and the maximum value is four, indicating a mistake in the morphometric characterization (Xu et al. 2016; Table 2). Because of this variability in their Antarctic population, Xu et al. (2016) followed Hemberger (1982) who synonymized *S. sterkii* with *P. muscicola*. By contrast, Garnjobst (1934, 1937) found *S. sterkii* specimens with 2–8, on average 4.5 (median 4.0, n = 15) macronuclear nodules (values from Fig. 10A–O in Garnjobst 1937). Dieckmann (1988a) reported invariably four nodules (n = 25). The data by Garnjobst (1934, 1937) and Dieckmann (1988a) indicate that *P. sterkii* is not identical with *P. muscicola*, but there is no doubt that more detailed studies are needed to support or disprove this hypothesis. Perhaps *P. sterkii* is a subspecies of *P. muscicola*.

Paraholosticha vitrea (Vörösváry, 1950) Berger, 2001 has six dorsal kineties according to the original description (vs. three in *P. muscicola* and other paraholostichids; Park et al. 2017; Vörösváry 1950). Hemberger (1982) discussed that *P. vitrea* is a valid species, when the increased number of dorsal kineties is confirmed; that is he synonymized this species with *P. muscicola*.

The fourth synonym proposed by Hemberger (1982), *P. pannonica*, was redescribed only recently (Park et al. 2017). It

[1] Görtz (1986, p. 176) designated the species as "*Paraholosticha sterkii*" referring to the paper by Görtz and Dieckmann (1987; as "Görtz and Dieckmann 1986"). Of course, this brief comment by Görtz (1986) cannot be a valid combination. Görtz and Dieckmann (1987) designated the present species as "*Paraholosticha sterkii* Dieckmann", which is incorrect in two respects, namely, (i) Dieckmann is not the author of the species and (ii) the "formal combination" made by Dieckmann (1988a) is also invalid because he transferred *Stylonethes sterkii* to the invalid genus *Paraholosticha* Kahl, 1932 and not to the valid genus *Paraholosticha* Wenzel, 1953 (Dieckmann 1988a, p. 227 wrote that "*P. sterkii* is a typical member of the genus *Paraholosticha* Kahl [11]" or "It appears justified to distinguish *P. sterkii* as a valid species of the genus *Paraholosticha* Kahl [11]"). Thus, neither Görtz (1986) and Görtz and Dieckmann (1987) nor Dieckmann (1988a) have made a reasoned and valid combination with *Paraholosticha* Wenzel, 1953. Consequently, the transfer of *S. sterkii* from *Stylonethes* to *Paraholosticha* Wenzel, 1953 is made in the present work.

can be easily distinguished from *P. muscicola* by the conspicuous, single micronucleus in-between the two macronuclear nodules (Gellért and Tamás 1959).

Paraholosticha muscicola sensu Shin (1994, p. 61) is relatively small (60–100 × 30–50 μm in vivo) and the frontoventral rows terminate about in mid-body. In addition, the arrangement of the cirri in the frontal area is rather irregular. Thus, this Korean population is provisionally classified as insufficient redescription. Studies on material from the same sample site (soil covered with moss from Namhansong in Kwangju-gun) will show whether *P. muscicola* or another species is present in this area.

Morphology of Korean brackish water population (Figs. 1A–F, 2A–G, 3A–C; Tables 1 and 2)

Body size in vivo 100–180 × 40–70 μm (n = 6), on average 101 × 59 μm in protargol preparations (Table 1). Body outline wide elliptical, both ends broadly rounded (Figs. 1A, 2A–C, 3A, B), slightly dorsoventrally flattened; protargol-prepared specimens ovoid to pyriform in outline (Figs. 1C, D, 3A, B). Body flexible, but not contractile. Cells grayish at low magnification (Fig. 2A–F). Nuclear apparatus composed of two ellipsoidal macronuclear nodules, arranged roughly in midline of cell and connected by thin (about 0.1 μm) thread (Figs. 1A, D, 2D, 3A, C); 1–6, on average three, spherical to slightly ellipsoidal micronuclei, usually located near macronuclear nodules, in specimens with one micronucleus it is never in-between the macronuclear nodules (Fig. 3C). Contractile vacuole about in mid-body near left cell margin, approximately 16 μm in diameter when fully extended, without distinct collecting canals (Figs. 1B, 2C). Cortical granules lacking. Locomotion without peculiarities, that is, glides moderately fast on bottom of Petri dish.

Adoral zone terminates at 45% of body length on average in protargol preparations, extends far posteriorly on right cell margin, DE-value on average 0.38 in protargol preparations, ventral portion roughly gonostomoid, composed of 38–55, an average of 46 membranelles of ordinary fine structure (Figs. 1A, C, 3A, Table 1); bases of largest membranelles about 8 μm wide on average; cilia 15–23 μm long in vivo. Buccal cavity narrow and shallow. Paroral and endoral arranged in parallel, both membranes slightly curved rightwards anteriorly, commence and terminate at different levels, paroral about 20% longer than endoral; paroral composed of obliquely arranged dikinetids, endoral made of monokinetids; pharyngeal fibers extend obliquely rightwards (Figs. 1A, C, E, F, 2D–F, 3A).

Cirri moderately fine, mostly 11–13 μm long in vivo. Cirral pattern rather constant and characteristic due to keronopsid frontal corona and two long frontoventral rows. Frontal corona (a mixed row composed of three true rows) composed of 17–24 cirri, arranged in parallel to distal portion of adoral zone, both ends at about same level; individual true rows not recognizable (Figs. 1A, 3A). Two to four buccal cirri in longitudinal row right of undulating membranes. Two frontal rows, always distinctly separated from buccal cirral row, composed of 2–6 (row 1) and 3–9 (row 2) cirri, respectively; row 1 never, row 2 sometimes overlapping with buccal row (Figs. 1C, E, F, 3A, Table 1). Left and right frontoventral row almost bipolar, composed of almost identical numbers of cirri (36; 37) on average (Figs. 1C, 3A, Table 1). Transverse cirri and pretransverse ventral cirri lacking. Left marginal row commences left of posterior end of adoral zone, terminates, like right row, at rear end of cell; marginal rows distinctly separated posteriorly. Right marginal row begins somewhat behind level of anterior end of right frontoventral row (Figs. 1A, 3A).

Three bipolar dorsal kineties, bristles about 3 μm long in vivo. Caudal cirri lacking (Figs. 1D, 2G, 3A, B).

Note on cell division (Fig. 4A–D)

Keronopsids are the sole hypotrichs which divide in reproduction cysts (e.g., Dieckmann 1989; Penard 1922). Very rarely, division in (resting) cysts can be observed in other hypotrichs (for details, see Benčat'ová et al. 2016).

One reproduction cyst of the brackish water population was studied in vivo. When we started to observe the cyst, it had a slightly ellipsoidal, about 2 μm thick wall. The dividing cell had a distinct equatorial constriction. A contractile vacuole was recognizable and the cell did not rotate in the cyst (Fig. 4A). After about 2 h, the cell began to rotate and likely divided (Fig. 4B). About 3 h after starting our observations, the rotation became irregular and few minutes later the two daughter cells hatched (Fig. 4C, D). An excystation vacuole was not observed. The body shape of the excysted specimens was very similar to that of interphasic cells, even immediately after the excystation (Fig. 4D).

Garnjobst (1937) and Dieckmann (1988a, 1989) studied the cell division of *P. sterkii* and *P. muscicola*. Accordingly, division ("separation of cells") takes place prior to the formation of the anlagen for the ciliature. By contrast, in *Keronopsis wetzeli* Wenzel, 1953 the division occurs after the complete formation of the ciliature (Dieckmann 1988b; 1989, p. 154). It has 1–3 transverse cirri (Berger and Foissner 1987; Grolière 1975; Wenzel 1953) and was fixed as type species of *Parakeronopsis* Shi, 1999. *Keronopsis wetzeli* differs from *K. helluo* Penard, 1922, type species of *Keronopsis*, inter alia, in the number of transverse cirri (1–3 vs. 8–13) and very likely also in the mode of their formation as explained in detail by Park et al. (2017, p. 115). These differences in the ciliature and its formation strongly suggest that *Parakeronopsis* is a valid genus or at least subgenus of either *Keronopsis* or *Paraholosticha*. However, as already explained by Park et al. (2017), ontogenetic data on *K. helluo* and molecular studies on *K. wetzeli* are needed to support or disprove this hypothesis.

Table 3. Uncorrected pairwise matrix of nuclear small subunit ribosomal DNA sequences of four keronopsids. Dissimilarity (bottom left) and number of nucleotide difference (above right).

Sequence		Sequence			
		1	2	3	4
1	*Paraholosticha muscicola* (MK287978, Korea)	–	15	14	14
2	*Paraholosticha muscicola* (KT003281, Antarctica)	1.0%	–	11	11
3	*Paraholosticha pannonica* (KY492517)	0.9%	0.7%	–	2
4	*Keronopsis helluo* (KY492516)	0.9%	0.7%	0.1%	–

Notes on the phylogeny of the keronopsids (Figs. 5 and 6)

The SSU rRNA of the Korean population from brackish water has a length of 1,537 bp and a GC content of 46.3% (GenBank accession number MK287978). It differs from that of the Antarctic population (KT003281; Jung et al. 2015) in 15 nucleotides which corresponds to about 1.0% (Table 3). The four keronopsids analyzed so far (present population of *P. muscicola* MK287978, *P. muscicola* KT003281, *P. pannonica* KY492517, *Keronopsis helluo* KY492516) do not form a monophyletic group in the maximum likelihood tree as one would expect on the basis of the rather unique morphological and ontogenetic apomorphies, namely, a frontal corona originating from anlagen I–III and the division in cysts (Fig. 5). However, at least all of them branch off rather basally as hypothesized from morphological/ontogenetic features because the keronopsids lack both dorsal kinety fragmentation and dorsomarginal rows, that is, they are part of the basal-branching "non-dorsomarginalian hypotrichs" (Berger 2008, p. 46). This simple dorsal kinety pattern (usually three bipolar kineties) is also present in the taxa branching off in the same part of the tree, namely *Uroleptoides magnigranulosa* (Berger 2008, p. 273), *Parabistichella variabilis* (Jiang et al. 2013), *Gonostomum* spp. (Berger 2011, p. 58), *Orthoamphisiella breviseries* (Berger 2011, p. 642), *Bistichella* (Berger 2008, p. 532), and *Cotterillia bromelicola* (Foissner and Stoeck 2011). To visualize and quantify phylogenetic conflicts hidden in conventional trees, we applied the phylogenetic network analysis (Vd'ačný 2017). In contrast to our conventional tree, these basal-branching species, excluding *Gonostomum strenuum* and *Cotterillia bromelicola*, were clustered in the phylogenetic network with a bootstrap value of 71.6%; however, any unique single nucleotide polymorphism was not detected in that cluster, which might be an evidence to support its monophyly (Fig. 6). Furthermore, as shown in the tree with low supporting values for these basal branches, the network was full of short parallelograms (e.g., star-like edges) in the non-dorsomarginalian hypotrichs and even in the central position of hypotrichs, indicating high radiation. Based on these results, we cannot explicitly accept/reject the monophyly of keronopsids because of the conflicts in the SSU rRNA gene. To test the homology, further studies should focus not only on the multigene/phylogenomic analysis (Gentekaki et al. 2017), but also on the reconstruction of morphological character evolution (Vd'ačný 2017). If the conflicts result from symplesiomorphic character states (= nucleotides), the problem cannot be overcome with an increased number of genes and taxa (Kück and Wägele 2016).

Morphological comparison of brackish water population with previously described populations, two synonyms, and with *P. algivora* (Table 2)

To date, nine populations of *P. muscicola* have been described, including the brackish water population from Korea. Furthermore, two species discovered in Hungary are classified as junior synonyms of *P. muscicola*, namely *P. ovata* and *P. lichenicola*. Seven populations are terrestrial and three were isolated from a limnetic habitat (including one from a glacier) while our population is the first recorded from brackish water (Table 2).

The total range of the most important morphometric features published by Jung et al. (2015, their Table 2) did not change distinctly in spite of the inclusion of three further populations (present work; Schmitz 1986; Xu et al. 2016). The variability within populations and the total variability of some characteristics is rather high, for example, number of buccal cirri, cirri forming frontal rows 1 and 2, adoral membranelles, and micronuclei. The sparse data available from the populations called *P. ovata* and *P. lichenicola* also fall within the total range of *P. muscicola* (Table 2). The number of macronuclear nodules was invariably two in most populations, so that we do not agree with Hemberger (1982) and Xu et al. (2016) who suggested synonymy of *P. muscicola* (usually two nodules) and *P. sterkii* (usually four nodules; see above). Perhaps gene sequence data of *P. sterkii*, that is, a brackish water population with, on average, four macronuclear nodules, can support or disprove this hypothesis.

Principal components analysis showed that the Korean population is more similar to the population from Danish soil than to the strain which we isolated from Antarctic soil (Fig. 7; Foissner 1987a; Jung et al. 2015). However,

Fig. 5. Maximum likelihood tree of nuclear small subunit ribosomal RNA gene. Asterisk marks *Paraholosticha muscicola* from Korea. The bootstrap values of maximum likelihood (ML) and posterior probabilities of Bayesian inference (BI) are shown for each interior branch. If they are less than 100 (ML)/0.50 (BI), they are excluded (dash/blank in the tree). The scale bar represents one nucleotide substitutions per 100 nt.

the ranges of the individual characters overlap more or less distinctly, that is, there is no feature that can be used for serious, classical species separation. The SSU rDNA sequence of the Korean and Antarctic population show a 1.0% (15 nucleotides) difference, which is significantly greater than the difference in only one nucleotide (position 325) between the sibling species *Stylonychia mytilus* and *S. lemnae* (Schmidt et al. 2006a, 2006b; for revision of *Stylonychia*, see Berger 1999). Interestingly, Schmidt et al. (2006a) also found a difference of one nucleotide (position 267) between *S. lemnae* populations from Eurasia and North America.

Paraholosticha algivora (Gellért, 1942) comb. nov.[2] was overlooked by almost all workers, for example, Borror (1966, 1972) and Jankowski (1979). Gellért (1956a) himself obviously did not remember that he has described this species when he established *P. nana*, which is so similar that it has to be classified as junior synonym of *P. algivora*, at least at

[2] Gellért (1942) established this species within the invalid genus *Paraholosticha* Kahl, 1932. Later workers (e.g., Stiller 1974) also referred to Kahl's invalid genus and therefore they cannot be accepted as combining authors. Consequently, we transfer *P. algivora* to the valid genus *Paraholosticha* Wenzel, 1953.

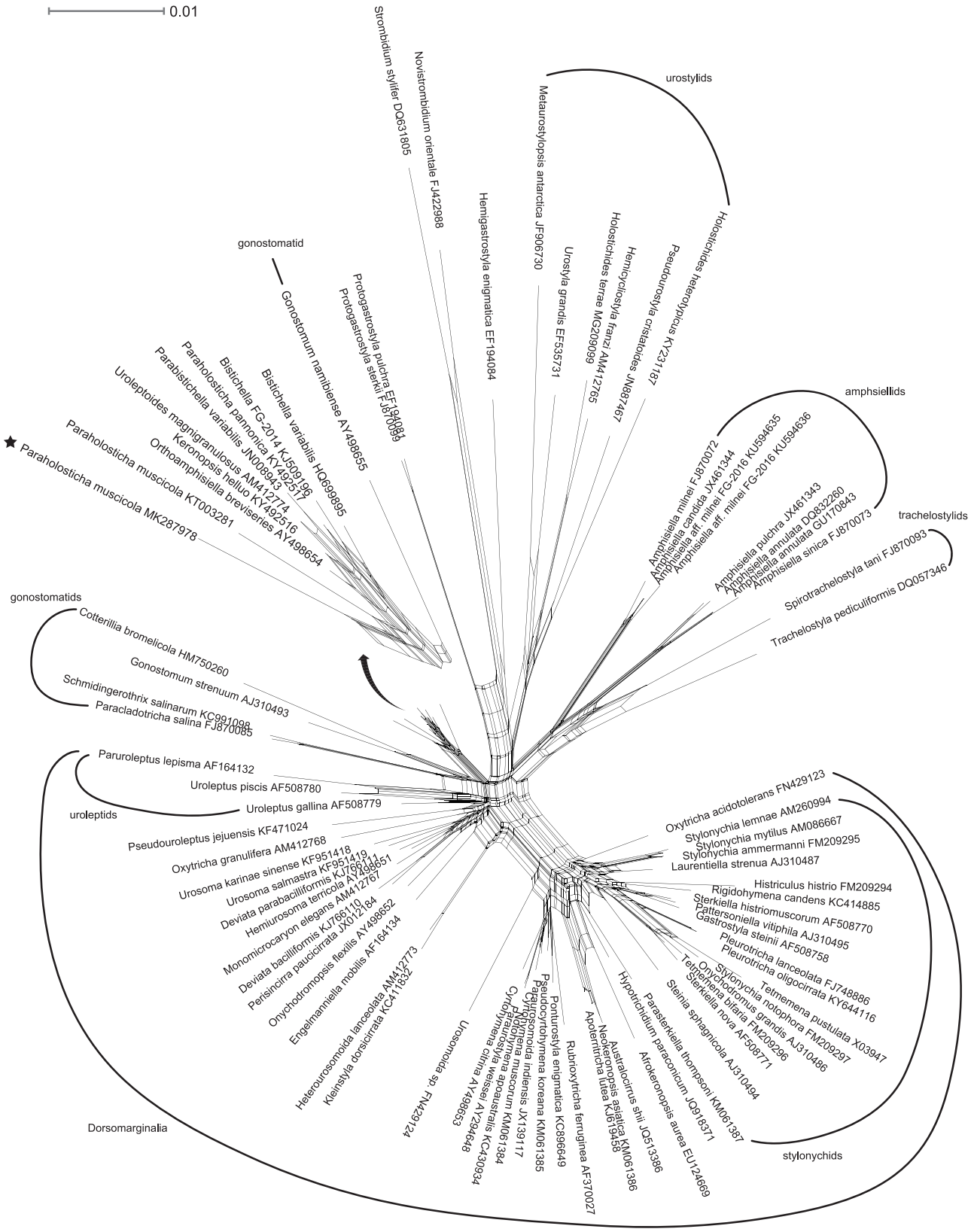

Fig. 6. Phylogenetic network based on neighbor-net analysis of nuclear small subunit ribosomal RNA gene. Asterisk marks *Paraholosticha muscicola* from Korea. The scale bar indicates one nucleotide substitution per 100 nt.

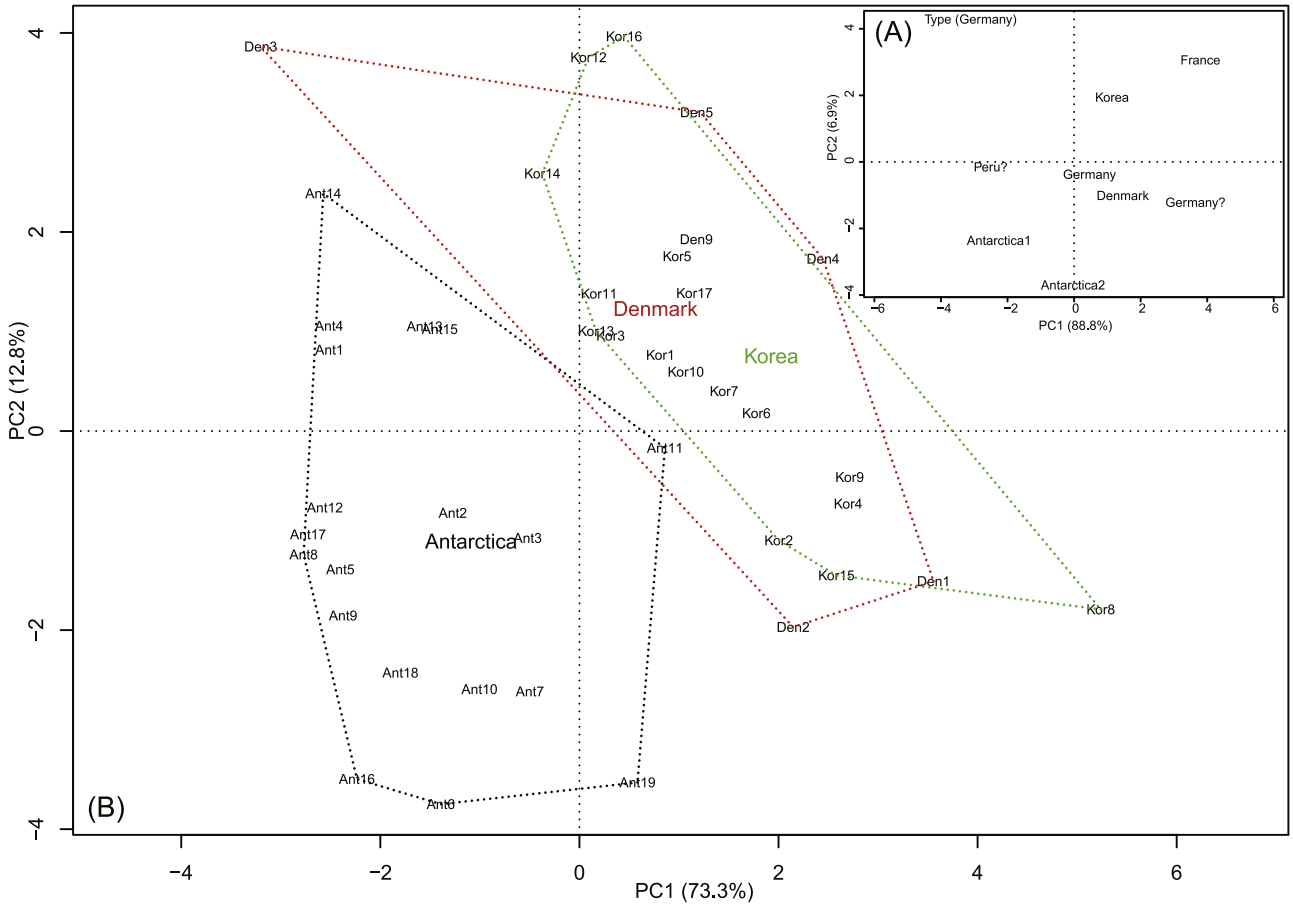

Fig. 7. A, B Plots of principal components analysis scores of morphometric data. **(A)** Multivariate morphometric data of nine populations of *Paraholosticha muscicola* based on the data in Table 2 (number of: adoral membranelles, macronuclear nodules, micronuclei, buccal cirri, cirri in frontal corona, in frontal rows 1 and 2, in left and right frontoventral row, and in right and left marginal row). **(B)** Multivariate morphometric data of Antarctic, Danish, and Korean population of *P. muscicola* based on each individual (body length, body width, distance from anterior body end to end of adoral zone, length of macronuclear nodule, width of macronuclear nodule, number of macronuclear nodules, length of micronuclei, width of micronuclei; number of: micronuclei, adoral membranelles, frontal and frontoventral rows, dorsal kineties, buccal cirri, cirri in frontal corona, cirri in frontal rows 1 and 2, cirri in left and right frontoventral row, cirri in right and left marginal row).

the present state of knowledge. The specimens of both populations are small (body length 60–90 μm and 60–70 μm, respectively) and have only one buccal cirrus and both frontal "rows" are composed of a single cirrus each. However, such values are also described for *P. muscicola* populations, so that the validity of *P. algivora* is not beyond doubt (Table 2). Hemberger (1982, p. 72) synonymised *P. algivora* with *P. ovata* Horváth, 1933, however, without justification. By contrast, we classify *P. ovata* as junior synonym of *P. muscicola* because it is somewhat larger (body length 70–100 μm) and very likely it has more cirri on the frontal field (for details, see above) than *P. algivora*. *Paraholosticha nana* has, like *P. algivora*, one micronucleus attached to each of the two macronuclear nodules (Gellért 1956a). By contrast, *P. nana* sensu Grolière (1975) is likely identical with *P. pannonica* because it has a single micronucleus in-between the two macronuclear nodules (further details, see Park et al. 2017, p. 108).

The available data show that the variability of important taxonomic features is rather high within and among populations of *P. muscicola*. An unambiguous separation is thus not possible indicating that it is a rather variable species. Further studies, comprising details on morphology, ontogenesis, cross conjugation, genetics, ecology, and molecular biology, are likely needed to show whether *P. muscicola* is indeed a highly variable species or a complex of distinct species which are indistinguishable at present. Thus, as a preliminary solution, we designate such populations as *Paraholosticha muscicola* complex. Foissner et al. (1992a) and Chen et al. (2016) made the same proposal for some vorticellids and uroleptids, respectively.

Key to species of the genus *Paraholosticha* Wenzel

When you know that your specimen/population belongs to *Paraholosticha* (keronopsid without transverse cirri), then the following features are important for identification of species: nuclear apparatus; body size; number of cirri in frontal rows; number of buccal cirri; number of dorsal kineties.

1 Nuclear apparatus composed of 2 macronuclear nodules with single micronucleus in-between 2
- Nuclear apparatus not as above (e.g., Fig. 1A, D) 3
2 Body length 150–190 μm; in total about 15 cirri in left and right frontal row and in buccal row (Fig. 88 in Kahl 1932) ..... *P. herbicola*
- Body length 80–90 μm (likely at least distinctly less than 150 μm); in total about 6 (likely at least less than 10) cirri in left and right frontal row and in buccal row (Fig. 1 in Gellért and Tamás 1959) ..... *P. pannonica*
3 Usually 4 macronuclear nodules; usually brackish water (e.g., Fig. 5, 6 in Garnjobst 1934) ..... *P. sterkii*
- Usually 2 macronuclear nodules; usually soil or freshwater (e.g., Fig. 1A, D, 3A, C)..... 4
4 Dorsal kineties 6 in number (Fig. 1b in Vörösváry 1950) ..... *P. vitrea*
- Dorsal kineties 3 in number (e.g., Fig. 1D) ..... *P. muscicola* complex 5
5 Body length 100–220 μm; frontal rows and buccal row usually composed of more than one cirrus (left frontal row 1–6 cirri, right frontal row 1–9, buccal row 0–6) ..... *P. muscicola*
- Body length 60–90 μm; frontal rows and buccal row composed of very few cirri, usually only one cirrus per row present (Fig. 21 in Gellért 1942) ..... *P. algivora*

Occurrence and ecology

So far, *Paraholosticha muscicola* was reliably recorded from terrestrial habitats (mosses, soil, lichens) and freshwater (details, see below). Our record is the first one from brackish water and Asia (for Korean population described by Shin 1994, see above). So far, the present species has been found in Europe, North America, Africa, New Zealand, Gough Island, and Antarctica. Not recorded from South America and Australia (Blatterer and Foissner 1988).

The type locality of *P. muscicola* is not described in detail. Kahl (1932) found it several times in mosses from Northern Germany; perhaps he discovered it in or near the city of Hamburg where he lived and worked. However, strictly speaking the type locality is not known. Kahl (1932) recorded it only once with high abundance, namely in moss from an old thatch roof. The population called *P. ovata* was discovered in a pond ("Cserepesisori tó" in the city of Szeged, Hungary; contains some sodium hydroxide), where Horváth (1933) collected it during late March 1932 when the water temperature was near the freezing point; the species lived only in markedly fresh (unpolluted) water. The type locality of the population called *P. lichenicola* is the south-western side of the Magoska hill (Tokaj-Eperjes mountains) north-east of the village of Boldogkõváralja, administrative district of Abauj-Torna, Hungary; Gellért (1956a) discovered it in the humus layer formed underneath the lichen *Parmelia saxatilis*.

Further records substantiated by morphological data (in chronological order): in September 1975 from moss collected in the "Foret de la Joux" (located at 46°50′21″N 06°00′33″E in the region Jura/Doubs according to fr.wikipedia.org), France (Tuffrau and Fryd-Versavel 1977); in infusions of a forest soil (Hemberger 1982; whether the soil is from Puerto Maldonado [about 12°36′S 69°12′W] in Peru or from a site in Germany remains unclear; Hemberger 1982, p. 2; Hemberger and Wilbert 1982, p. 262 did not make a comment on the sample site); during summer with low abundance on exposed plates in the River Rhine near the village of Beuel, Germany (Schmitz 1986, p. 88); with low abundance in a sample (collected on 26.09.1985) with lichens, moss, and soil from a granite rock at the village Sandkäs, north-eastern coast of the Danish island Bornholm in the Baltic Sea (Foissner 1987a, p. 188; interestingly, the sample was collected by Fritz Wenzel who established the genus *Paraholosticha* and fixed *P. muscicola* as type species); likely in Germany (Dieckmann 1989; did not mention the sample site of his populations); moss-covered soil collected near King Sejong station in southwestern part (62°13′49.1″S 58°42′37.3″W) of King George Island, Antarctica in December 2013 (Jung et al. 2015); melt-water, slush, and algae from Collins glacier (62°10′S 58°54′W) close to Russian station, Bellinghausen in Fildes Peninsula, King George Island, Antarctica (Xu et al. 2016).

Records of *P. muscicola* not substantiated by morphological data: not to distinctly compacted humus-rich as well as fertilized (thomasphosphate, compound fertilizer) soil of an alpine pasture from the Schlossalm area (47°08′54″N 13°03′29″E; altitude 1965 m), Bad Hofgastein, Salzburg, Austria (Berger et al. 1985, p. 107; 1986, p. 268); alp (Palfner Alm, Mittleres Seidlwinkltal) in Salzburg, Austria (Gros et al. 2012, p. 43); soil (alpine brown earth; partly treated with organic or mineral fertilizer; altitude 2800 m) in Tyrolean Central Alps (Obergurgel, Festkogel), Austria (Lüftenegger et al. 1986, p. 153); polysaprobic running waters in Bulgaria (Detcheva 1979, p. 364; 1992, p. 102; Russev et al. 1976, p. 62); river Iskar, Bulgaria (Detcheva 1993, p. 34); during cold season scattered in road puddles in Bavaria, Germany (Dingfelder 1962, p. 609; pH 6.2–7.2, acid binding capacity 1.5–4.9); river Amper and/or a small tributary (Windach) in Bavaria, Germany (Foissner et al. 1992b, p. 102); two mesosaprobic tributaries (Maisach, SI = 3.0; Würm, SI = 2.5) of the river Amper, Bavaria, Germany (Foissner et al. 1992c, p. 50); dry mosses, leaf litter, lichens, and foam of spittlebugs (Aphrophoridae) collected in suburbs of city of Erlangen, Germany (Wenzel 1953, p. 104; 1954, p. 123); salt-loaded running waters near the city of Lippstadt, Germany (Mihailowitsch 1989, p. 166; some autecological data

provided, see below); Germany? (Dieckmann 1995, p. 373); leaf litter from the Hosszúbérc mountains in Hungary (Varga 1959, p. 458); small alkaline ponds and salinized soils in Hortobágy National Park, Hungary (Szabó 1999a, p. 229; 1999b, p. 248); terrestrial mosses from near the city of Pisa, Italy (Verni and Rosati 2000, p. 68); soil from the Macaulay Land Use Research Institute, Sourhope Research Station, near Kelso, Southern Scotland (Esteban et al. 2006, p. 142; Finlay et al. 2001, p. 363); submerged, wet, and moist, but not dry mosses from the area of Slovensky raj, Slovakia (Tirjaková and Matis 1987a, p. 9); dry mosses from various sites in Bratislava, Slovakia (Tirjaková and Matis 1987, p. 22); agricultural soils near Pieštany, Slovakia (Tirjaková 1988, p. 500); bark and decaying wood masses (in contact with soil) of *Pinus sylvestris* and *Populus tremula* in Slovakia (Bartošová and Tirjaková 2008, p. 180; for records from Slovakia, see also Matis et al. 1996, p. 16); litter and soil from Olympic National Park, Washington State, USA (Bamforth 2010, p. 365); thick, flaky bark from *Colophospermum mopane* trees at Bambetsi Guest Farm between towns of Khorixas and Outjo, altitude 1150 m, Mopane savannah, Namibia (Foissner et al. 2002, p. 61a, b); with a frequency of 3.7% in mire vegetation, that is, almost pure moss with very few soil particles (pH 4.5, altitude 500 m) from Tafelkop, Gough Island (40°21′S 09°53′E; Foissner 1996, p. 284); moss and soil from Antarctica (Sudzuki 1979, p. 123); ornithogenic soil from margin of a penguin (*Pygoscelis adeliae*) rookery at Shirley Island (66°17′S 110°29′E; Budd Coast), a small rocky island about 100 m offshore from the continental coast and some other sites near Casey Station, Antarctica (Petz 1997, p. 39; Petz and Foissner 1996, p. 259; 1997, p. 309).

Records of synonym *P. ovata* not substantiated by morphological data: Germany? (Dieckmann 1995, p. 373); ponds in the Hortobágy National Park, Hungary (Szabó 1999a, p. 229).

Record of synonym *P. lichenicola* not substantiated by morphological data: tussock leaves from Waiouru, New Zealand (Stout 1960, p. 238; see also Foissner et al. 2012, p. 230).

Paraholosticha muscicola feeds on algae and bacteria (Kahl 1932; Schmitz 1986); according to Foissner (1987a), it also ingests ciliates (*Colpoda steinii*), zooflagellates, fungal spores, and green algae. Tuffrau and Fryd-Versavel (1977) cultured their specimens in mineral water (Volvic) and fed it with *Chlorogonium* sp. The food vacuoles of the Korean brackish water population contained bacteria and euglenids. Biomass of 10^6 specimens about 210 mg (Foissner 1987b, p. 125; Foissner 1998, p. 207); biomass of synonym *P. lichenicola* 63 mg per 10^6 specimens (Foissner 1998, p. 207). According to Lüftenegger et al. (1988, p. 99), *P. muscicola* is easily recognized when studying soil samples directly.

For description of a culture medium, see Dieckmann (1989, p. 144). He fed *P. muscicola* every 2–3 d with a suspension of *Chlorogonium elongatum* or with fresh baker's yeast. The hypotrich is very sensitive to pollution due to high concentration of bacteria; resting cysts are formed immediately. Thus, Dieckmann transferred the ciliate in fresh culture medium every two or three days. Some strains were very temperature-sensitive. At > 16 °C they no longer fed and encysted. Thus, all cultures were kept at 12 ± 1 °C (Dieckmann 1989). According to Horváth (1933) and Gellért (1956a), the populations called *P. ovata* and *P. lichenicola* fed on globular species of algae and on plant debris; Horváth (1933) reported *Euglena* as further food item.

Mihailowitsch (1989, p. 163) provided the following autecological data from salt-loaded running waters in Germany (n = 6 or 7; identification not substantiated by morphological data): 11.5–13.5 °C; pH 7.03–7.49; 34.5–70.1 mg l^{-1} CO_2; 4.7–7.1 mg l^{-1} O_2; 0.5–2.2 mg l^{-1} NH_4^+-N; 0.03–0.10 mg l^{-1} NO^{-2}-N; 1.5–13.4 mg l^{-1} NO_3^--N; 108–2890 mg l^{-1} Cl^-; 90–1030 µS cm^{-1} conductivity (threshold value for drinking water in Europe, 2500 µS cm^{-1}; Rat der Europäischen Kommission 1998). Detcheva (1993) found *P. muscicola* once in a Bulgarian river at following conditions: 23 °C; pH 8.0; 2.1 mg l^{-1} O_2 (= 25% saturation); 2.0 mg l^{-1} NH_3.

Authors contributions

J.J. collected the samples and carried out all laboratory work (preparations, analyses, illustrations, micrographs, calculations, etc.). H.B. provided the monographic data of *P. muscicola* and its synonyms and suggested the new combinations. H.B. and J.J. wrote the manuscript.

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