

Brief description of newly recorded eight ciliate species (Protozoa, Ciliophora) collected from South Korea

Ji Hye Kim¹ and Jae-Ho Jung^{2,*}

¹Natural Science Research Institute, Gangneung-Wonju National University, Gangneung, Republic of Korea

²Department of Biology, Gangneung-Wonju National University, Gangneung, Republic of Korea

*Correspondent: jhjung@gwnu.ac.kr

We collected ciliate species from diverse habitats (brackish water, marine water, and terrestrial ecosystems) composed of estuarine, coast, and moss samples during 2017–2018 in Korea. The morphology and infraciliature of these species were examined and identified based on the observations of living cells and protargol impregnated specimens. Of the ciliates we identified, we described eight unrecorded species in Korea as followings: class Heterotrichea - *Condylostoma vorax*, *Peritromus kahli*; class Protocruzia - *Protocruzia labiata*; class Spirotrichea - *Hemiamphisiella granulifera*; class Litostomatea - *Loxophyllum chinense*; class Phyllopharyngea - *Trochiloides recta*; and class Oligohymenophorea - *Cohnilembus verminus*, *Uronemella filificum*. Among them, *Protocruzia labiata* is a poorly known species. In this paper, we provide the improved diagnoses and brief descriptions of the species with their microphotographs. The taxonomic classifications of the eight species are summarized as six classes, eight orders, five families, and eight genera. These voucher slides with protargol impregnated specimens have been deposited in the National Institutes.

Keywords: infraciliature, protargol impregnation, *Protocruzia*, redescription, taxonomy

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DOI:10.12651/JSR.2019.8.4.380

INTRODUCTION

The phylum Ciliophora is one of the most diverse groups of protists, up to data, about 8,000 species were described. The expected diversity of the undescribed ciliates should be 27,000–40,000 free-living species worldwide as calculated by Foissner *et al.* (2008). In Korea, researchers have discovered more than 450 species but still many species are under discovery (Kwon *et al.*, 2019).

We have collected eight species from marine, brackish water, and soil habitats of South Korea. *Protocruzia labiata* Kahl, 1932 is described here for the first time after the discovery of the type population by Kahl (1932) who used only live observation.

MATERIALS AND METHODS

The eight ciliate species were collected from different habitats such as marine water, brackish water, and terrestrial moss. The specific information of locality is described in 'Material examined' section of each species. The samples were collected and transferred to our

laboratory. The soil samples were air-dried for at least two weeks, then cultured using the non-flooded Petri dish method (Foissner *et al.*, 2002). The marine and brackish water samples were cultured in Petri dishes and two or three rice grains were added to increase bacteria as food source at room temperature (about 20°C).

The morphology was investigated using a stereomicroscope (Olympus SZ11, Japan), an optical microscope (Olympus BX53, Japan) at low ($\times 40$ –200) to high ($\times 400$ –1000) magnifications, and digital camera (Olympus DP74, Japan). The synthesized protargol powder was prepared by using the method of Pan *et al.* (2013c) and the cells were impregnated using the 'procedure A' protargol method (Foissner, 2014). The differential through-focal images of the protargol impregnated specimens were merged using the software of Helicon Focus 6.8.0 (HeliconSoft Ltd, Ukraine). The terminology and taxonomic classification follow Berger (2008) and Lynn (2008).

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901

Class Heterotrichea Stein, 1859
 Order Heterotrichida Stein, 1859
 Family Condylomatidae Kahl in Doflein and Reichenow, 1929
 Genus *Condylostoma* Bory de St. Vincent, 1824

1. *Condylostoma vorax* Villeneuve-Brachon, 1940 (Fig. 1)

Material examined. Brackish water (salinity 17.4‰) collected from Taehahang Port, Hyeonpo-ri, Buk-myeong, Ulleung-gun, Gyeongsangbuk-do, Korea (37°31'24"N, 130°48'52"E) on 30 August 2018.

Diagnosis. Body size 200–315 × 55–80 μm in vivo; body contractile and flexible with elongated ellipsoidal shape; buccal cavity 33–38% of body length; cortical granules slightly yellowish and size about 2.5 × 1.6 μm; macronucleus moniliform with 13 nodules; 73–87 adoral membranelles; 4 or 5 frontal cirri; 25–30 somatic kineties (n = 8).

Distribution. Korea and France.

Remarks. The Korean population of *Condylostoma vorax* corresponds well with the type population (Villeneuve-Brachon, 1940), except for the number of somatic kineties (25–30 vs. 30–34) and the habitat (brackish vs. marine water). *Condylostoma vorax* is also similar to *C. curva* Burkovsky, 1970 but differs mainly in having an elongated ellipsoidal (vs. oval) body shape (Villeneuve-Brachon, 1940; Kim *et al.*, 2012).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109482, NIBRPR0000109483) and two slides were deposited at the National Marine

Biodiversity Institute of Korea (MABIK PR00043125, PR00043126).

Family Peritromidae Stein, 1867

Genus *Peritromus* Stein, 1863 Bory de St. Vincent, 1824

2. *Peritromus kahli* Villeneuve-Brachon, 1940 (Fig. 2)

Material examined. Brackish water (salinity 17.4‰) collected from Taehahang Port, Hyeonpo-ri, Buk-myeong, Ulleung-gun, Gyeongsangbuk-do, Korea (37°31'24"N, 130°48'52"E) on 30 August 2018.

Diagnosis. Body size 50–150 × 40–80 μm in vivo; body broad elliptical to oval shape, strongly flattened dorso-ventrally, whole body folding when stimulated; cytoplasm slightly yellowish; spine-like external cilia surrounded body outline; cortical granules linearly arranged between somatic kineties on ventral side and slightly yellowish; numerous peculiar chalice-like structure densely arranged on whole dorsal side; 2 ellipsoidal macronuclei; 80–93 adoral membranelles; 30–38 somatic kineties.

Distribution. Korea and France.

Remarks. *Peritromus kahli* differs from the most similar congener, *P. faurei* Kahl, 1932, in the absence (vs. presence) of the protrusions on dorsal surface and in the presence (vs. absence) of the peculiar chalice-like structure on dorsal side (Rosati *et al.*, 2004; Shao *et al.*, 2009).

Voucher slides. One slide with protargol-impregnated specimens was deposited at National Institute of Biological Resources, Korea (NIBRPR0000109479) and one slide was deposited at the National Marine Biodiversity

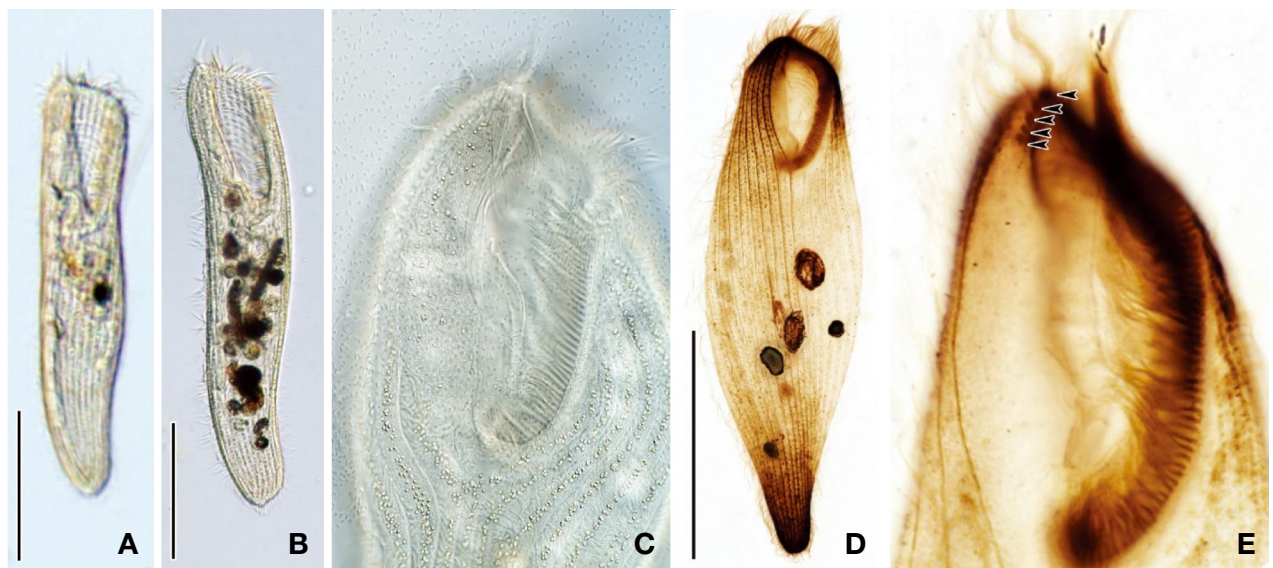


Fig. 1. *Condylostoma vorax* in vivo (A–C) and after protargol impregnation (D, E). A, B. Ventral views of typical individuals, showing the buccal cavity and food vacuoles. C. Buccal cavity and cortical granulation. D. Ventral view showing the oral and somatic infraciliature. E. Details of oral apparatus and frontal cirri (arrowheads). Scale bars: A, B, D = 100 μm.

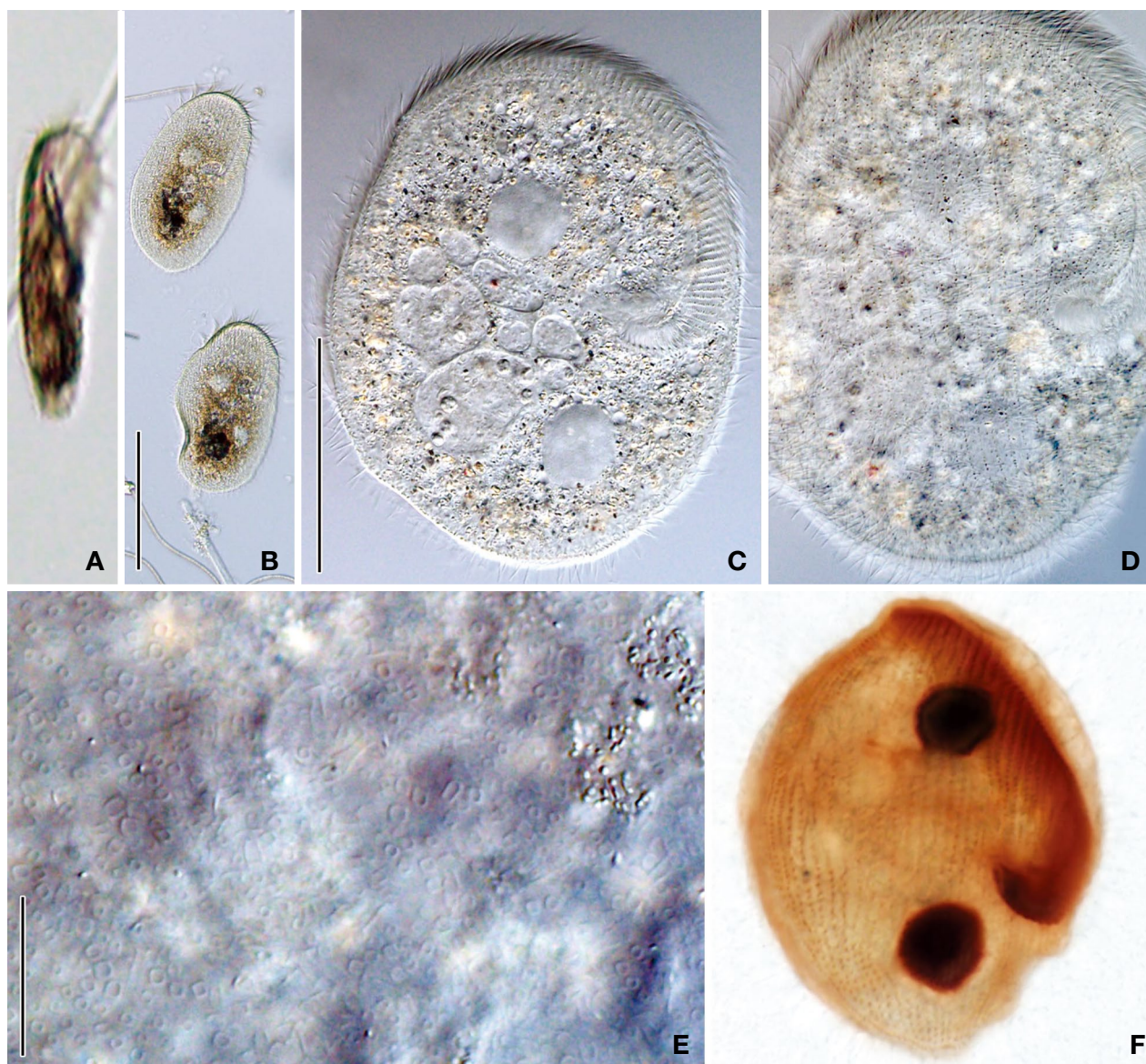


Fig. 2. *Peritromus kahli* in vivo (A–E) and after protargol impregnation (F). A. Lateral side view to show flattened body. B. Original body shapes. C. Squashed specimen showing two macronuclei. D. Cortical granules on ventral side. E. Details of peculiar chalice-like structures on dorsal side. Scale bars: B = 100 μ m; C = 50 μ m; E = 30 μ m.

Institute of Korea (MABIK PR00043127).

Class Protocruzidea Gao, Warren, Zhang, Gong, Miao, Sun, Xu, Huang, Yi and Song, 2016
 Order Protocruziida Jankowski, 1980
 Family Protocruzidae Jankowski, 1980
 Genus *Protocruzia* Faria, da Cunha and Pinto, 1922

3. *Protocruzia labiata* Kahl, 1932 (Fig. 3)

Material examined. Brackish water (salinity 12.5‰) collected from Taehahang Port, Taeha-ri, Seo-myeong, Ulleung-gun, Gyeongsangbuk-do, Korea (37°30'45.72"N,

130°47'44.76"E) on 30 August 2018.

Diagnosis. Body size 40–50 \times 15–20 μ m in vivo; slightly contractile; elliptical to ovoid with rounded posterior end; bipartite adoral zone of membranelles recognizable only in vivo, continuous after protargol impregnation; extrusomes scattered throughout the whole body, about 3–5 μ m in length; cortical granules sparsely arranged and 0.3–0.4 μ m in size; blood cell-like cytoplasmic granules on right side and about 1.2 μ m in diameter; 8–11 somatic kineties including loosely arranged single somatic kinety on left side; 6–8 adoral membranelles; paroral membrane curved and composed of dikinetids; single macronuclear complex; one tiny micronucleus near macronucleus.

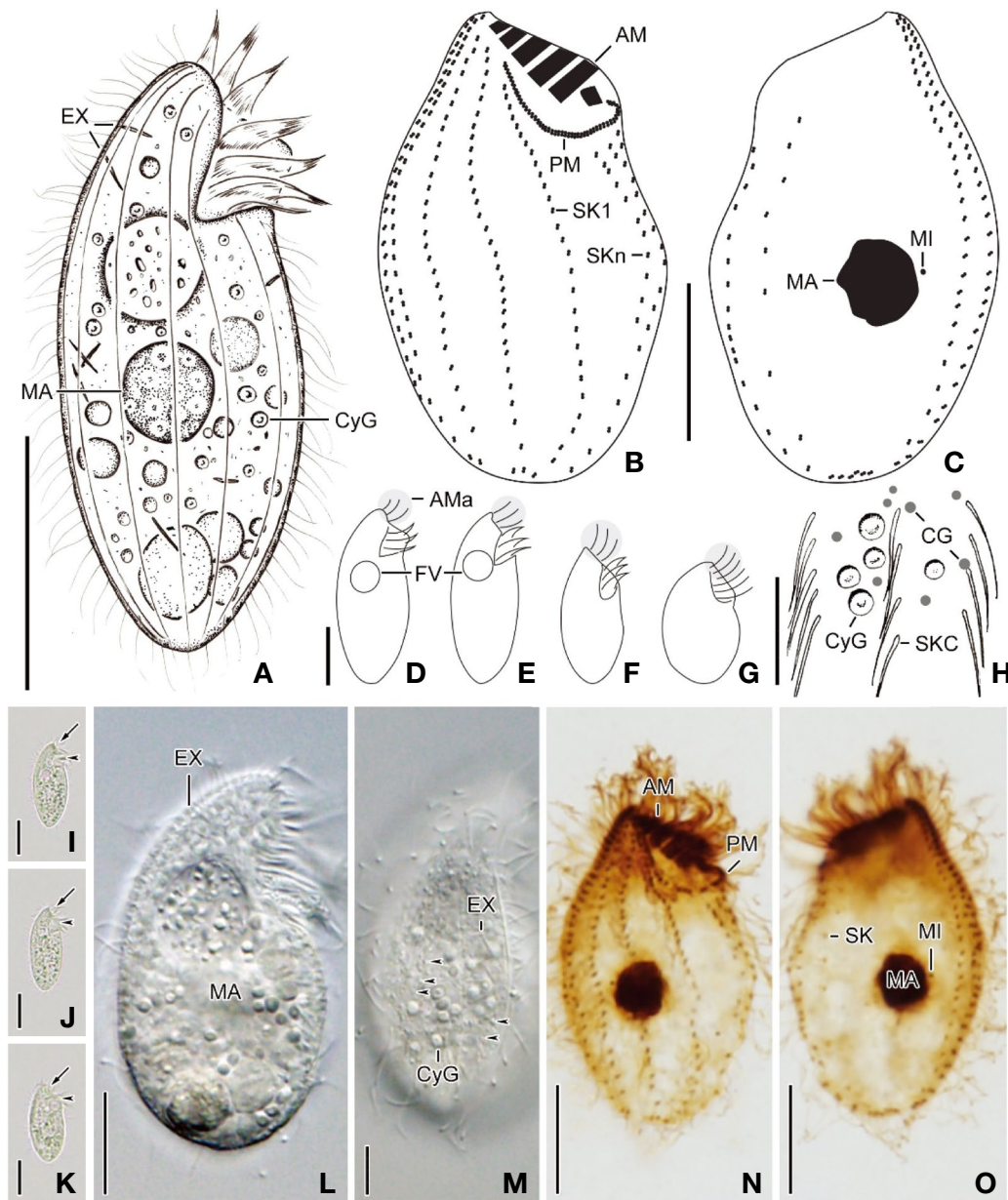


Fig. 3. *Protocruzia labiata* in vivo (A, D–M) and after protargol impregnation (B, C, N, O). A. Typical individual showing body shape and extrusomes. B, C. Right and left side view showing somatic, oral infraciliature, and nuclear apparatus. D–G. Various body shapes, gray circles denote anterior portion of adoral membranelles, specimen in (G) slightly squashed. H. Schematic drawing of cortical and cytoplasmic granules. I–K. Various body shapes (arrows and arrowheads indicate anterior and posterior portion of AM, respectively). L, M. Right side views showing extrusomes, cortical granules (arrowheads), and cytoplasmic granules (blood cell-like shaped granules). N, O. Right and left side view showing somatic, oral infraciliature, and nuclear apparatus. AM, adoral membranelles; AMa, adoral membranelles on anterior portion; CG, cortical granules; CyG, cytoplasmic granules; EX, extrusomes; FV, food vacuoles; MA, macronuclei; MI, micronuclei; PM, paroral membrane; SK1-n, somatic kinety1-n; SKC, somatic dikinetid cilium. Scale bars: A, D, I–K = 20 μ m; C, L, N, O = 10 μ m; H, M = 5 μ m.

Distribution. Korea and Germany.

Remarks. *Protocruzia labiata* Kahl, 1932 is one of the rarely known species in *Protocruzia* group. It was characterized by the bipartite adoral zone of membranelles (Kahl, 1932). However, the bipartite adoral zone of membranelles can be recognized only in live observations.

According to Kahl (1932), the body shape of the squashed cells became similar to that of *P. contrax*. The Korean population corresponded with the original description in this view. However, the nuclear apparatus and the cortical granulation differ from those of the type population, which has an inconspicuous nuclear apparatus and corti-

cal granules easily confused with the cytoplasmic granules (Kahl, 1932).

Voucher slides. One slide with protargol-impregnated specimens was deposited at National Institute of Biological Resources (NIBRPR0000109484) and two slides (GUC001588, GUC001591) were deposited in the National Marine Biodiversity Institute of Korea (MABIK).

Class Spirotrichea Bütschli, 1889
Order Stichotrichida Fauré-Fremiet, 1961
Family Amphisiellidae Jankowski, 1979
Genus *Hemiamphisiella* Foissner, 1988

4. *Hemiamphisiella granulifera* (Foissner, 1987)

Foissner, 1988 (Fig. 4)

Material examined. Terrestrial moss collected from Mt. Odaesan, Yeongok-myeon, Ganungneung-si, Gangwon-do, Korea (37°44'8"N, 128°35'16"E) on 16 December 2017.

Diagnosis. Body size about 120 × 30 μm after protargol impregnation; body elongated elliptical shape; 2 ellipsoidal macronuclear nodules; 1 spherical micronucleus; 29–36 adoral membranelles; 3 frontal cirri; 1 buccal cirrus; 40–46 cirri in amphisiellid median cirral row; about 5 cirri in right ventral row; transverse cirri lacking; 2(?) caudal cirri; 3 dorsal kineties.

Distribution. Korea, Kenya, and Portugal.

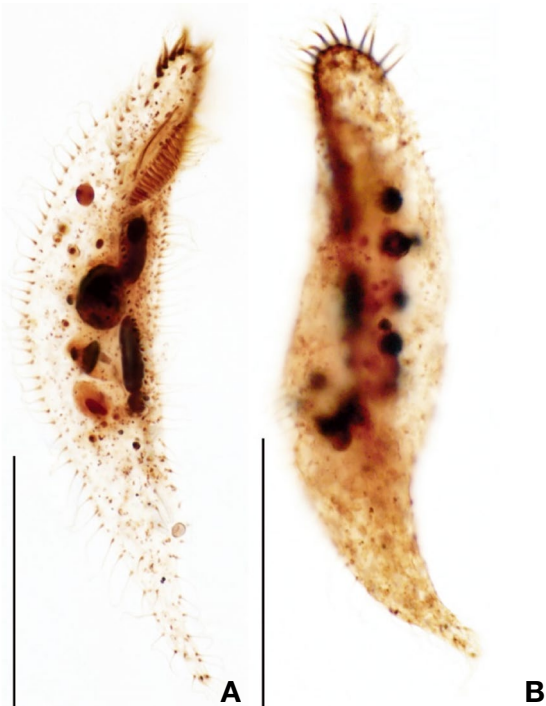


Fig. 4. *Hemiamphisiella granulifera* after protargol impregnation. A. Ventral view showing somatic, oral infraciliature and macronuclear nodules. B. Dorsal view showing dorsal kineties. Scale bars = 50 μm.

Remarks. The Korean population of *H. granulifera* corresponds with the type population, except for the number of right ventral cirri (five vs. three) (Berger, 2008). Regarding the caudal cirri, the number is seemingly two in our population as in the original description by Foissner (1987); however, as Foissner (1987) and Berger (2008) mentioned, it is difficult to distinguish from the left marginal row.

Voucher slides. Two slides of protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109478, NIBRPR0000109477).

Class Litostomatea Small and Lynn, 1981
Order Pleurostomatida Schewiakoff, 1896
Family Litostomatidae Kent, 1882
Genus *Loxophyllum* Dujardin, 1841

5. *Loxophyllum chinense* Pan, Gao, Lin, Warren and Song, 2013 (Fig. 5)

Material examined. Brackish water (salinity 3‰) collected from Taehwagang river, Samsan-dong, Nam-gu, Ulsan-si, Korea (35°32'55"N, 129°20'12"E) on 26 September 2018.

Diagnosis. Body size 110–130 × 30–45 μm in vivo; body shape lanceolate; highly contractile; cytoplasm colorless; extrusomes ventrally scattered, size about 6.0–7.0 × 0.5 μm; about 7–9 warts on dorsal side; 2 or 3 contractile vacuoles located subterminal to ventral side; 1 micronucleus in between 2 macronuclei; 12 or 13 right and 5 or 6 left somatic kineties; 3 perioral kineties; 2 left side furrows present; brackish water habitat.

Distribution. Korea and China.

Remarks. *Loxophyllum* is a speciose taxon with more than 60 nominal species (Pan *et al.*, 2013a). The Korean population of *Loxophyllum chinense* differs from the type population only in the habitat salinity (3‰ vs. ca. 20‰) (Pan *et al.*, 2013a). *L. chinense* can be distinguished from the most similar congener, *L. perihoplophorum* Buddenbrock, 1920, in the body length (110–130 μm vs. about 400 μm) and the location of contractile vacuoles (ventral vs. dorsal) (Kim and Min, 2015).

Voucher slides. Two slides of protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109489, NIBRPR0000109490).

Class Phyllopharyngea de Puytorac *et al.*, 1974
Order Dysteriida Deroux, 1976
Family Hartmannulidae Poche, 1913
Genus *Trochilioides* Chen *et al.*, 2011

6. *Trochilioides recta* (Kahl, 1928) Chen *et al.*, 2011 (Fig. 6)

Material examined. Brackish water (salinity 3‰) collec-

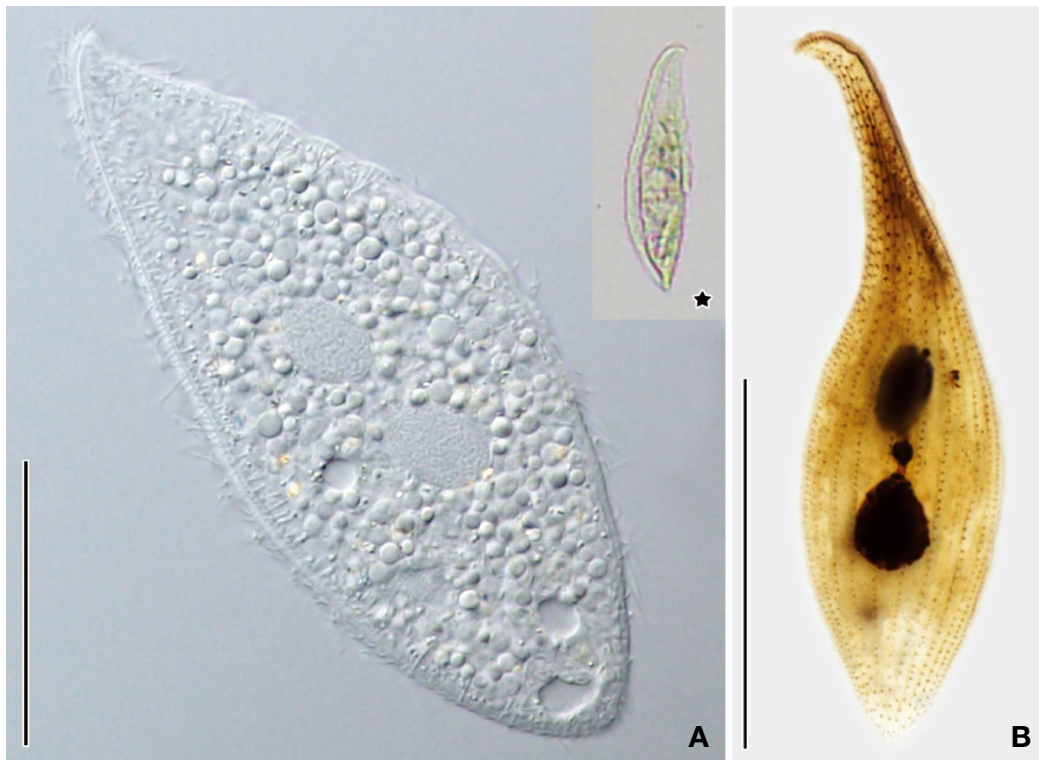


Fig. 5. *Loxophyllum chinense* in vivo (A) and after protargol impregnation (B). A. Left side view showing body shape, warts on dorsal side, and contractile vacuoles. Asterisk indicates original body shape under low magnification. B. Right side view showing somatic kineties. Scale bars = 50 μ m.

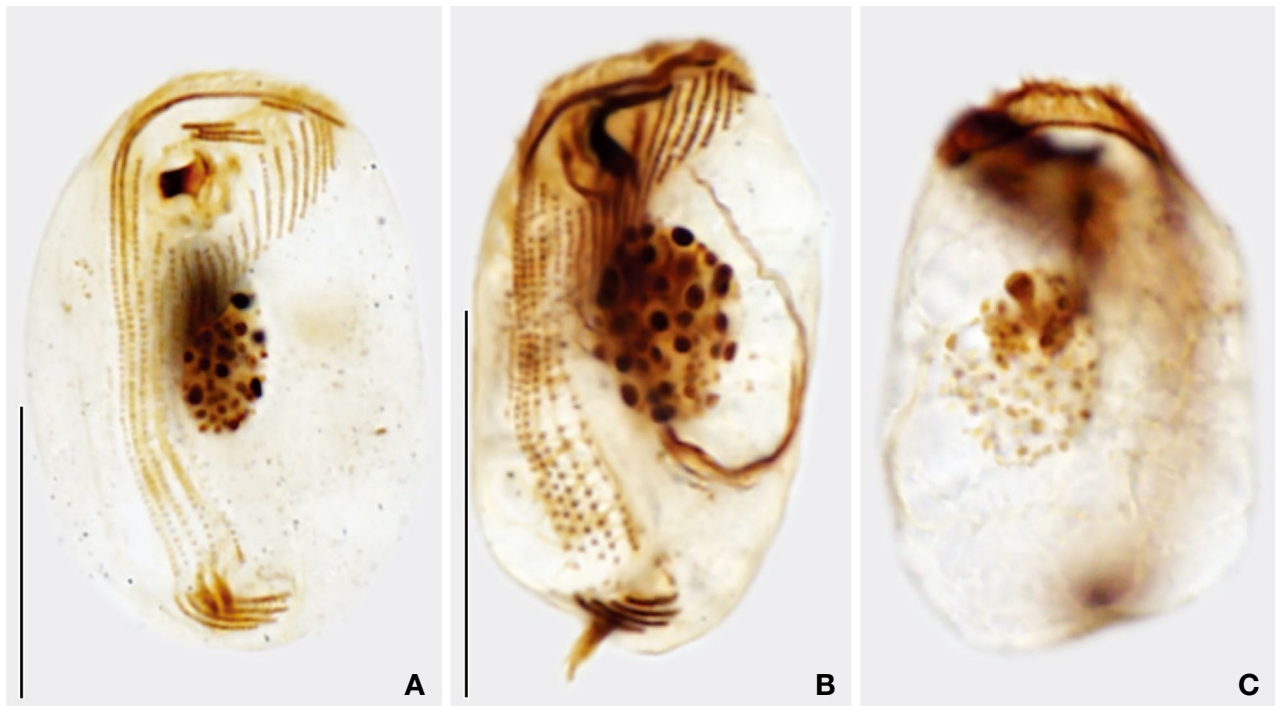


Fig. 6. *Trochiloides recta* after protargol impregnation. A, B. Ventral views showing somatic kineties, three rows of circumoral kineties, macronucleus, and the strongly curved cytopharyngeal basket. C. Dorsal view showing dorsally extended right kinety. Scale bars = 20 μ m.

ted from Taehwagang river, Samsan-dong, Nam-gu, Ulsan-si, Korea (35°32'55"N, 129°20'12"E) on 26 September 2018.

Diagnosis. Body size 31–42 × 21–27 μm after protargol impregnation; body shape oval; cytostome in anterior 1/5 of cell; 6 nematodesmal rods; 5 right kineties; 7–9 left kineties; 5 or 6 postoral kineties; 2 rightmost kineties extending to anterior left side; 2 or 3 circumoral kineties; 4 terminal fragments arranged almost horizontally; and single ellipsoidal macronucleus.

Distribution. Korea, Austria, China, Germany, and North America.

Remarks. The Korean population of *Trochilioides recta* is peculiar in the number of circumoral kineties (two or three). Of the specimens examined, only one specimen had three circumoral kineties, but the number of circumoral kineties are almost invariable in the specific level (Pan *et al.*, 2013b).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109480, NIBRPR0000109481).

Class Oligohymenophorea de Puytorac *et al.*, 1974
Order Philasterida Small, 1967
Family Cohnilembidae Kahl, 1933
Genus *Cohnilembus* Kahl, 1933

7. *Cohnilembus verminus* (Müller, 1786) Kahl, 1933 (Fig. 7)

Material examined. Mud flat with marine water (salinity 24‰) collected from Sunam-ri, Goseong-gun, Gyeongsangnam-do, Korea (34°57'40"N, 128°19'03"E) on 25 September 2018.

Diagnosis. Body size 58–112 × 7–8 μm after protargol impregnation; spindle-like body shape; buccal field narrow and length about 40–60% of body length; single triangular to ellipsoidal macronucleus; 10 or 11 somatic kineties; of membranelle 1 consists of a single row and occupies about 80% of buccal field, membranelles 2 and 3 close to each other and near posterior part of buccal area.

Distribution. Cosmopolitan.

Remarks. Of the genus *Cohnilembus*, *C. verminus* is the only species described using standard methods (Song, 2000; Hu *et al.*, 2009). The Korean population corresponds with the Chinese population morphologically by the slender body shape, the oral and somatic ciliary pattern, and the habitat (Hu *et al.*, 2009).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109485, NIBRPR0000109486) and one slide was deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00043191).

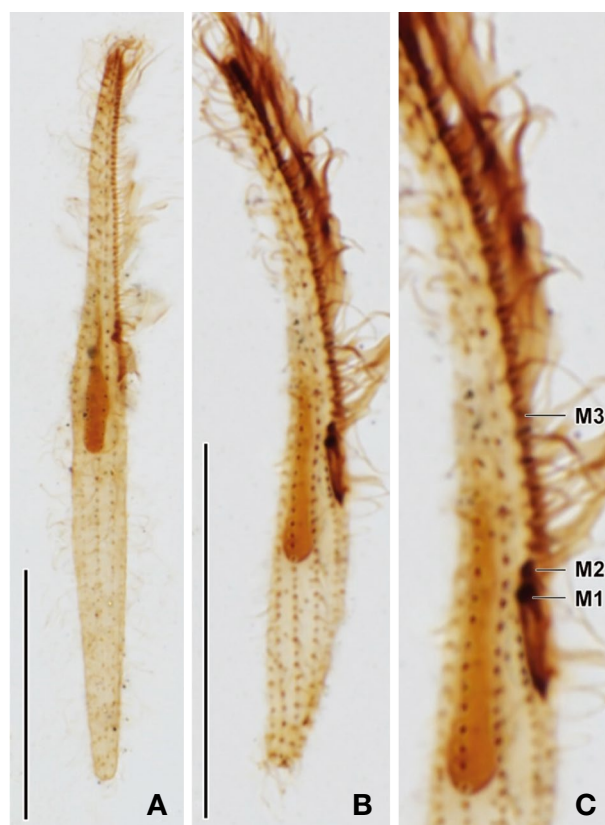


Fig. 7. *Cohnilembus verminus* after protargol impregnation. A, B. Right side views showing somatic, oral infraciliature, and nuclear apparatus. C. right side view showing details of buccal field. M1–3, membranelle 1–3. Scale bars = 30 μm.

Family Uronematidae Thompson, 1964
Genus *Uronemella* Song and Wilbert, 2002

8. *Uronemella filificum* (Kahl, 1931) Song and Wilbert, 2002 (Fig. 8)

Material examined. Marine water (salinity 24‰) collected from Cheonbuhang Port, Cheonbu-ri, Buk-myeon, Ulleng-gun, Gyeongsangbuk-do, Korea (37°32'27"N, 130°52'22"E) on 30 August 2018.

Diagnosis. Body size about 30 × 15 μm in vivo; body shape obpyriform to broadly oval; frontal end slightly oblique; cytostome slightly beyond mid-body; single globular macronucleus; spindle shape extrusomes rarely observed about 2.5 × 0.6 μm; cytoplasm colorless; single contractile vacuole at posterior end; 21–23 somatic kineties; caudal cilium about 13 μm long; membranelle 1 consists of a long right row and a shorter left row; membranelle 2 and 3 close to each other, each consists of 3 rows of basal bodies.

Distribution. Cosmopolitan.

Remarks. *Uronemella filificum* is similar to *U. para-*

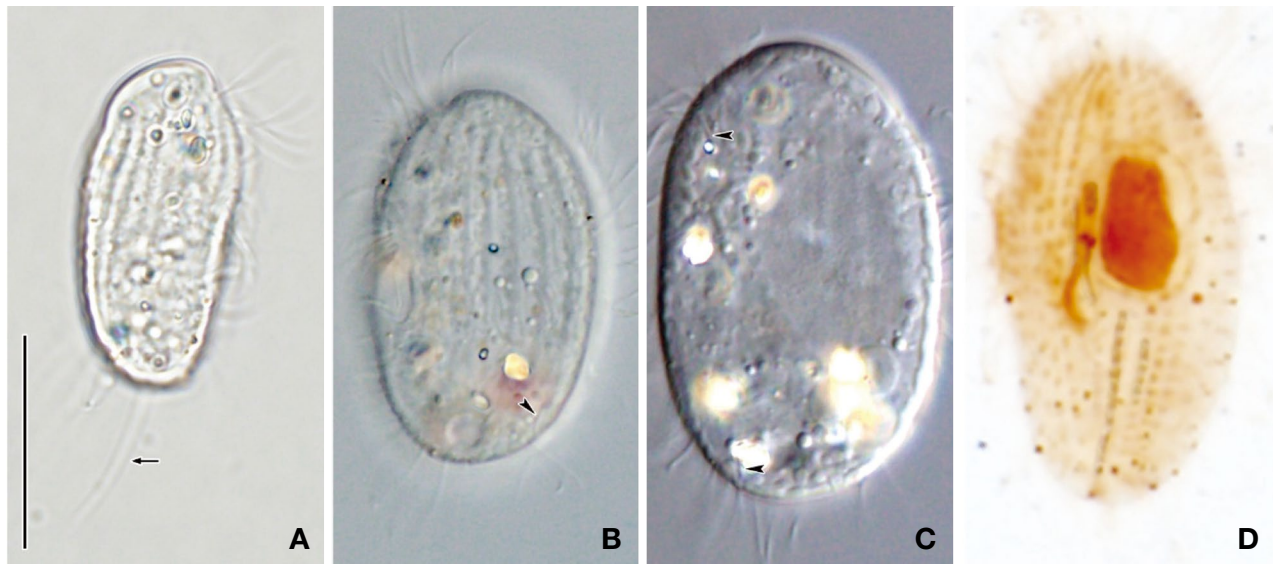


Fig. 8. *Uronemella filificum* in vivo (A–C) and after protargol impregnation (D). A. Right side view showing body shape (arrow indicates caudal cilium). B, C. Left side views showing oral apparatus, macronucleus, and extrusomes (arrowheads). D. Ventral view showing oral, somatic infraciliature, and nuclear apparatus. Scale bar = 20 μ m.

filificum Gong *et al.*, 2007 but they differ mainly in the number of somatic kineties (21–23 vs. 16 or 17) and the absence (vs. presence) of the elongated, posteriorly located somatic cilia (Gong *et al.*, 2007; Hu *et al.*, 2009).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at National Institute of Biological Resources (NIBRPR0000109487, NIBRPR0000109488) and four slides were deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00043192–PR00043195).

ACKNOWLEDGEMENTS

We are grateful to Dr. Atef Omar for improving this manuscript. This study was supported by grants from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (ME) of the Republic of Korea (NIBR201801202), the Marine Biotechnology Program of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) (No. 20170431).

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Submitted: August 22, 2019

Revised: September 17, 2019

Accepted: September 24, 2019