

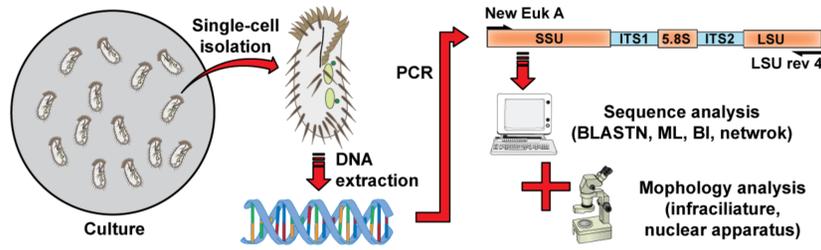
Monographic treatment of *Paraholosticha muscicola* (Ciliophora, Keronopsidae), including morphological and molecular biological characterization of a brackish water population from Korea

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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 fromanlagen I–III; division in cysts). However, the SSU rRNA sequence of the Korean population does not cluster with that of the Antarctic population, but both branch off consecutively and immediately before a mixture of other non-dorsomarginalian hypotrichs, including two further keronopsids. However, the keronopsids cluster in the phylogenetic network, indicating phylogenetic conflicts, which cannot be exemplified in the conventional 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 preliminary accept only *P. lichenicola* and *P. ovata*. *Paraholosticha algivora* is likewise very similar and thus we propose to summarize them as species of the *P. muscicola* complex. *Stylonethes sterkii* and *P. algivora* are transferred to *Paraholosticha* Wenzel. A key to the *Paraholosticha* species is provided.

Materials & Methods



Results

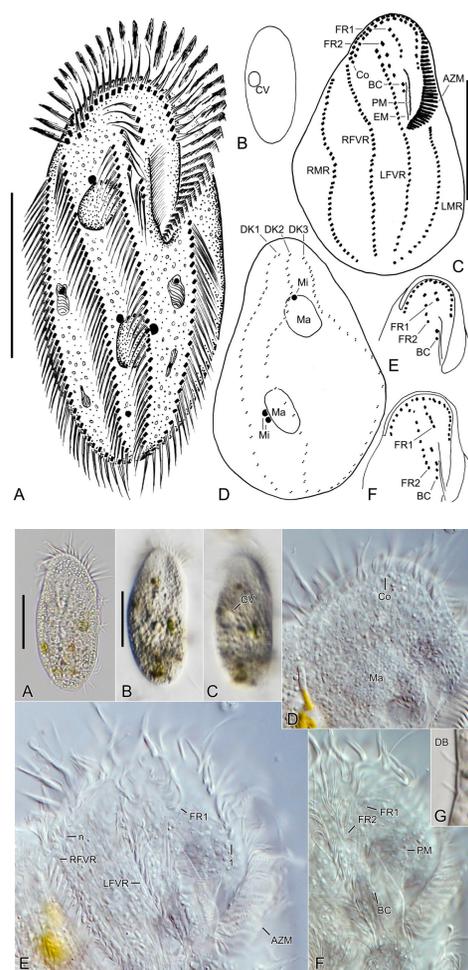


Fig. 1A–F. *Paraholosticha muscicola*, Korean population from life (A, B) and after protogal preparation (C–F). (A) Ventral view of a representative specimen. (B) Dorsal view showing position of contractile vacuole. (C, D) Ciliature of ventral and dorsal side and nuclear apparatus of same specimen. (E, 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; LFRV, 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.

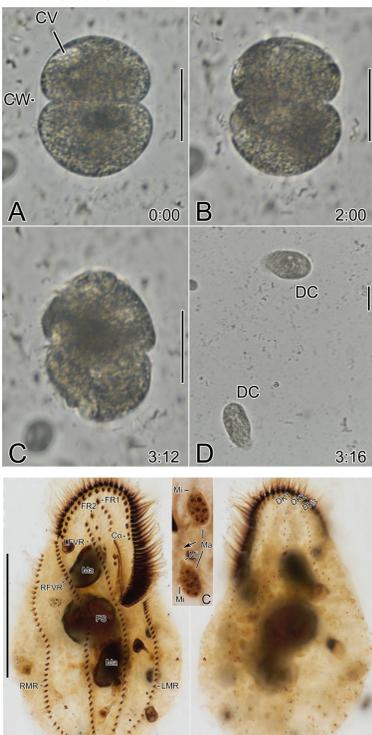


Fig. 2A–G. *Paraholosticha muscicola*, Korean population from life (A, B) and after protogal preparation (C–F). (A, B) Ventral and dorsal view of representative specimen. (C) Nuclear apparatus in dorsal view; arrow denotes thin thread connecting macronuclear nodules. Co, frontal corona; DK1–3, dorsal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

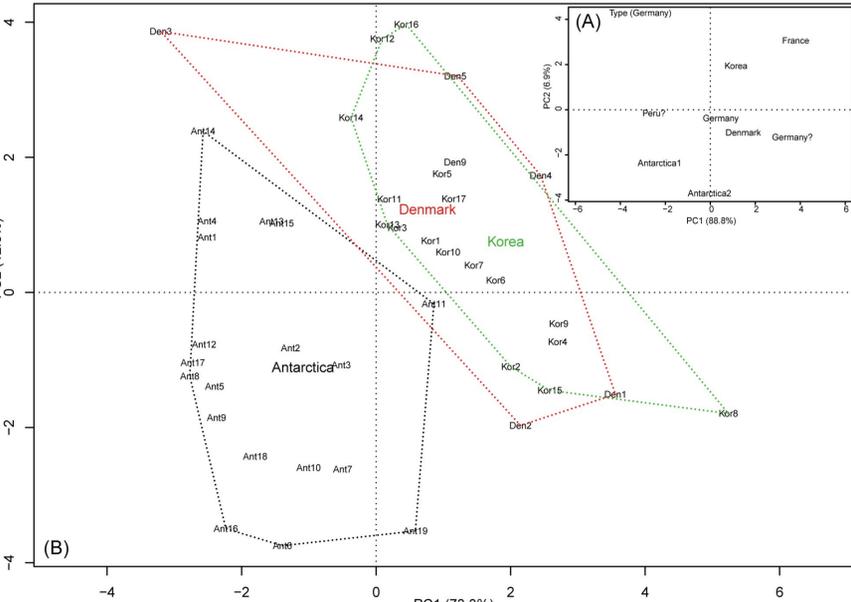


Fig. 7A, B. Plots of principal components analysis scores of morphometric data. (A) Multivariate morphometric data of nine populations of *Paraholosticha muscicola* based on the data in Table 2 (number of: adoral membranelles, macronuclear nodules, micronuclei, buccal cirri, frontal corona, in frontal 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).

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

Characteristic*	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	2.0	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	38.0	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	4.0	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	3.0	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

* Data based on protogal 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.

Fig. 4A–D. Chronological sequence of division in cyst of *Paraholosticha muscicola* from life. CV, contractile vacuole; CW, cyst wall; DC, daughter cell. Scale bars: 50 µm.

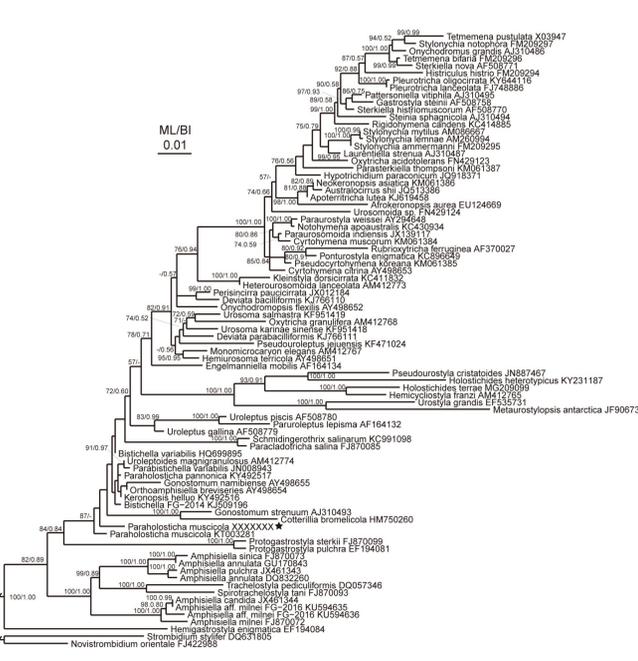


Fig. 5. Maximum likelihood tree of nuclear small subunit ribosomal RNA gene. Asterisk marks *Paraholosticha muscicola*. The bootstrap values of maximum likelihood (ML) and posterior probabilities of Bayesian inference (BI) are shown for each interior branch. If they are less than 100 (ML)/0.50 (BI),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

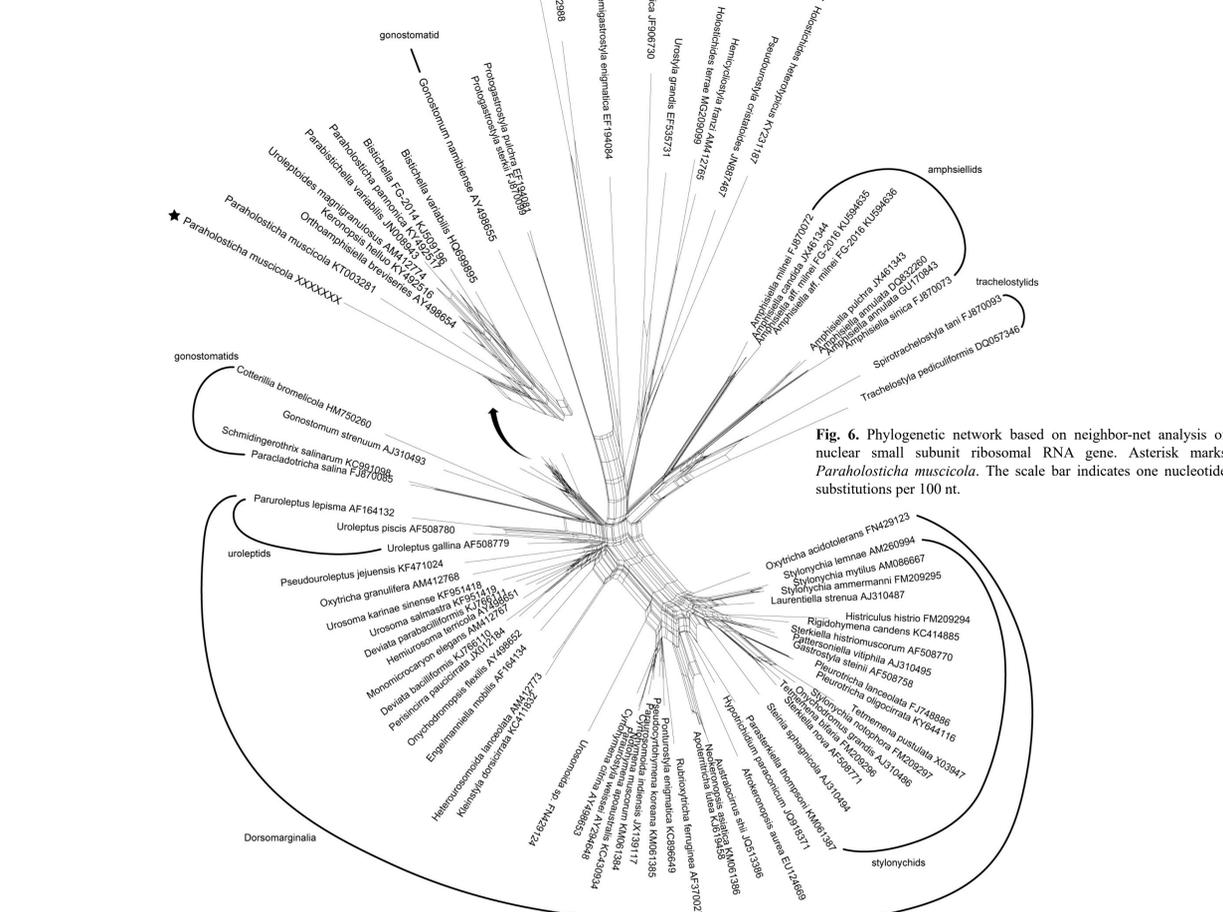


Fig. 6. Phylogenetic network based on neighbor-net analysis of nuclear small subunit ribosomal RNA gene. Asterisk marks *Paraholosticha muscicola*. The scale bar indicates one nucleotide substitutions per 100 nt.

Discussion (Key to species of the genus *Paraholosticha* Wenzel)

- 1 Nuclear apparatus composed of 2 macronuclear nodules with single micronucleus in-between ----- 2
- Nuclear apparatus not as above (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 (1) Usually 4 macronuclear nodules; usually soil or freshwater (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*