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New records of nine ciliates (Protozoa: Ciliophora) from Korea: Brief descriptions and remarks

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Based on morphological observation, we identified nine ciliates previously unrecorded in Korea, *Euplotidium itoi* Ito, 1958, *Anteholosticha verrucosa* (Foissner & Schade, 2000) Berger, 2008, *Holostichides dumonti* Foissner, 2000, *Amphisiella australis* Blatterer & Foissner, 1988, *Terricirra matsusakai* Berger & Foissner, 1989, *Uroleptoides binucleatus binucleatus* Hemberger, 1985, *Uroleptoides magnigranulosus* (Foissner, 1988) Berger, 2008, *Leptopharynx costatus gonohymen* Omar & Foissner, 2012, *Pseudochilodonopsis mutabilis* Foissner, 1981, from three sites in Korea, which were isolated from three environmental samples, consisting of one seawater and two soil samples. These ciliates were assigned to three classes, five orders, five families, and eight genera. In this study, we provide photographs and brief descriptions and remarks of the nine species.

Keywords: morphology, protargol-impregnated specimen, South Korea, unrecorded ciliates

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Introduction

Since the study of Korean ciliates began, around 420 species have been reported in South Korea (Dovgal and Pešić, 2014; Jung *et al*., 2017b; Kim and Jung, 2018; Park *et al*., 2017). This number constitutes only about 10% of the total number of free-living ciliate species estimated worldwide in 2008 (Foissner, 2008). Thus, many Korean species are likely still awaiting discovery. From a project to discover Korean indigenous species, we identified nine previously unrecorded ciliate species from South Korea. Herein we provide and morphological descriptions of these nine species.

Materials and Methods

All samples were cultured at room temperature (18-20°C). For soil samples, we used the non-flooded Petri dish culture method (Foissner *et al*., 2002). For seawater, samples were filtered with 200 μm nylon mesh before transferring to Petri dishes. Rice grains were added to enrich the growth of bacteria and bacteriovorous flagellates.

Both living and stained specimens were observed using a stereo microscope (SZH10; Olympus, Tokyo, Japan) and light microscope (DM2500; Leica, Wetzlar, Germany) at magnifications ranging from ×50 to ×1,000. Protargol impregnation was performed according to method of Foissner (2014). Classification and terminology follow Berger (2006; 2008), Foissner (1981), Lynn (2008), and Omar and Foissner (2012).

Results and Discussion

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Euplotida Small & Lynn, 1985
Family Gastrocirrhidae Fauré-Fremiet, 1961
Genus *Euplotidium* Noland, 1937

1. *Euplotidium itoi* Ito, 1958 (Fig. 1)

Material examined. Seawater (salinity 30.7‰) taken from Lake Yeongnangho, Geumho-dong, Sokcho-si, Gangwon Province, South Korea (38°12′58″N 128°34′25″E) on June 2012.
Diagnosis. Cell size 55-80×30-45 μm in protargol preparations, rigid, bell-shaped; 56-61 adoral zone of membranelles; 2 elongated macronucleus nodules; 12 frontoventral cirri; 6 transverse cirri; 1 left marginal cirrus; 5 dorsal kineties.
Remarks. Regarding its congener in terms of the cirral

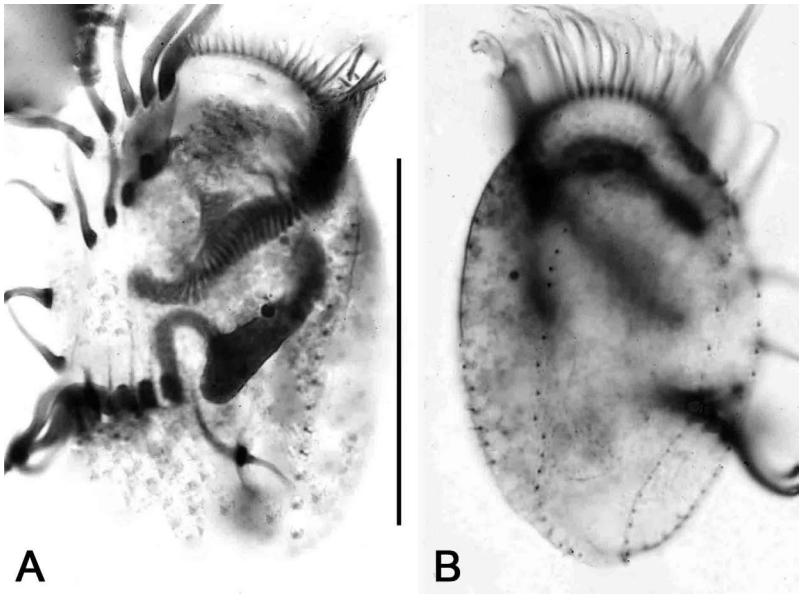

Fig. 1. Photomicrographs of *Euplotidium itoi* from after protargol impregnation (A, B). (A) Ventral view. (B) Dorsal view. Scale bar = 50 μm.

pattern on the ventral and dorsal sides, *Euplotidium itoi* should be compared with two congeners, *E. arenarium* and *E. rosati*. *Euplotidium arenarium* can be distinguished from *E. itoi* based on the number of frontal cirri (10 vs. 12), the number of transverse cirri (5 vs. 6), and the number of macronucleus nodules (5-10 vs. 2). *Euplotidium rosati* has similar ventral ciliature to *E. itoi*. However, the former species differs from *E. itoi* in the left marginal cirrus (absent vs. present) (Ito, 1958; Lei *et al*., 2002; Modeo *et al*., 2013).

Deposition. One voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109444).

Order Urostylida Jankowski, 1979
Family Urostylidae Bütschli, 1889
Genus *Anteholosticha* Berger, 2003

2. *Anteholosticha verrucosa* (Foissner & Schade, 2000) Berger, 2008 (Fig. 2)

Material examined. Soil sample taken from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E) on February 2018.

Diagnosis. Cell size 60-70 × 15-20 μm in protargol preparations, elongated ellipsoidal, flexible; adoral zone 27% of body length; contractile vacuole at left mid-body; conspicuous cortical granules, colorless, arranged around dorsal bristles; 11-14 macronuclear nodules with 2 or 3 micronuclei; 3 frontal and 2 frontoterminal cirri; 1 buccal cirrus; 8-10 cirri in midventral complex slightly zigzagging; 3 or 4 transverse cirri; 1 left (14-16 cirri) and 1 right (15-18 cirri) marginal cirral row; 4 dorsal kineties (including dorsal marginal kineties) with 2 basal bodies in kinety 4; caudal cirri lacking.

Remarks. The Korean population is highly similar to the populations in Tenerife (type) and Berlin based on the number and the disposition of cirri and macronuclear nodules, though it differs from other populations based on the pretransverse ventral cirrus (absent vs. present) (Berger, 2008).

Deposition. Two voucher slides with protargol-impregnated specimens are deposited in the National Institute of Biological Resources in Korea (NIBRPR000010445, NIBRPR000010446).

Genus *Holostichides* Foissner, 1987

3. *Holostichides dumonti* Foissner, 2000 (Fig. 3)

Material examined. Soil sample taken from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E) on February 2018.

Diagnosis. Cell size 130-140 × 30-35 μm in protargol preparations, elongated ellipsoidal, flexible; adoral zone about 30% of body length, 36-40 adoral membranelles; 60-62 macronuclear nodules; contractile vacuole at left mid-body; cortical granules colorless, arranged as longitudinal rows; 3 frontal and 2 frontoterminal cirri; 1 buccal cirrus; about 15 cirri in midventral pairs; 2 or 3 additional midventral row; transverse cirri lacking; 1 left (47-49 cirri) and 1 right (44-47 cirri) marginal cirral

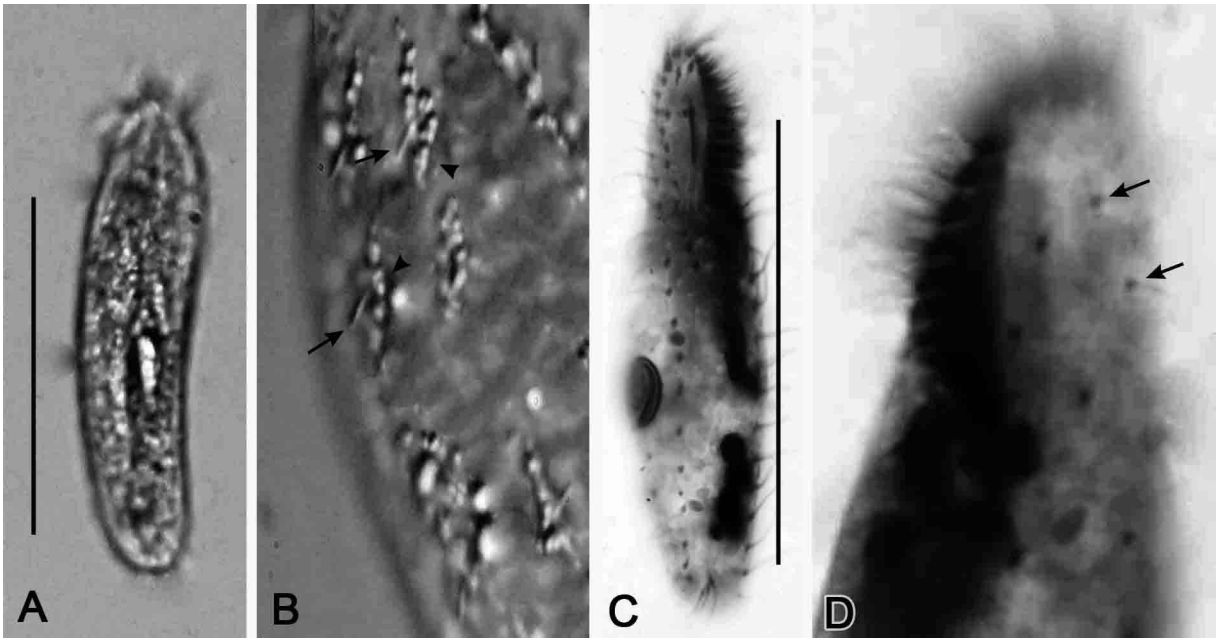

Fig. 2. Photomicrographs of *Anteholosticha verrucosa* from life (A, B) and after protargol impregnation (C, D). (A, C) Ventral views. (B) Dorsal cilia (arrows) and cortical granules (arrowheads) on dorsal surface. (D) Arrows indicate basal bodies on dorsal surface. Scale bars = 50 μm.

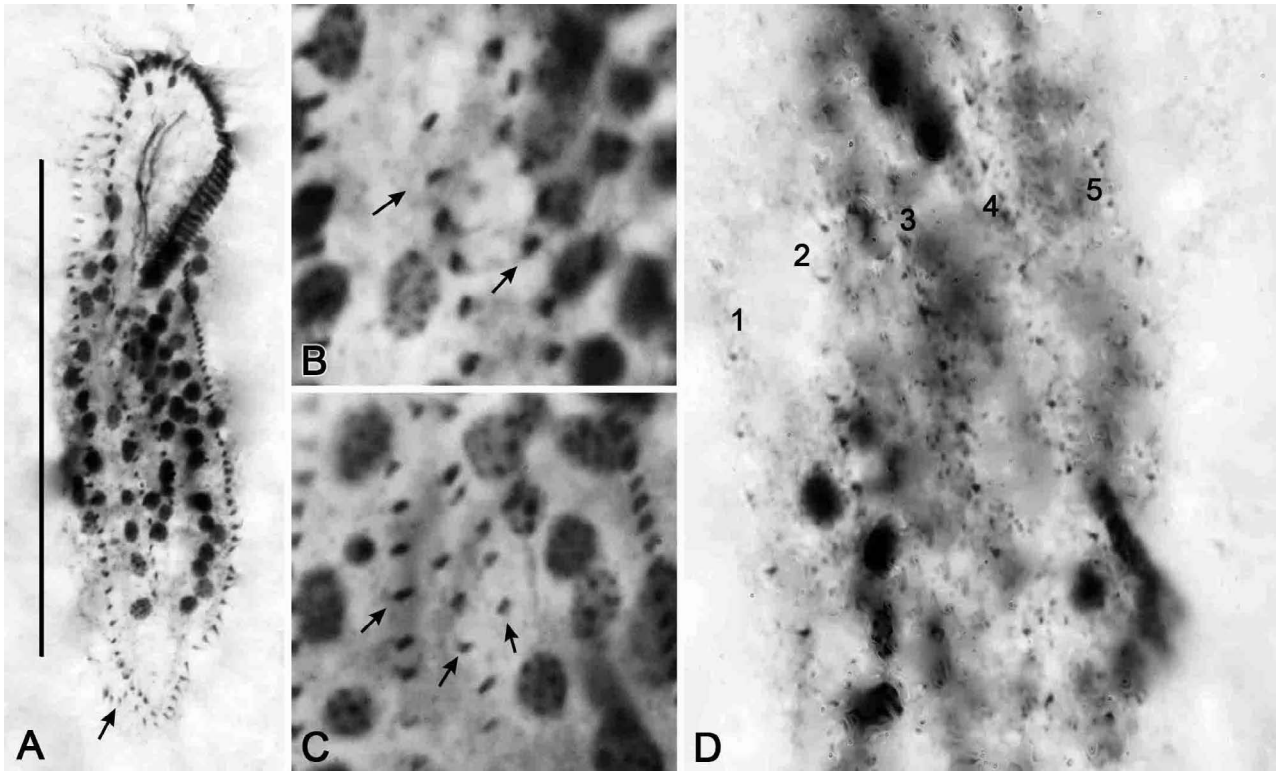

Fig. 3. Photomicrographs of *Holostichides dumonti* from after protargol impregnation (A-D). (A) Arrow indicates caudal cirri. (B, C) Arrows indicate midventral cirral rows. (D) Dorsal view. 1-5, dorsal kineties. Scale bar = 100 μm.

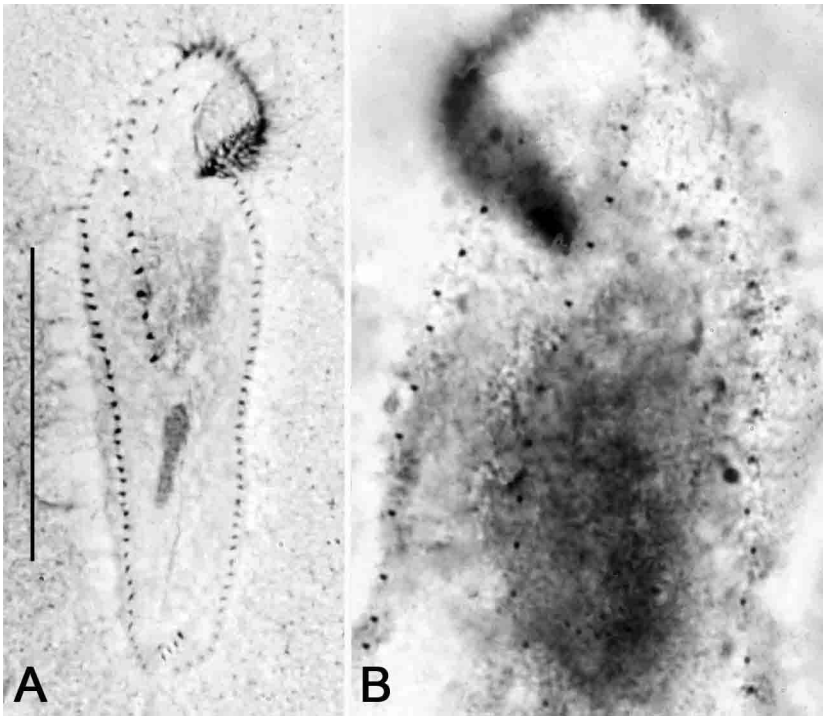

Fig. 4. Photomicrographs of *Amphisiella australis* from after protargol impregnation (A, B). (A) Ventral view. (B) Dorsal view. Scale bar = 50 μm.

row; invariably 5 dorsal kineties.

Remarks. The Korean population follows most morphological features of the Finnish (type) population, though the former only differs from the latter by the number of macronuclear nodules (60-84 vs. 100-150) (Berger, 2006). In comparison with its congeners, *H. terrae* can be separated from *H. dumonti* by the number of midventral rows (2 or 3 vs. 2) and the rod-shaped structure in the wall of the pharynx (present vs. absent) (Jung *et al*., 2017a). The other *Holostichides* species (*H. chardezi*, *H. heterotypicus* and *H. typicus*) have a shorter undulating membrane compared to *H. dumonti* (Kim *et al*., 2017).

Deposition. Three voucher slides with protargol-impregnated specimens are deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109441-NIBRPR0000109443).

Order Stichotrichida Fauré-Fremiet, 1961
Family Amphisiellidae Jankowski, 1979
Genus *Amphisiella* Gourret & Roeser, 1888

4. *Amphisiella australis* Blatterer & Foissner, 1988 (Fig. 4)

Material examined. Soil sample taken from Bangeo-dong, Ulsan, South Korea (35°28′56″N, 129°25′54″E) on November 2015.

Diagnosis. Cell size in protargol preparations 95-110 × 30-35 μm, flexible, elliptical; adoral zone about 26% of body length, 22-25 adoral membranelles; contractile vacuole at slightly ahead of mid-body near left cell margin; 2 macronuclear nodules with 2 or 3 micronuclei; 3 frontal cirri; 3 or 5 cirri in left of anterior end of amphisiellid median cirral row; 13-15 cirri in amphisiellid median cirral row; 1 buccal cirrus; 5 or 6 transverse cirri; 1 left (37-40 cirri) and 1 right (41-43 cirri) marginal cirral row; 3 dorsal kineties; caudal cirri lacking.

Remarks. Morphologically, *Amphisiella australis* is most similar to *Lamtostyla australis*, although they are assigned to different genera. *Lamtostyla australis* differs from *Amphisiella australis* by the following features: number of transverse cirri (usually 2 vs. usually 5); length of amphisiellid median cirral row (38-46% of body length vs. 50%); shape of undulating membranes (slightly curved vs. distinctly curved); position of buccal cirrus (at anterior end of the paroral vs. distinctly behind the anterior end of the paroral) (Berger, 2008). However, as these features showed ambiguous differences, additional data and molecular phylogenetic analysis are needed clarification.

Deposition. One voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109447).

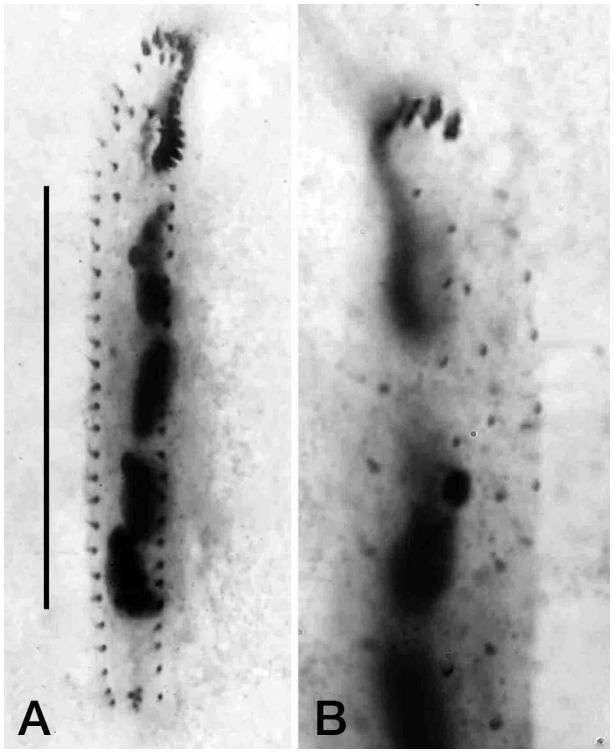

Fig. 5. Photomicrographs of *Terricirra matsusakai* from after protargol impregnation (A, B). (A) Ventral view. (B) Dorsal view. Scale bar = 50 μm.

Genus *Terricirra* Berger & Foissner, 1989

5. *Terricirra matsusakai* Berger & Foissner, 1989 (Fig. 5)

Material examined. Soil sample taken from Wangsan-ri Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E) on February 2018.

Diagnosis. Cell size about 80 × 15 μm in protargol preparations, elongated ellipsoidal body shaped, flexible; adoral zone about 25% of body length; contractile vacuole at left mid-body; 4 macronuclear nodules with 2 or 3 micronuclei; 3 frontal and 2 frontoterminal cirri; 1 buccal cirrus; 4 cirri in left of anterior end of amphisiellid median cirral row; 1 pretransverse ventral cirrus and 3 transverse cirri; 1 left (about 26 cirri) and 1 right (about 25 cirri) marginal cirral row; 4 dorsal kineties (including dorsal marginal kineties); caudal cirri lacking.

Remarks. The Korean population only differs from the type population (Japan) in pretransverse ventral cirri (present vs. absent) (Berger and Foissner, 1989). This species can be separated from *Hemisincirra quadrinucleata*, by the adoral zone pattern of membranelles (continuous vs. discontinuous) and the number of dorsal kineties (4 vs. 3) (Berger, 2008).

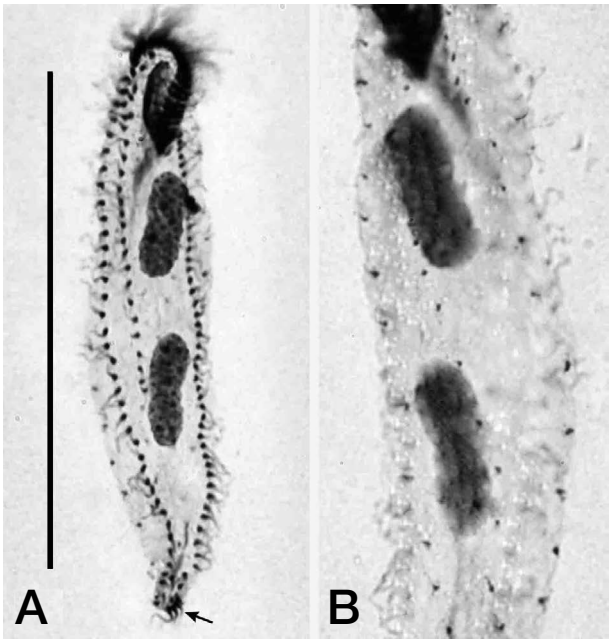

Fig. 6. Photomicrographs of *Uroleptoides binucleatus binucleatus* from after protargol impregnation (A, B). (A) Arrow indicates transverse cirri. (B) Dorsal view. Scale bar = 100 μm.

Deposition. One voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109448).

Genus *Uroleptoides* Wenzel, 1953

6. *Uroleptoides binucleatus binucleatus* Hemberger, 1985 (Fig. 6)

Material examined. Soil sample taken from Bangeo-dong, Ulsan, South Korea (35°28′56″N, 129°25′54″ E) on November 2015.

Diagnosis. Cell size 105-125 × 20-30 μm in protargol preparations, slenderly elliptical body shaped, flexible; adoral zone about 23% of body length, 22-25 adoral membranelles; contractile vacuole at slightly ahead of mid-body near left cell margin; 2 macronuclear nodules with 2 micronuclei; 3 frontal cirri1 buccal cirrus; 3 cirri left of anterior end of amphisiellid median cirral row; 20-23 cirri in amphisiellid median cirral row; 4 transverse cirri; 1 left (42-50 cirri) and 1 right (36-42 cirri) marginal cirral row; 3 dorsal kineties; caudal cirri lacking.

Remarks. Berger (2008) found a different number of cirri between the European and the Namibian populations, and suggested that *Uroleptoides binucleatus* should be divided into two subspecies *U. binucleatus binucleatus* (European population) and *U. binucleatus multicirratus* (Namibian population). The morphology

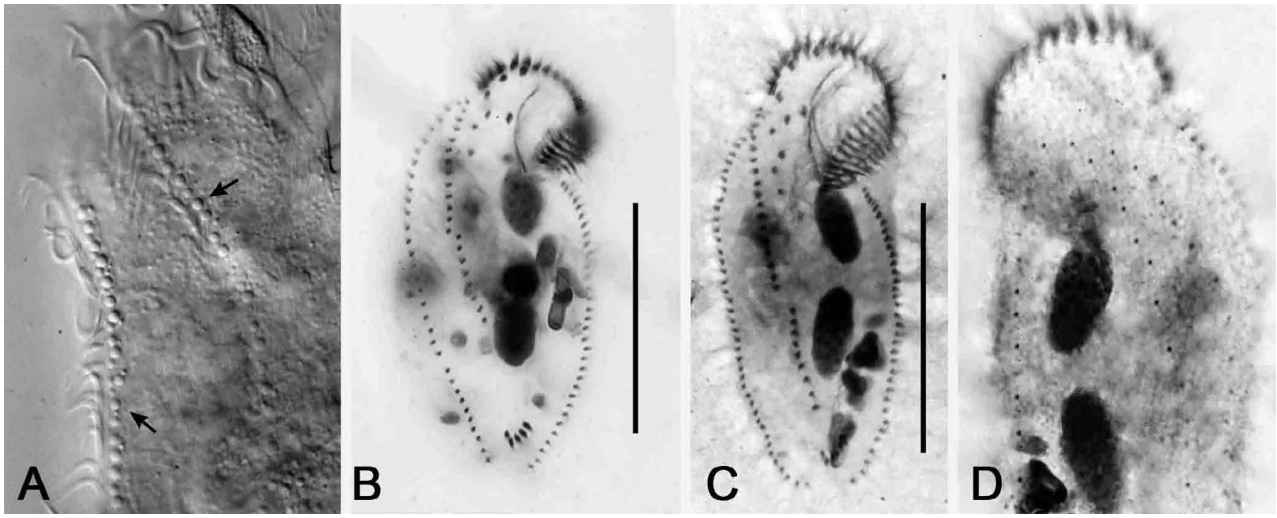

Fig. 7. Photomicrographs of *Uroleptoides magnigranulosus* from life (A) and after protargol impregnation (B-D). (A) Arrows indicates cortical granules on ventral surface. (B, C) ventral views. (D) Dorsal view. Scale bars = 50 μm.

of the Korean population follows that of *U. binucleatus binucleatus*, and mainly differs from *U. binucleatus multicirratus* by the following two features: numbers of adoral membranelles (22-25 vs. more than 38) and cirri in amphisiellid median cirral row (20-23 vs. more than 50).

Deposition. One voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109449).

7. *Uroleptoides magnigranulosus* (Foissner, 1988) Berger, 2008 (Fig. 7)

Material examined. Soil sample taken from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E) on February 2018.

Diagnosis. Cell size in protargol preparations 75-100 × 35-40 μm on average, elliptical body shaped, flexible; adoral zone about 28% of body length, 21-28 adoral membranelles; contractile vacuole at slightly ahead of mid-body near left cell margin; conspicuous cortical granules arranged near cirri, colorless; 2 macronuclear nodules with 2 micronuclei; 3 frontal cirri; 2 amphisiellid median cirral rows; 1 buccal cirrus; 3 or 5 cirri left of anterior end of amphisiellid median cirral row; 5 or 6 transverse cirri; 1 left (32-35 cirri) and 1 right (32-43 cirri) marginal cirral row; 3 dorsal kineties; caudal cirri lacking.

Remarks. The major distinguishing features of *Uroleptoides magnigranulosus* from its congeners are the following combination of two features: having distinct cortical granules and amphisiellid median cirral rows that are arranged in two parts. The morphological features of the Korean population are very similar to those of the type population (Kenya), and the former only differs from the latter by the number of cirri in amphisiellid median cirral row (21-26 vs. 12-19) (Foissner, 1988).

Deposition. Two voucher slides with protargol-impregnated specimens are deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109450, NIBRPR0000109451).

Class Nassophorea Small & Lynn, 1881
Order Microthoracida Jankowski, 1967
Family Leptopharyngidae Kahl, 1926
Genus *Leptopharynx* Mermod, 1914

8. *Leptopharynx costatus gonohymen* Omar & Foissner, 2012 (Fig. 8)

Material examined. Soil sample taken from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E) on February 2018.

Diagnosis. Cell size 30-40 × 28-35 μm in protargol preparations; 9 somatic and 4 preoral ciliary rows with an average of 183; 2 contractile vacuole; kinety 1 consisting of widely spaced dikinetids; adoral membranelle 1 absent; membranelles 2 and 3 right-angled to each other.

Remarks. Omar and Foissner (2012) found the uniqueness of the Floridian population within the *Leptopharynx costatus* and suggested its subspecies, *L. costatus gonohymen*. *Leptopharynx costatus gonohymen* can be distinguished from *L. costatus costatus* by the numbers of dikinetids (7-8 vs. 11-15) and monokinetids (1 vs. 0-2) in kinety 1, while the Korean population has 7 dik-

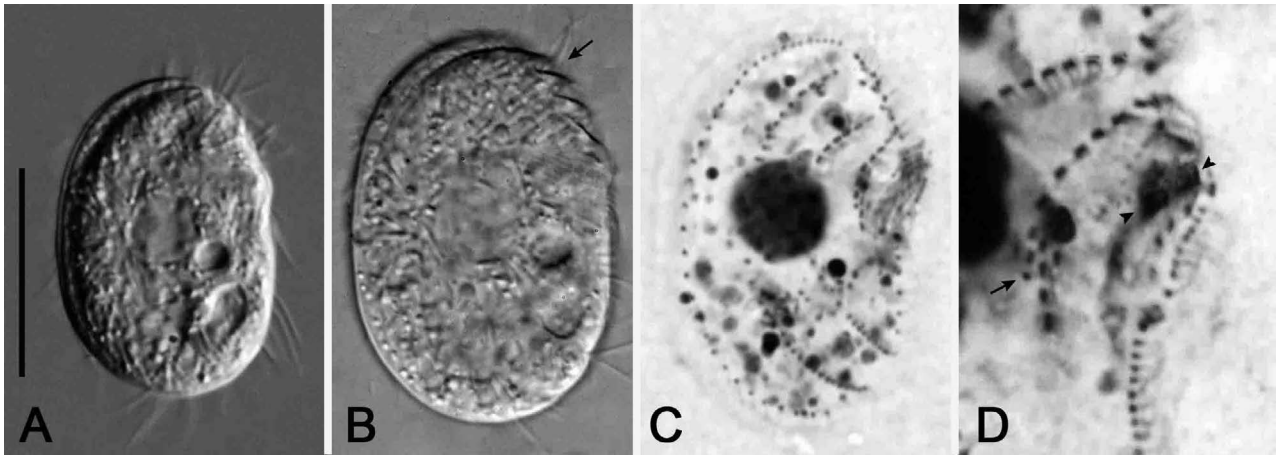

Fig. 8. Photomicrographs of *Leptopharynx costatus gonohymen* from life (A, B) and after protargol impregnation (C, D). (A) Right side view. (B) Arrow indicates preoral kineties. (C) Right side view. (D) Arrow indicates monokinetid in kinety 1, and arrowheads mark adoral membranelles. Scale bar = 20 μm.

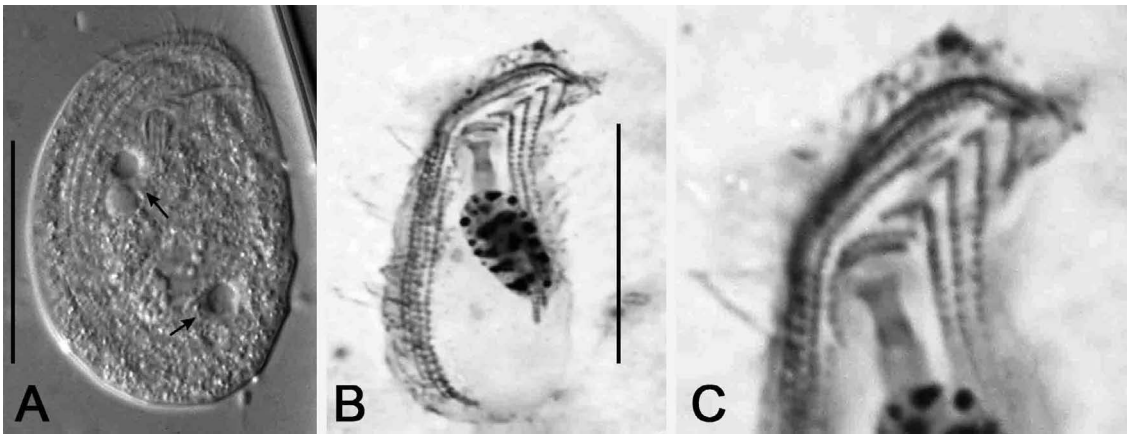

Fig. 9. Photomicrographs of *Pseudochilodonopsis mutabilis* from life (A) and after protargol impregnation (B, C). (A) Arrows indicate contractile vacuoles. (B) Ventral view. (C) Anterior portion of ventral side. Scale bars = 20 μm.

inetids and 1 monokinetid in kinety 1 (Fig. 8D) (Omar and Foissner, 2012).

Deposition. Two voucher slides with protargol-impregnated specimens are deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109452, NIBRPR0000109453).

Class Phyllopharyngea de Puytorac *et al*., 1974
Order Chlamydodontida Deroux, 1970
Family Chilodonellidae Deroux, 1970
Genus *Pseudochilodonopsis* Foissner, 1979

9. *Pseudochilodonopsis mutabilis* Foissner, 1981 (Fig. 9)

Material examined. Soil sample taken from Bangeo-dong, Ulsan, South Korea (35°28′56″N, 129°25′54″E) on November 2015.

Diagnosis. Cell size 30-40 × 20-35 μm in protargol preparations; oval body shaped, posterior end rounded; 2 contractile vacuoles; 1 macronucleus size 10 × 5 on average; 12 or 13 nematodesmal rods; 2 circumoral kineties; 5 right and 6 left somatic kineties; 4 fragments in preoral kinety.

Remarks. The Korean population of *Pseudochilodonopsis mutabilis* shows high consistency with the type (Austria) and the Chinese populations based on morphological information (contractile vacuoles and somatic kineties pattern). However, the left somatic kineties of the Korean population are shorter than the Chinese population (innermost one behind the midbody level vs. innermost one at the posterior 1/4 of cell length), and these

two population have different habitats (soil vs. brackish water) (Foissner, 1981; Qu *et al*., 2015).

Deposition. One voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000109454).

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