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Brief morphological description of stichotrichid ciliates (Ciliophora: Stichotrichia) from Korea

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Ten stichotrichid ciliates were identified from six environmental samples, consisting of one freshwater, two seawater, and three soil samples. Morphological assessments were performed based on observations of both living and stained specimens. These species were assigned to two orders, three families, and nine genera, and we provide photographs and brief descriptions and remarks for each species: *Parabirojimia similis* Hu *et al*., 2002, *Arcuseries warreni* (Song & Wilbert, 1997) Huang *et al*., 2014, *Gonostomum lajacola* Foissner, 2016, *Paragonostomoides xianicum* Wang *et al*., 2017, *Australocirrus oscitans* Blatterer & Foissner, 1988, *Monomicrocaryon crassicirratum* Foissner, 2016, *Monomicrocaryon euglenivorum fimbricirratum* Foissner, 2016, *Notohymena rubescens* Blatterer & Foissner, 1988, *Sterkiella multicirrata* Li *et al*., 2018, *Stylonychia ammermanni ammermanni* Gupta *et al*., 2001.

Keywords: freshwater, marine, soil, South Korea, stichotrich, unrecorded ciliate

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Introduction

The class Spirotrichea is one of the most species-rich group of the Ciliophora Doflein (1901) (Lynn, 2008). To resolve taxonomic ambiguities among spirotrich members, their relationships have been revised on the basis of traditional and molecular phylogenetic analyses. Of seven spirotrich subclasses, the Stichotrichia Small & Lynn, 1985 is especially difficult to study due to its high diversity and unresolved taxonomy (Schmidt *et al*., 2007; Lynn, 2008). Although stichotrichs are one of the most heavily researched ciliate groups in Korea, about one third of Korean reported, many unrecorded species are still waiting to be discovered (Dovgal and Pešić, 2014; Jung *et al*., 2017a; 2017b; 2017c; Park *et al*., 2017; Choi *et al*., 2018; Kim and Jung, 2018).

In the present study, we provide a brief diagnosis and remarks of 10 ciliate species belonging to the subclass Stichotrichia previously unrecorded in Korea. The ciliates were identified based on the observation of both live and stained specimens.

Materials and Methods

For soil samples, we used the non-flooded Petri dish culture method (Foissner *et al*., 2002). Freshwater and seawater samples were filtered with 200 μm nylon mesh and then were transferred to Petri dishes with rice or wheat grains to enrich growth of bacteria and bacteriovorous flagellates recognized as a food source for ciliates. All samples were cultured at 18-20°C.

For species identifications, Protargol impregnation was performed according to Foissner (2004). Living and stained specimens were observed under a stereo microscope (SZH10; Olympus, Tokyo, Japan) and a light microscope (DM2500; Leica, Wetzlar, Germany) at magnifications ranging from 50× to 1,000×. Classification and terminology follow Berger (2006), Foissner (2016), Kumar and Foissner (2015), and Huang *et al*. (2014).

Results and Discussion

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Urostylida Jankowski, 1979
Family Urostylidae Bütschli, 1889
Genus *Parabirojimia* Hu *et al*., 2002

1. *Parabirojimia similis* Hu *et al*., 2002 (Fig. 1)

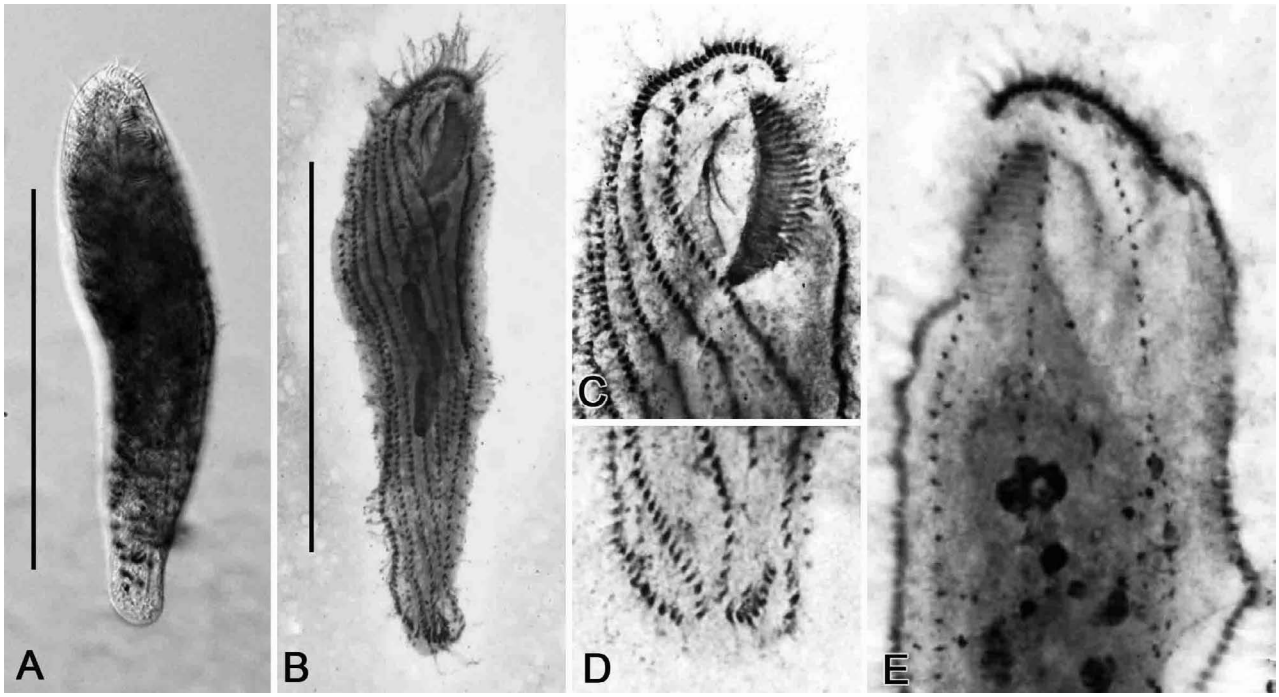

Fig. 1. Photomicrographs of *Parabirojimia similis* from life (A) and after protargol impregnation (B-E). (A-D) Ventral views. (E) Dorsal view. Scale bars = 100 μm.

Material examined. Seawater (32‰) from Oho breakwater, Gangwon Province, Korea (38°19′33″N, 128°31′47″E), on June 2018.
Diagnosis. Cell size 130-155 × 40-55 μm in protargol preparations, body outline roughly elongated elliptical, widest in anterior body third, left and right margin converging posteriorly, and flexible; adoral zone 25% of body length, 37-54 adoral membranelles, bipartite; 4 or 5 macronuclear nodules with about 3 micronuclei; 3 frontal and 2 frontoterminal cirri; 1 buccal cirrus; 5-7 cirri in midventral pairs with 37-48 additional midventral cirri; 5-8 transverse cirri; 1 left and 5 or 6 right marginal cirral rows; 3 dorsal kineties; caudal cirri lacking.
Remarks. Two described *Parabirojimia* species, *P. multinucleata* and *P. similis*, are different by the following combination of features: numbers of macronuclear nodules (43-70 vs. 3-6), right marginal cirral rows (invariably 5 vs. 5-8), ventral cirri (44-69 vs. 27-45), and adoral membranelles (52.4 vs. 46.7 on average) (Chen *et al*., 2010). The Korean population of *P. similis* corresponds very well with the Chinese population (Berger, 2006).
Deposition. Four slides (NIBRPR0000109455-NIBRPR0000109458) have been deposited at the National Institute of Biological Resources (NIBR), Korea.

Genus *Arcuseries* Huang *et al*., 2014

2. *Arcuseries warreni* (Song & Wilbert, 1997) Huang *et al*., 2014 (Fig. 2)

Material examined. Seawater (28.8 ‰) from Incheon Port, Korea (37°26′57″N, 126°35′37″E), on May 2018.
Diagnosis. Cell size 65-80 × 25-30 μm in protargol preparations, oval shape, and flexible; adoral zone 32% of body length, 28-30 adoral membranelles; 43-60 macronuclear nodules; conspicuous cortical granules arranged around dorsal bristles, erythrocytes-like granules densely distributed in cytoplasm; 3 frontal and 2 frontoterminal cirri; 1 buccal cirrus; 7-9 midventral pairs; 10-12 transverse cirri and 2 pretransverse ventral cirri; 1 left (20-25 cirri) and 1 right (20-27 cirri) marginal cirral row; 3 dorsal kineties; hair-like ejected granules present; caudal cirri lacking.
Remarks. *Arcuseries warreni* can be easily distinguished from other *Arcuseries* species by conspicuous erythrocyte-like granules and hair-like ejected granules. In the Korean population of *A. warreni*, cortical granules were mainly arranged along with cirri, and around dorsal bristles (Fig 2B, C).
Deposition. Two slides (NIBRPR0000109459, NIBRPR0000109450) have been deposited at the National Institute of Biological Resources (NIBR), South Korea.

Order Sporadotrichida Fauré-Fremiet, 1961
Family Gonostomatidae Small & Lynn, 1985
Genus *Gonostomum* Sterki, 1878

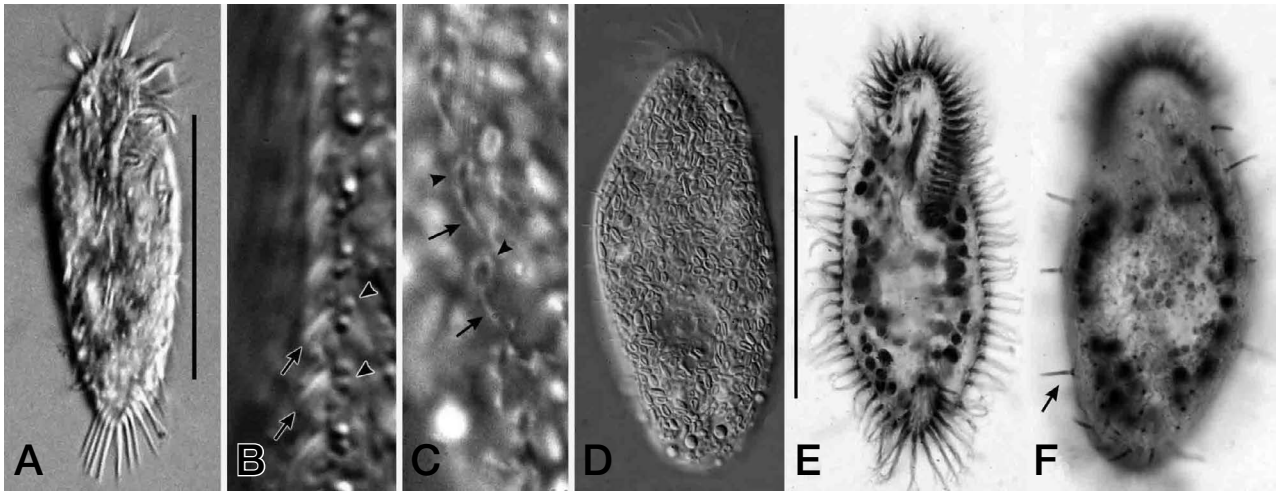

Fig. 2. Photomicrographs of *Arcuseries warreni* from life (A-D) and after protargol impregnation (E, F). (A) Ventral view. (B) Cirri (arrows) and cortical granules (arrowheads) on ventral surface. (C) Bristles (arrows) and cortical granules (arrowheads) on dorsal surface. (D) Erythrocytes-like granules. (E) Arrow indicates hair-like ejected granules. Scale bars = 50 μm.

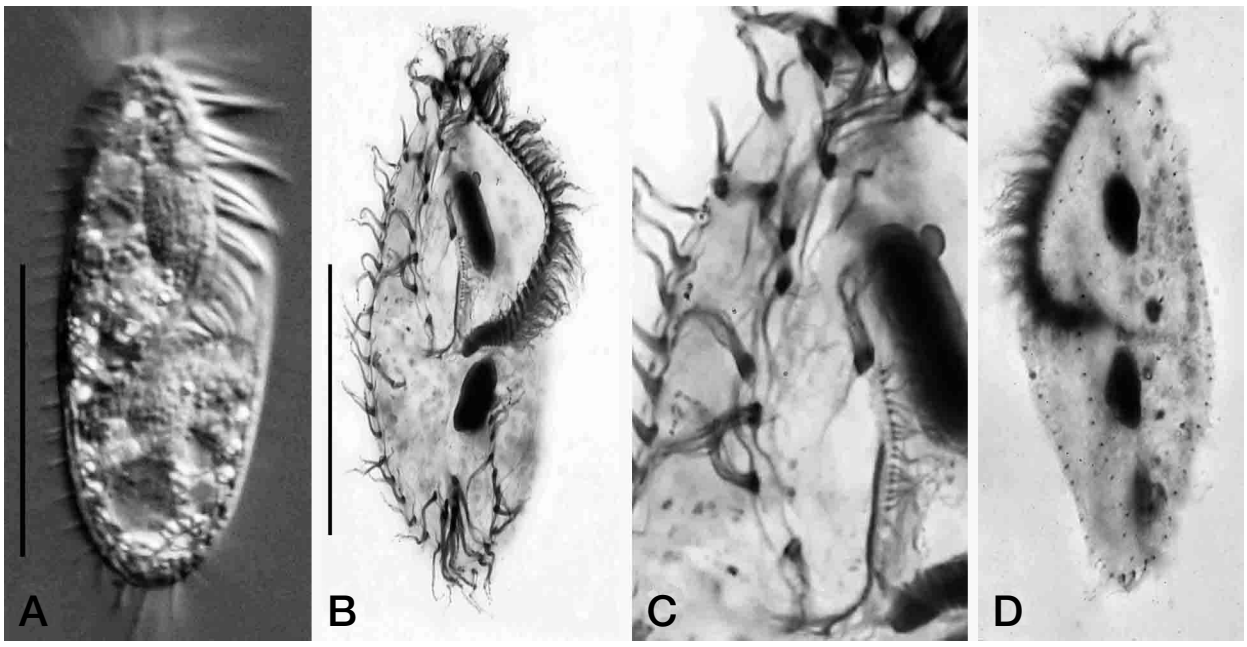

Fig. 3. Photomicrographs of *Gonostomum lajacola* from life (A) and after protargol impregnation (B-D). (A-C) Ventral views. (D) Dorsal view. Scale bars = 50 μm.

3. *Gonostomum lajacola* Foissner, 2016 (Fig. 3)

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

Diagnosis. Cell size 70-95 × 25-40 μm in protargol preparations; 33-40 adoral zone of membranelles; cortical granules present, colorless; 2 macronuclear nodules with 2 or 3 micronuclei; 3 frontal cirri; 2 frontoterminal cirri; 1 buccal cirrus; 17-24 paroral; 2 or 3 frontoventral cirral pairs; 6 or 7 pretransverse and transverse cirri; 1 left (10-14 cirri) and 1 right (20-23 cirri) marginal cirral row; 3 dorsal kineties; 3 caudal cirri.

Remarks. *Gonostomum lajacola* is highly similar to three congeners, *G. bromelicola*, *G. fraterculus*, and *G. singii* (Foissner, 2016). *Gonostomum bromelicola* and *G. fraterculus* can be separated from *G. lajacola* by numbers of frontoventral (4 vs. 5-7), and transverse cirri (in-

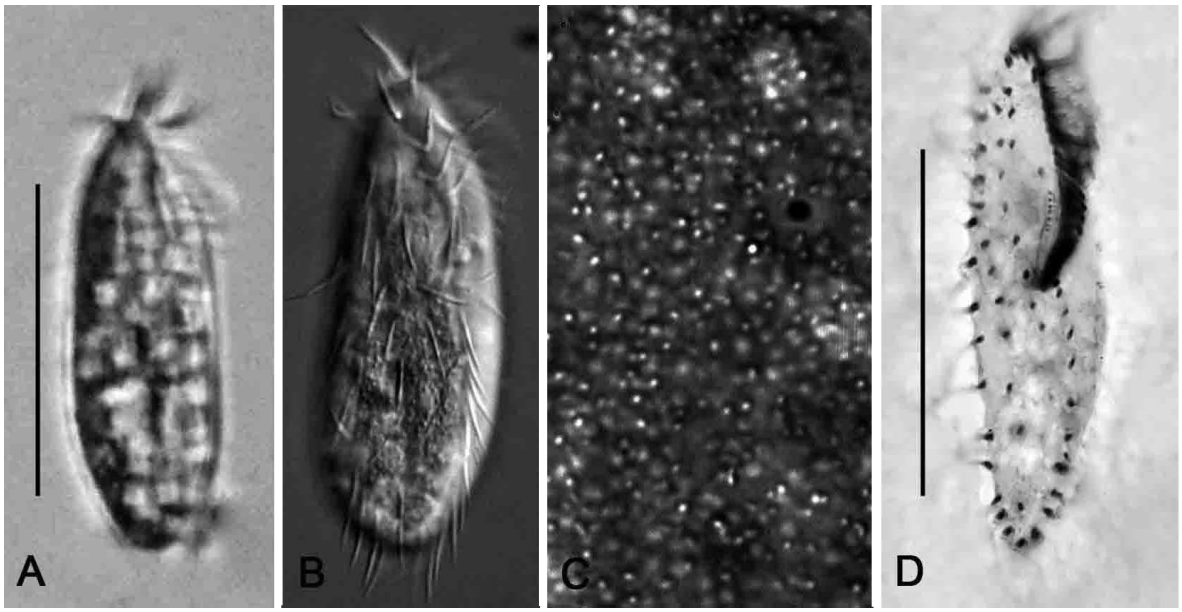

Fig. 4. Photomicrographs of *Paragonostomoides xianicum* from life (A, B) and after protargol impregnation (C, D). (A-B) Ventral views. (C) Cortical granules. (D) Ventral view. Scale bars = 50 μm.

variably 5 vs. 4 or 5). Furthermore, *G. bromelicola* has a distinct break in dorsal kinety 2 (vs. no break). *Gonostomum singii* differs from *G. lajacola* by cortical granules (sparse vs. dense) and the number of frontoventral cirral pairs (2 vs. variably 2 or 3) (Foissner, 2016).

Deposition. One slide (NNIBR2018113IV1939) has been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Genus *Paragonostomoides* Foissner, 2016

4. *Paragonostomoides xianicum* Wang *et al.*, 2017 (Fig. 4)

Material examined. Soil sample from Gyeokpo-ri, Buan-gun, Jeollabuk Province, Korea (35°38′22″N, 126° 27′59″E), on October 2015.

Diagnosis. Cell size 70-85 × 25-35 μm in protargol preparations; 25-28 adoral zone of membranelles; mitochondrion-like cortical granules present, colorless; 2 macronuclear nodules with about 2 micronuclei; 3 frontal cirri; 1 buccal cirrus; 3 or 4 cirri in frontoventral cirral row III; 6-8 cirri in frontoventral cirral row IV; 10 or 11 cirri in frontoventral cirral row VI; about 6 paroral; pretransverse and transverse cirri lacking; 1 left (10-12 cirri) and 1 right (16-18 cirri) marginal cirral rows; 3 dorsal kineties; 3 caudal cirri.

Remarks. The Korean population of *Paragonostomoides xianicum* can be separated from *P. minutus* by body length (75 μm vs. 33 μm on average) in protargol preparations, the numbers of macronuclear nodules (2 vs. 7 or 8), left marginal cirri (10 vs. 4 on average), frontal, buccal and frontoventral cirri (15 vs. 7 on average), and basal bodies in paroral (5-8 vs. 2-3) (Kamra *et al.*, 2008; Wang *et al.*, 2017).

Deposition. Three slides (NNIBR2018113IV1940-NNIBR2018113IV1942) have been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Family Oxytrichidae Ehrenberg, 1830
Genus *Australocirrus* Blatterer & Foissner, 1988

5. *Australocirrus oscitans* Blatterer & Foissner, 1988 (Fig. 5)

Material examined. Soil sample from Obong-ri, Gangwon Province, Korea (38°20′11″N, 128°31′01″E), on July 2012.

Diagnosis. Cell size 140-185 × 90-110 μm in protargol preparations, flexible; 26-34 adoral membranelles; cortical granules lacking; 2 macronuclear nodules with about 2 micronuclei; 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 5 transverse cirri; 1 left (18-25 cirri) and 1 right (18-25 cirri) marginal cirral row; 8 dorsal kineties (4 dorsal and 4 dorsomarginal kineties); 3 caudal cirri.

Remarks. The genus *Australocirrus* consists of five species. Three species namely, *Australocirrus zechmeister-*

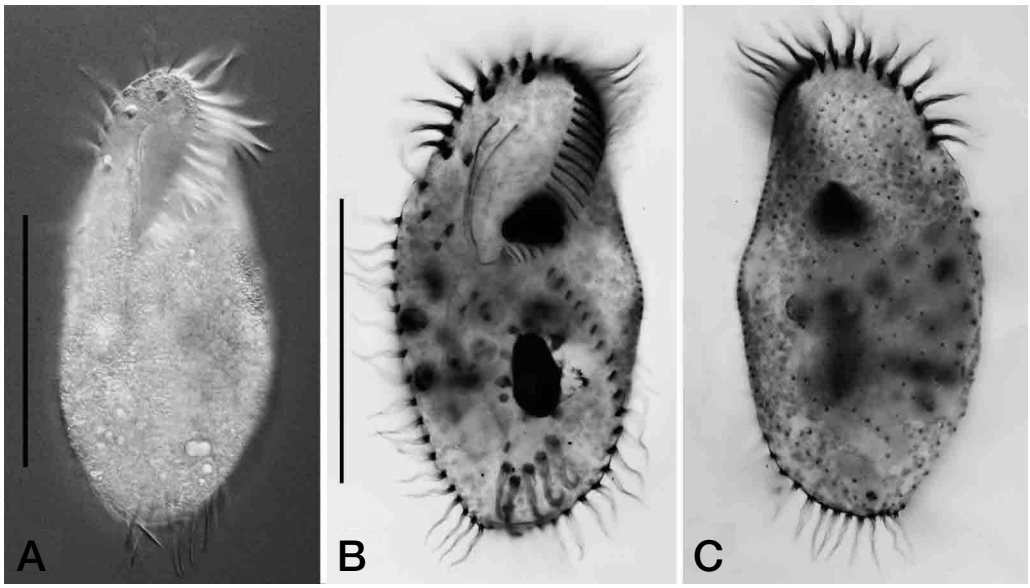

Fig. 5. Photomicrographs of *Australocirrus oscitans* from life (A) and after protargol impregnation (B, C). (A-B) Ventral views. (C) Dorsal view. Scale bars = 100 μm.

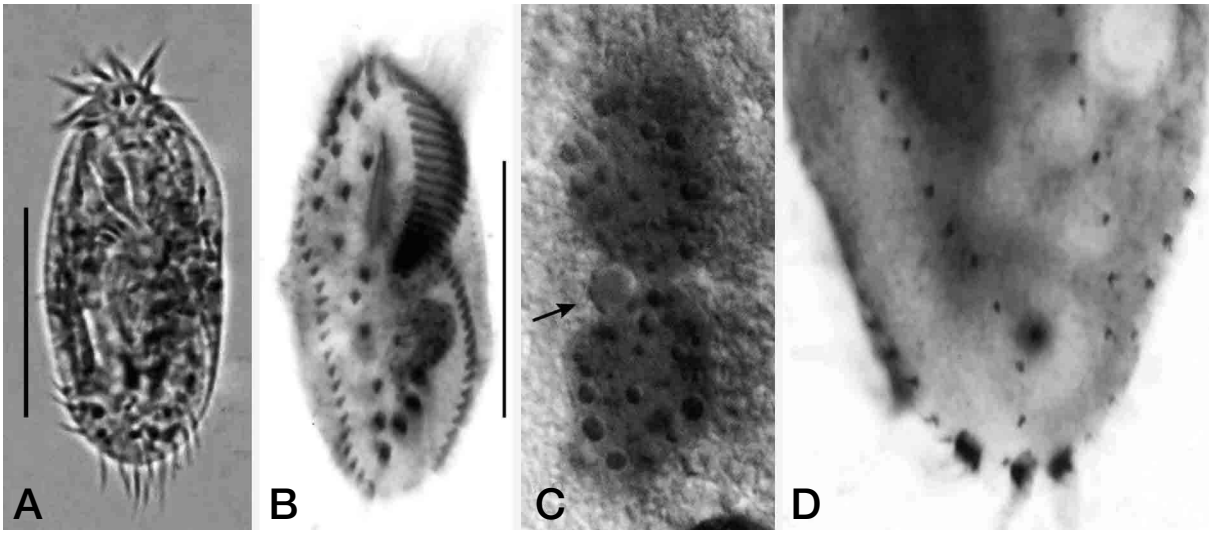

Fig. 6. Photomicrographs of *Monomicrocaryon crassicirratum* from life (A) and after protargol impregnation (B-D). (A-B) Ventral views. (C) Arrow indicates micronuclei. (D) Dorsal view. Scale bars = 50 μm.

ae, *A. oscitans*, and *Rigidocortex octonucleatus*, exhibit similar morphological characteristics. *Rigidocortex octonucleatus* previously belonged to the genus *Australocirrus*, but it was transferred to the genus *Rigidocortex* due to its rigid body (Berger 1999). *Australocirrus zechmeisterae* differs from *A. oscitans* and *R. octonucleatus* mainly by macronuclear nodules (4 in *A. zechmeisterae* vs. 2 in *A. oscitans* vs. 8 in *R. octonucleatus*).

Deposition. Two slides (NIBRPR0000104093, NIBRPR 0000104094) have been deposited at the National Institute of Biological Resources (NIBR), South Korea.

Genus *Monomicrocaryon* Foissner, 2016

6. *Monomicrocaryon crassicirratum* Foissner, 2016 (Fig. 6)

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

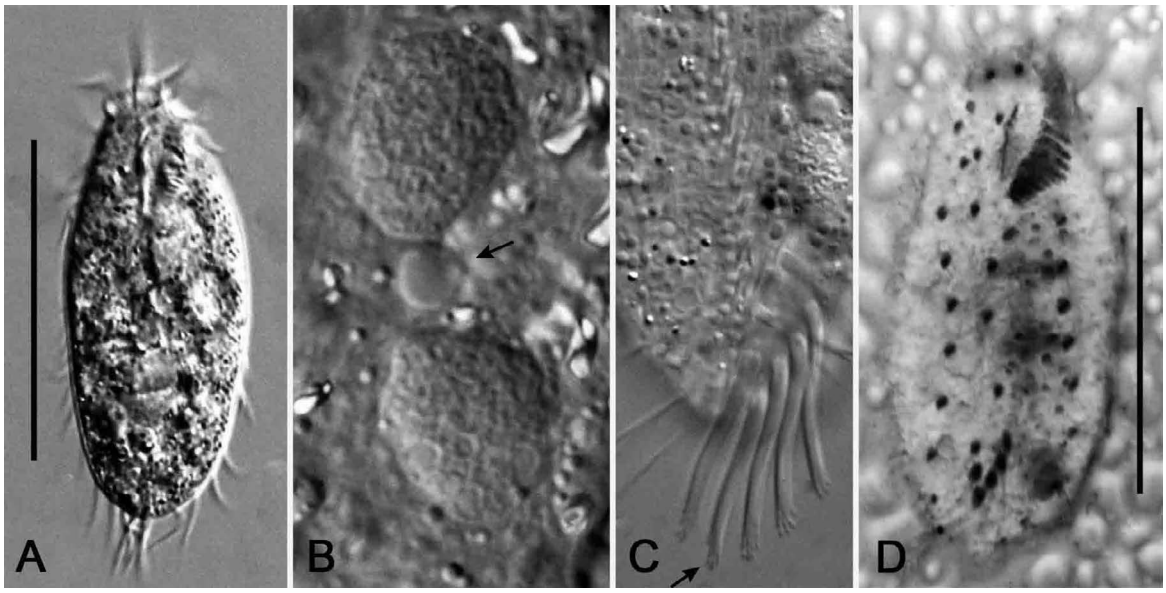

Fig. 7. Photomicrographs of *Monomicrocaryon euglenivorum fimbricirratum* from life (A-C) and after protargol impregnation (D). (A) Ventral view (B) Arrow indicates micronuclei. (C) Arrow indicates transverse cirri. (D) Ventral view. Scale bars = 50 μm.

Diagnosis. Cell size 85-100 × 40-45 μm in protargol preparations, oval shape; 24-27 adoral membranelles; cortical granules lacking; 2 macronuclear nodules, 1 micronucleus between macronuclear nodules; 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 5 transverse cirri; 1 left (16-19 cirri) and 1 right (16-20 cirri) marginal cirral row; 6 dorsal kineties (4 dorsal and 2 dorsomarginal kineties); 3 conspicuous caudal cirri.
Remarks. *Monomicrocaryon crassicirratum* can be distinguished from other *Monomicrocaryon* species by the following combination of features: moderate body size; conspicuous large cirri and caudal cirri; adoral zone of membranelles that occupies more than 40% of the body length; dorsal bristles that are 5-10 μm long; paroral and endoral membrane, parallel and almost straight; left pretransverse cirrus near the level of the last postoral cirrus (Fig. 6) (Foissner, 2016).
Deposition. Two slides (NNIBR2018113IV1943, NNIBR2018113IV1944) have been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), South Korea.

7. *Monomicrocaryon euglenivorum fimbricirratum* Foissner, 2016 (Fig. 7)

Material examined. Soil sample from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N, 126°23′40″ E), on February 2018.
Diagnosis. Cell size 55-65 × 30-35 μm in protargol preparations, shape ellipsoid; about 19 adoral membranelles; cortical granules lacking; 2 macronuclear nodules, 1 micronucleus between macronuclear nodules; 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 5 transverse cirri; 1 left (about 10 cirri) and 1 right (about 9 cirri) marginal cirral row; 5 dorsal kineties; 3 caudal cirri.
Remarks. *Monomicrocaryon euglenivorum* comprises two subspecies, *M. euglenivorum fimbricirratum and M. euglenivorum euglenivorum*. *Monomicrocaryon euglenivorum fimbricirratum* can be distinguished from *M. euglenivorum euglenivorum* as follows: body length (less than 70 μm vs. 80-90 μm); number of left marginal cirri (less than 11 vs. 15-20); transverse cirri (fringed distally vs. acicular) (Foissner, 2016).
Deposition. Three slides (NNIBR2018113IV1945-NNIBR2018113IV1947) have been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Genus *Notohymena* Blatterer & Foissner, 1988

8. *Notohymena rubescens* Blatterer & Foissner, 1988 (Fig. 8)

Material examined. Soil sample from Wangsan-ri, Jung-gu, Incheon, South Korea (37°26′47″N 126°23′40″ E), in February 2018.
Diagnosis. Cell size 90-105 × 35-45 μm in protargol preparations, shape ellipsoid; 27-30 adoral membranelles; cortical granules reddish color; paroral mem-

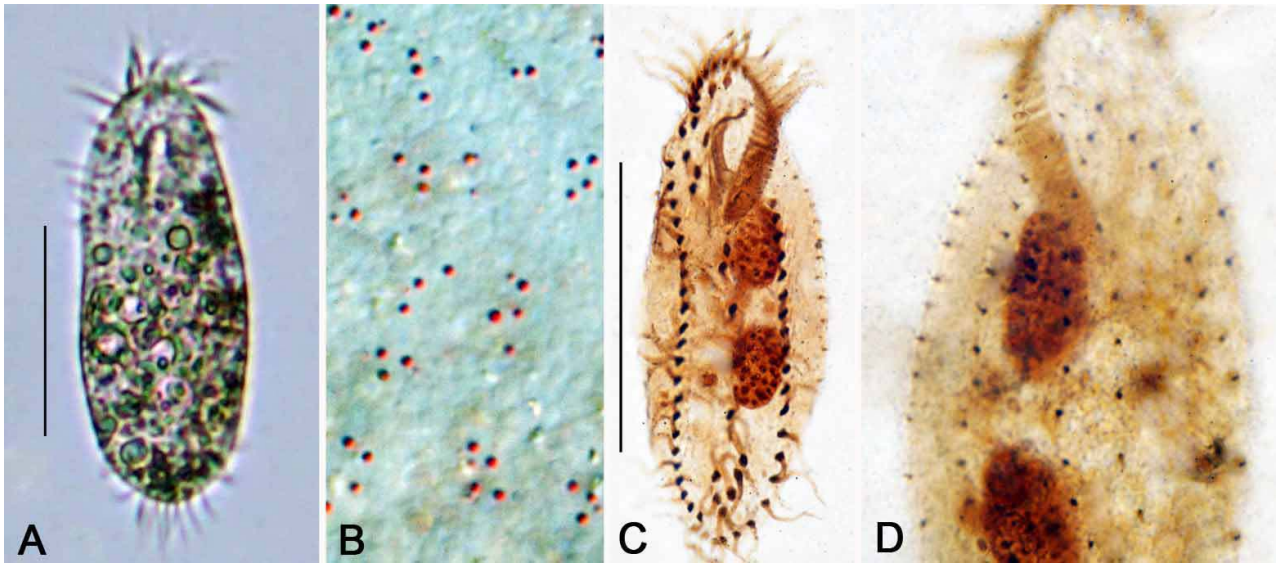

Fig. 8. Photomicrographs of *Notohymena rubescens* from life (A, B) and after protargol impregnation (C, D). (A) Ventral view. (B) Cortical granules. (C) Ventral view. (D) Dorsal view. Scale bars = 50 μm.

brane with hooked distal end in the typical *Notohymena*-pattern; 2 macronuclear nodules with 2 or 3 micronuclei; 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 5 transverse cirri; 1 left (18-20 cirri) and 1 right (20-22 cirri) marginal cirral row; 5 dorsal kineties; 3 caudal cirri.

Remarks. *Notohymena rubescens* mainly differs from other *Notohymena* species because of the reddish color of its cortical granules (*N. antarctica*, yellowish; *N. limus* and *N. pampasica*, colorless; *N. quadrinucleata*, yellowish to citrine; *N. saprai*, dark green) (Berger, 1999; Kuppers *et al*., 2007; Kamra and Kumar, 2010; Foissner, 2016; Naqvi *et al*., 2016).

Deposition. Three slides (NNIBR2018113IV1948-NNIBR2018113IV1950) have been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Genus *Sterkiella* Blatterer *et al*., 1991

9. *Sterkiella multicirrata* Li *et al*., 2018 (Fig. 9)

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

Diagnosis. Body broadly elliptical, size in vivo 120-165 × 50-90 μm, semirigid; cortical granules lacking; 4 macronuclear nodules with about 4 micronuclei; about 50 adoral membranelles; more than 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 1 parabuccal, 1 IV/3, 1 or no postoral ventral, 1 or no pretransverse ventral, and 4 or 5 transverse cirri; 2 frontoventral cirral rows (FVR) (3 or 4 cirri in FVR1; 5 or 6 cirri in FVR2); 1 left (about 26 cirri) and 1 right (about 30 cirri) marginal cirral rows; 6 dorsal kineties (4 dorsal and 2 dorsomarginal kineties); 3 caudal cirri.

Remarks. The Korean population of *Sterkiella multicirrata* differs from its congeners by having the more than 20 frontal-ventral-transverse (FVT) cirri (vs. 17 or 18) (Chen *et al*., 2015; Kumar *et al*., 2015). The increased number of FVT cirri is a representative feature of the genus *Gastrostyla*, and the phylogenetic tree showed that *S. multicirrata* has a close relationship with the *G. steinii* group (Kumar *et al*., 2015; Li *et al*., 2018). These two genera share many morphological traits (inflexible body, lack of cortical granules, and six dorsal kineties), and only differ in the number of FTV cirri (usually 18 in *Sterkiella* vs. more than 18 in *Gastrostyla*). Based on the above mentioned findings, we supposed that the location of *S. multicirrata* is intermediated to these two genera regarding evolution.

Deposition. Two slides (NIBRPR0000107255, NIBRPR0000107269) have been deposited at the National Institute of Biological Resources (NIBR), Korea.

Genus *Stylonychia* Ehrenberg, 1830

10. *Stylonychia ammermanni ammermanni* Gupta *et al*., 2001 (Fig. 10)

Material examined. Freshwater taken from Danam-dong, Incheon, Korea (37°33′42″N 126°43′37″E) on April 2018.

Diagnosis. Cell size 110-120 × 55-60 μm in protargol

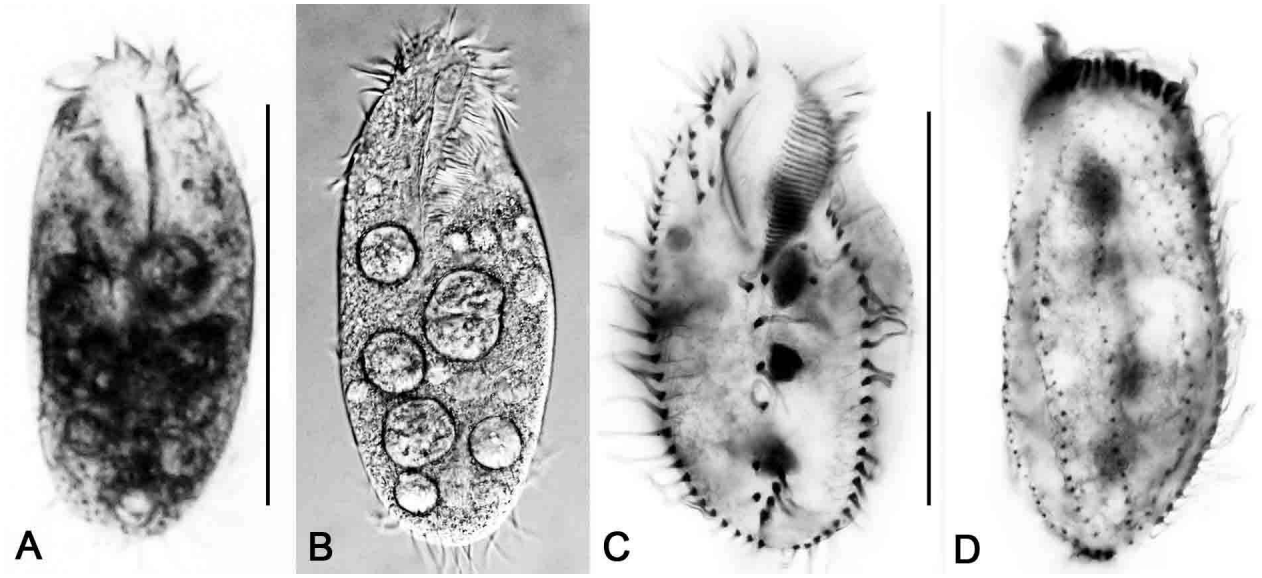

Fig. 9. Photomicrographs of *Sterkiella multicirrata* from life (A, B) and after protargol impregnation (C, D) specimens. (A-C) Ventral views. (D) Dorsal view. Scale bars = 100 μm.

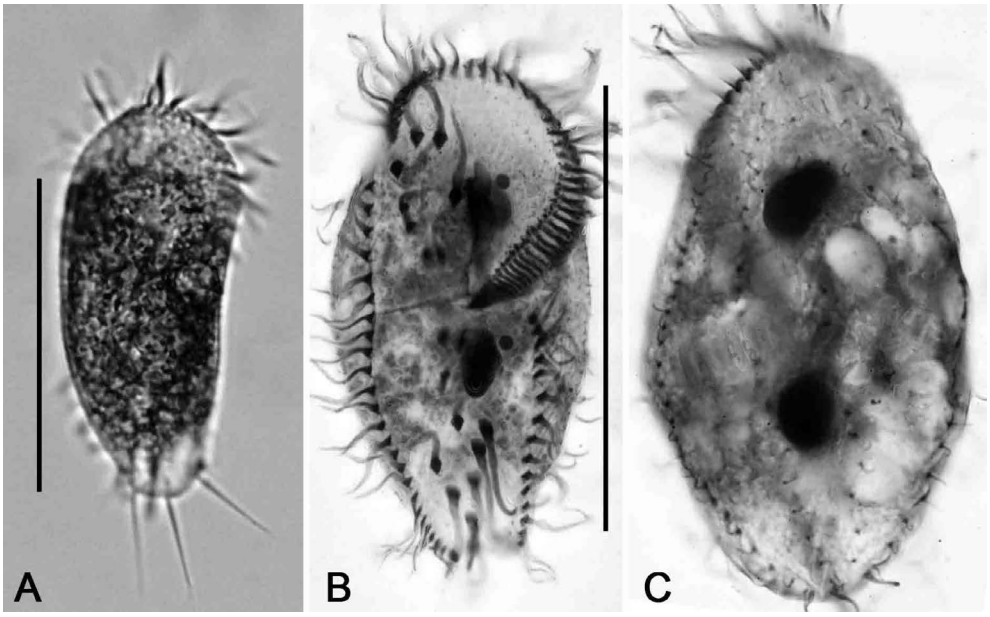

Fig. 10. Photomicrographs of *Stylonychia ammermanni ammermanni* from life (A) and after protargol impregnation (B, C). (A, B) Ventral views. (C) Dorsal view. Scale bars = 100 μm.

preparations, typical shape striking bulge in region of peristome, rigid; 44-48 adoral membranelles; cortical granules lacking; 2 macronuclear nodules, 2 or 3 micronuclei; 18 frontal-ventral-transverse cirri, 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 5 transverse cirri; 1 left (14-16 cirri) and 1 right (23-25 cirri) marginal cirral row; 6 dorsal kineties, 16-20 bristles in kinety 4; 3 caudal cirri.

Remarks. The subspecies called *Stylonychia ammermanni koreana* was established by Kumar *et al*. (2016). *Stylonychia ammermanni koreana* mainly differs from *S. ammermanni ammermanni* by the following two features: number of adoral membranelles (less than 44 vs. more than 44) and number of bristles in dorsal kinety 4 (less than 15 vs. more than 16) (Kumar *et al*., 2016). Our Korean population resembles *S. ammermanni ammer-*

manni mainly due to the number of adoral membranelles (44-48) and number of bristles in kinety 4 (16-20)**.**
Deposition. Two slides (NNIBR2018113IV1951, NNIBR2018113IV1952) have been deposited at the Nakdonggang National Institute of Biological Resources (NNIBR), South Korea.

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