

New record of five ciliates (Protozoa, Ciliophora) collected in eastern Gangwon-do Province, South Korea

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We investigated five ciliate species from soil, estuary and marine habitats in eastern Gangwon-do Province, South Korea. Morphology was studied through observation of living specimens as well as silver preparations. The species were classified into five genera, five families, five orders and four classes. *Aspidisca aculeata*, *Hemiurosoma similis*, *Litonotus alpestris*, *Stentor roeselii* and *Vorticella infusionum* are first records in Korea, and among them, the genus *Hemiurosoma* is recorded for the first time. Here, we provide short descriptions with microphotographs of these species.

Keywords: infraciliature, protargol impregnation, redescription, taxonomy

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INTRODUCTION

Since species diversity studies of Korean ciliates began in 1931, more than 400 ciliate species have been identified (Huruyama, 1931; Jang *et al.*, 2017; Jung *et al.*, 2017; Kim and Jung, 2017; Moon *et al.*, 2017; Park *et al.*, 2017). In this study, we observed five ciliate genera, including four that were previously described in Korea: *Aspidisca* Ehrenberg, 1830, *Litonotus* Wresniowski, 1870, *Stentor* Oken, 1815, *Vorticella* Linnaeus, 1767 (Shin, 1988; Shin and Kim, 1988; Moon *et al.*, 2004; Kim and Min, 2009; Shazib *et al.*, 2014; Kim and Shin, 2016). Of the five genera, *Hemiurosoma* Foissner, Agatha and Berger, 2002 is a first record in Korea. Here, we provide brief taxonomical re-descriptions of the five species including *Stentor roeselii* Ehrenberg, 1835 which is the species available only for the gene sequences of Korean population without its morphology (Shazib *et al.*, 2014).

MATERIALS AND METHODS

Aspidisca aculeata, *Hemiurosoma similis*, *Litonotus alpestris*, *Stentor roeselii* and *Vorticella infusionum* were collected from soil, marine water, brackish water and freshwater habitats. Information related to collection sites, dates and habitat attributes can be found in the section 'Material examined'.

The samples were transported to a laboratory and then cultured on Petri dishes. In all culture plates, two or three rice grains were added to encourage bacterial growth as a food supply for the ciliates. Silver impregnated specimens were prepared by a protargol method (procedure A) and 'dry' silver nitrate method (*Vorticella infusionum* only) (Foissner, 2014; Kim and Jung, 2017).

Observation of living and stained cells was performed under a stereomicroscope (Olympus SZ11, Japan) and an optical microscope (Olympus BX53, Japan) at low ($\times 40$ -200) to high ($\times 400$ -1,000) magnifications. Microphotographs were captured by a digital camera (Olympus DP74, Japan) and sequential through-focal images of stained individuals were combined by Helicon Focus (HeliconSoft Ltd, Ukraine).

The terminology and taxonomical classification follow Lynn (2008).

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Subphylum Postciliodesmatophora Gerassimova and Seravin, 1976
Class Heterotrichea Stein, 1859
Order Heterotrichida Stein, 1859
Family Stentoridae Carus, 1863
Genus *Stentor* Oken, 1815

1. *Stentor roeselii* Ehrenberg, 1835 (Fig. 1)

Material examined. Freshwater collected from the River Gyeongpochen, Unjeong-dong, Gangneung-si, Gangwon-do, Korea (37°47'20"N, 128°54'34"E) on May 12, 2017.

Diagnosis. Body size 265-410 × 60-110 μm on free living form; attached cell trumpet shape about 500-1,000 μm long in vivo; body colorless; flexible, highly contractile body; weakly loricated; 178-187 adoral membranelles; 56-62 somatic kineties; about 13 buccal kineties includ-

ing 1 peristomial kinety; macronucleus band-like shaped.

Distribution. Cosmopolitan (Austria, Benin, Black Sea, Caspian Sea, Danube River, Gdańsk Bay, Germany, Japan, Korea).

Remarks. *Stentor roeselii* highly resembles *S. muelleri* Ehrenberg, 1831 in the body size, the body shape, the color of cortical granules, the number of somatic kineties and the number of buccal kineties (Foissner *et al.*, 1992). However, it differs from *S. muelleri* in the shape of the macronucleus (band-like shape vs. moniliform).

Voucher slides. Two slides of protargol impregnated spe-

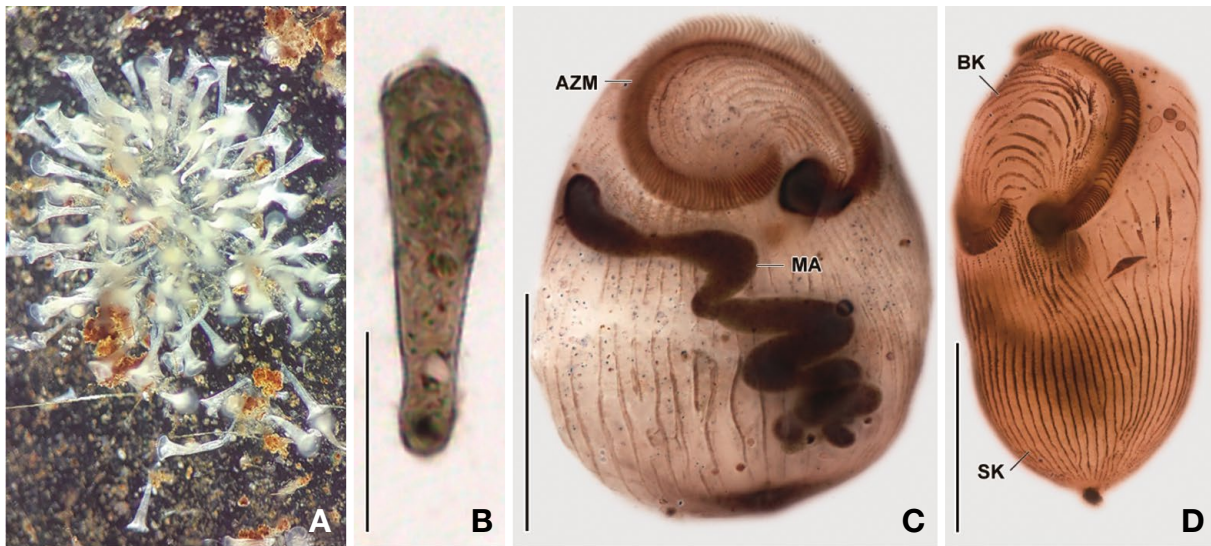


Fig. 1. *Stentor roeselii* in vivo (A, B) and after protargol impregnation (C, D). A. Living cells attached to substrates. B. Free swimming cell. C, D. Somatic and oral ciliature with band-like macronucleus. AZM, adoral zone of membranelles; BK, buccal kinety; MA, macronucleus; SK, somatic kinety. Scale bars: B = 200 μm, C, D = 50 μm.

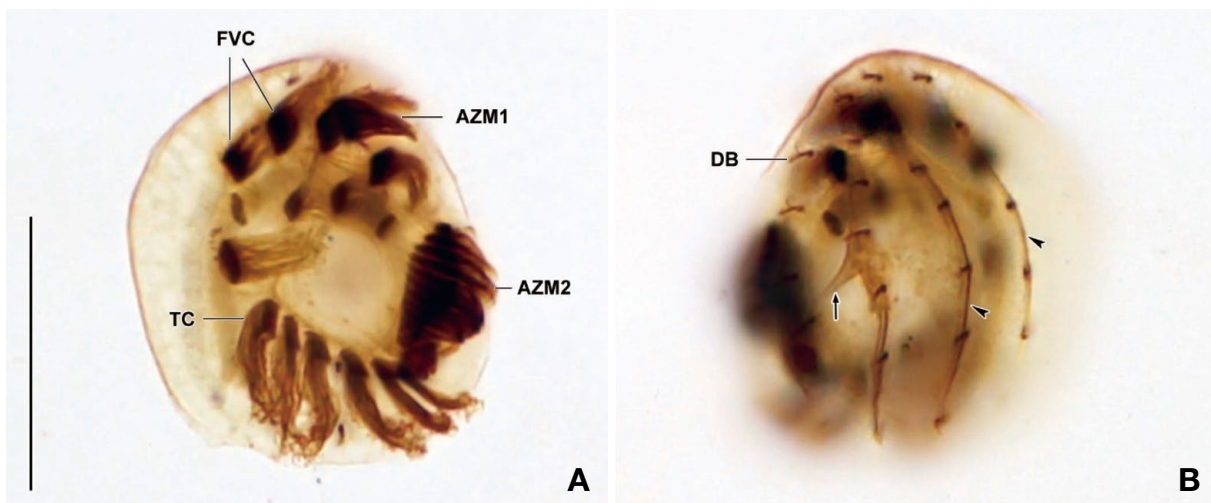


Fig. 2. *Aspidisca aculeata* after protargol impregnation. A, B. Ventral and dorsal view of typical individual. Arrow marks a curved and pointed thorn. Arrowheads indicate dorsal ridges. AZM1, 2, adoral zone of membranelles 1, 2; DB, dorsal bristle; FVC, frontoventral cirri; TC, transverse cirrus. Scale bar: 20 μm.

cimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000107960, NIBRPR0000107961).

Class Spirotrichea Bütschli, 1889
Order Euplotida Small and Lynn, 1985
Family Aspidiscidae Ehrenberg, 1830
Genus *Aspidisca* Ehrenberg, 1830

2. *Aspidisca aculeata* (Ehrenberg, 1982) Kahl, 1932 (Fig. 2)

Material examined. Brackish water (salinity 10.4‰) collected from Songji Lake, Oho-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea (38°20'11"N, 128°30'58"E) on April 28, 2017.

Diagnosis. Marine *Aspidisca*, body about 30 × 30 μm in vivo, with 4 dorsal ridges and 1 thorn on second dorsal ridge; 7 frontoventral cirri polystyla-arrangement; 6 transverse cirri; 4 dorsal kineties; adoral zone of membranelles 1 composed of 4 membranelles; adoral zone of membranelles 2 composed of 13 membranelles.

Distribution. Cosmopolitan (China, Germany, Korea).

Remarks. The Korean population of *A. aculeata* is slightly different from the Chinese population in the shape of the dorsal thorn (curved and pointed vs. keel-like). *Aspidisca aculeata* differs from the most similar species *A. turrita* (Ehrenberg, 1831) in the dorsal ridges (present vs. absent) (Wu and Curds, 1979; Li *et al.*, 2008).

Voucher slides. Two slides of protargol impregnated spe-

cimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000107964).

Subclass Stichotrichida Fauré-Fremiet, 1961
Order Sporadotrichida Fauré-Fremiet, 1961
Family Oxytrichidae Ehrenberg, 1830
Genus *Hemiurosoma* Foissner, Agatha and Berger, 2002

3. *Hemiurosoma similis* (Foissner, 1982) Foissner, Agatha and Berger, 2002 (Fig. 3)

Material examined. Terrestrial soil collected from Boonsu Park, Gyo-dong, Gangneung-si, Gangwon-do, Korea (37°45'48"N, 128°52'29"E) on October 6, 2016.

Diagnosis. Body size 150-190 × 20-25 μm in vivo; body cylindrical shape; pointed posterior body end; dorsoventrally flattened, flexible; cytoplasm colorless, 2 types of colorless subpellicular granules, larger ellipsoidal granules size 2-3 × 0.7-0.9 μm, smaller spherical granules diameter about 0.5 μm; 1 left and 1 right marginal row; about 20 adoral membranelles; 3 frontal cirri; 1 buccal cirrus; 4 frontoventral cirri; undulating membranes in *Gonostomum* pattern; 3 transverse cirri; 3 caudal cirri; 2 ellipsoidal macronuclei; 1-3 spherical micronuclei; 3 dorsal kineties.

Distribution. Austria, Costa Rica, Czech, Korea.

Remarks. *Hemiurosoma similis* is easily distinguished from *H. terricola* Foissner *et al.*, 2002 in the number of macronuclear nodules (2 vs. 4), and the number of transverse cirri (3 vs. constantly 2) (Berger, 1999; Foissner *et al.*, 2002).

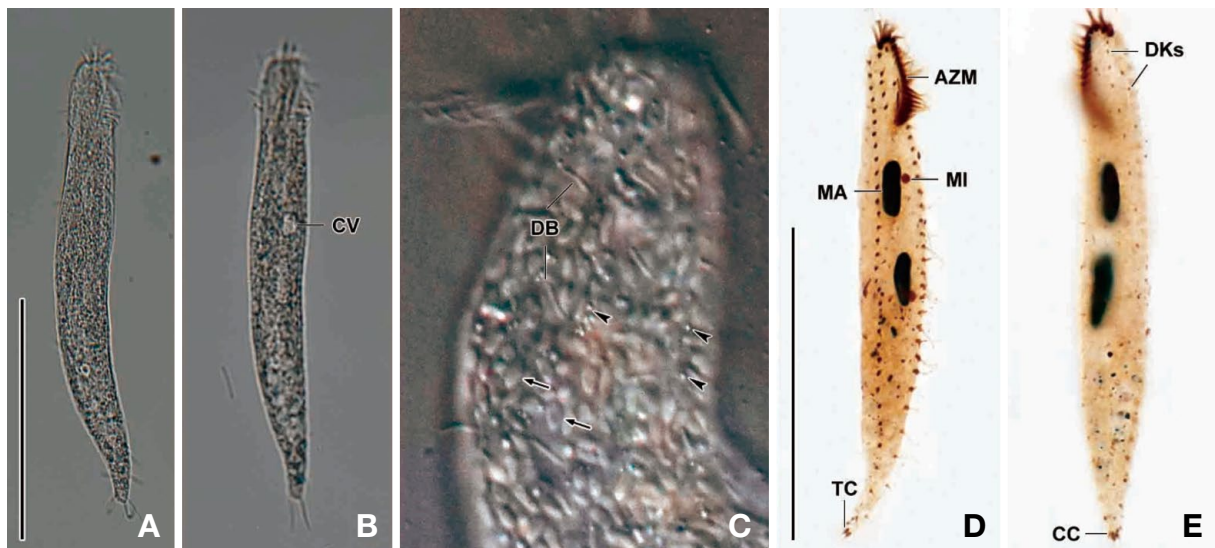


Fig. 3. *Hemiurosoma similis* in vivo (A-C) and protargol impregnated specimens (D, E). A. Typical individual in vivo. B. Location of contractile vacuole. C. Cortical granules and dorsal kineties on dorsal side. Arrows indicate ellipsoidal granules. Arrowheads mark small spherical granules. D. Ventral view showing oral and somatic ciliatures with nuclear apparatus. E. Dorsal kineties and caudal cirri on dorsal side. AZM, adoral zone of membranelles; CC, caudal cirrus; CV, contractile vacuole; DB, dorsal bristles; DKs, dorsal kineties; MA, macronucleus; MI, micronucleus; TC, transverse cirrus. Scale bars: A, D = 100 μm.

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000107967, NIBRPR0000107968).

Class Litostomatea Small and Lynn, 1981
Order Pleurostomatida Schewiakoff, 1896
Family Litonotidae Kent, 1882
Genus *Litonotus* Wresniowski, 1870

4. *Litonotus alpestris* Foissner, 1978 (Fig. 4)

Material examined. Brackish water (salinity 7.9‰) collected from the River Gyeongpochen, Unjeong-dong, Gangneung-si, Gangwon-do, Korea (37°47'20"N, 128°54'34"E) on August 24, 2017.

Diagnosis. Body size 25-35 × 10-20 μm in vivo; body lanceolate shape, colorless; cortical granules colorless, 0.5-0.7 μm in diameter, irregularly arranged between so-

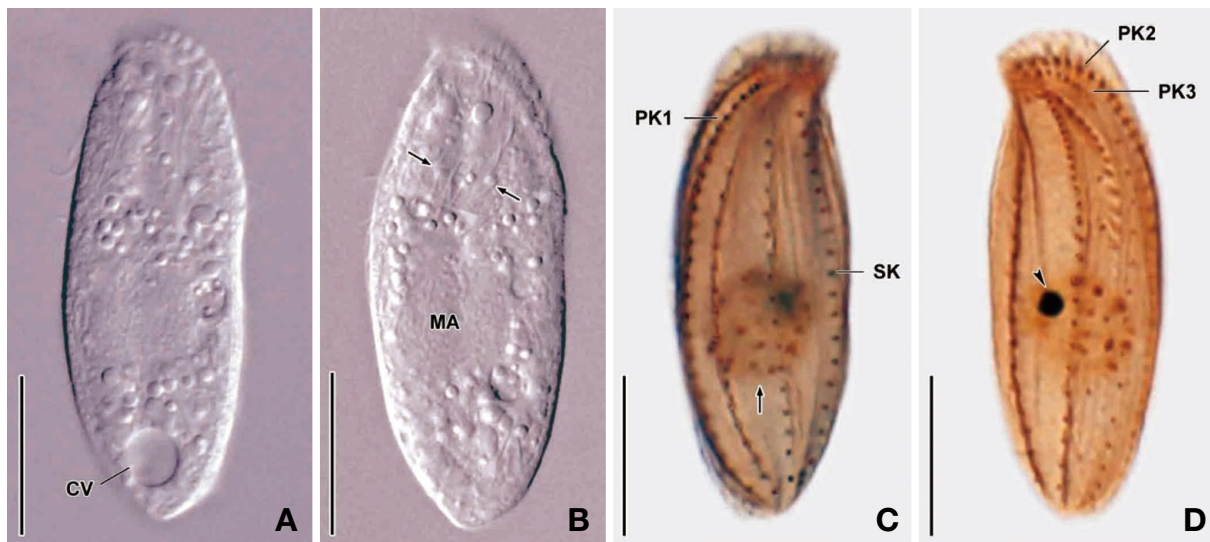


Fig. 4. *Litonotus alpestris* in vivo (A, B) and protargol impregnated specimens (C, D). A. An individual showing contractile vacuole and cytoplasmic inclusions. B. Extrusomes (arrows) and macronucleus. C. Perioral kinety 1 and somatic kineties on left side (arrow marks spherical macronucleus). D. Perioral kinety 2, 3 and somatic kineties on right side (arrowhead indicates spherical micronucleus). CV, contractile vacuole; MA, macronucleus; PK1-3, perioral kinety 1-3; SK, somatic kinety. Scale bars: A-D = 10 μm.

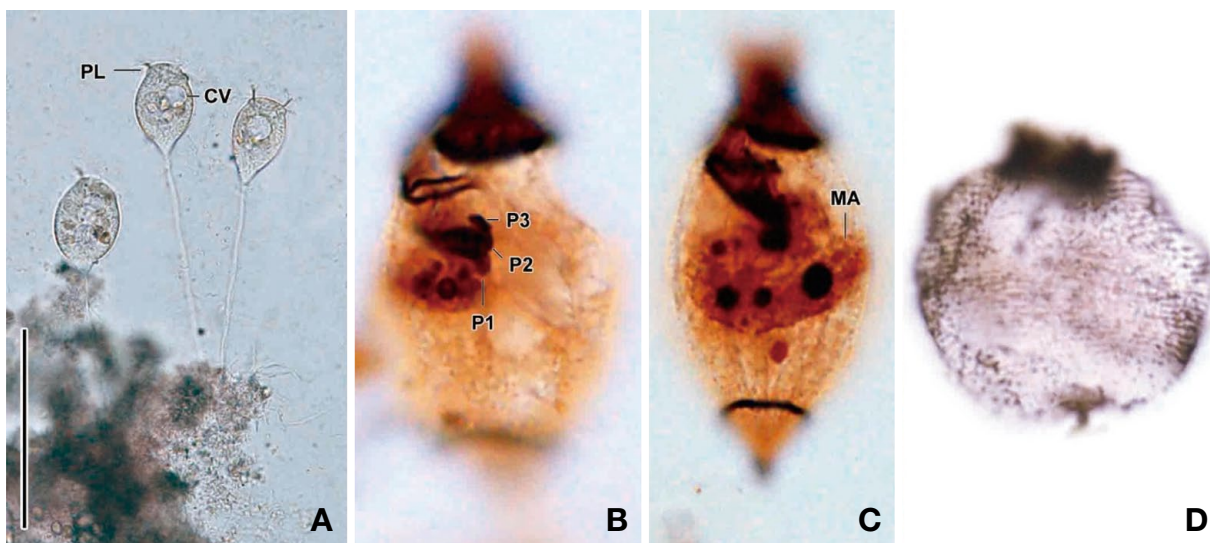


Fig. 5. *Vorticella infusionum* in vivo (A), and protargol (B, C) and silver nitrate (D) impregnated specimens. A. Zooid shapes in vivo. B. Oral ciliature. C. Horizontal C-shaped macronucleus. D. Silverline system. CV, contractile vacuole; MA, macronucleus; PL, peristomial lip; P1-3, infundibular polykinety 1-3. Scale bar: 100 μm.

matic kineties; 1 contractile vacuole subterminally located; 1 globular macronucleus size about $7 \times 6 \mu\text{m}$; 1 micronucleus size about $1.6 \times 1.7 \mu\text{m}$; 3 right side somatic kineties; 3 left side somatic kineties; perioral kinety (PK) 1-3, PK1 on left side, PK2, 3 on right side.

Distribution. Austria, Germany, Korea.

Remarks. The Korean population of *L. alpestris* is slightly different from the Austrian population in the body length (25-35 μm vs. 30-50 μm), the macronucleus length (7 μm vs. 10 μm), the number of right somatic kineties (3 vs. 3 or 4) and number of left somatic kineties (3 vs. 3 or 4) (Foissner *et al.*, 1995). *Litonotus alpestris* differs from *L. bonnensis* Song and Wilbert, 1989 in the number of right somatic kineties (3 vs. 6) and the number of left somatic kineties (3 vs. 6) (Song and Wilbert, 1989). *Litonotus alpestris* differs from *L. uninucleatus* (Kahl, 1931) in the body length (25-35 μm vs. 30-60 μm) and the anterior and posterior ends (broadly rounded vs. sharply rounded) (Song and Wilbert, 1989). *Litonotus alpestris* is distinguished from *L. crystallinus* Vuxanovici, 1960 in the number of macronuclear nodules (1 vs. 2) (Foissner *et al.*, 1995).

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000107965, NIBRPR0000107966).

Class Oligohymenophorea de Puytorac *et al.*, 1974
Subclass Peritrichia Stein, 1859
Order Sessilida Kahl, 1933
Family Vorticellidae Ehrenberg, 1838
Genus *Vorticella* Linnaeus, 1767

5. *Vorticella infusionum* complex (Fig. 5)

Material examined. Brackish water (salinity 1.0‰) collected from the River Gyeongpochen, Unjeong-dong, Gangneung-si, Gangwon-do, Korea ($37^{\circ}47'20''\text{N}$, $128^{\circ}54'34''\text{E}$) on July 20, 2017.

Diagnosis. Zooid size 45-55 \times 26-34 μm in vivo; bell-like shape; peristomial lip single layered and diameter 17-22 μm ; 1 contractile vacuole ventrally located; macronucleus horizontal C-shaped; transverse silverlines about 25 rows between peristomial lip to aboral ciliary wreath and about 10 rows between aboral ciliary wreath to scopula; infundibular polykinety 3 (P3) slightly shortened adstomally.

Distribution. Cosmopolitan (Alaska, Antarctica, Argentina, Austria, China, Germany, Iceland, Italy, Poland, Russia, U.S.A., Korea).

Remarks. *Vorticella infusionum* complex differs from *V. microstoma* complex in the shape of macronucleus (horizontal C vs. longitudinal band) (Warren, 1986; Foissner *et al.*, 1992).

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000107962, NIBRPR0000107963).

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