

New records of terrestrial and freshwater ciliates (Protozoa: Ciliophora) from Korea: Brief descriptions with notes

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Eight samples, collected from six moss-covered soil and two freshwater samples, were examined to determine ciliate diversity in Korea. Based on the morphology of living and stained specimens, thirteen ciliate species, previously unrecorded in Korea, were identified. These specimens were classified to four classes, seven orders, ten families, and twelve genera. Of these genera, the following six were first records in Korea: *Bresslauides*, *Dexiotricha*, *Glaucoma*, *Paragastrostyla*, *Sathrophilus*, and *Trithigmotoma*. Here, we provide a brief description of the morphology of these 13 taxa along with microphotographs and notes.

Keywords: infraciliature, morphology, nuclear apparatus, protargol impregnation

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INTRODUCTION

To date, more than 330 ciliate species have been recorded in Korea (National Institute of Biological Resources, 2012; Choi *et al.*, 2016; Jung and Min, 2016; Kim and Jung, 2016; Kim and Choi, 2016; Kim and Lee, 2016; Shin *et al.*, 2016; Jung *et al.*, 2017). Of these, approximately ten percent were new to science. Since most of these species have only recently been described in Korea, it is likely that many more are yet to be discovered.

Our research group focused on ciliates that are found in terrestrial and freshwater habitats. Here, we describe thirteen ciliates, previously unrecorded in Korea, based on observations of live and stained specimens.

MATERIALS AND METHODS

Eight samples were collected and cultured at room temperature or 18°C. Of the samples, six were moss-covered soil and two were freshwater samples: DC4-moss-covered soil sample from the entrance of a trail on Chorock Peak (37°32'N/129°5'E; March, 2016); GP-freshwater sample from Gyeongpocheon (37°47'N/128°54'E; August, 2016); GS-freshwater sample from Gasiyeonseupji (37°47'N/128°54'E; August, 2016); HG-moss-covered soil sample from Haegan Island (34°51'N/128°28'E;

June, 2016); JM-moss-covered soil sample from Maebong Mountain (34°25'N/126°21'E; July, 2016); and TB1, TB6, and TB9-moss-covered soil samples from a trail on Taebaek Mountain (37°6'N/128°54'E; May, 2016). Moss-covered samples were air-dried for days in a laboratory, and raw cultures were maintained using the non-flooded Petri dish culture method (Foissner *et al.*, 2002). For freshwater samples, we added rice grains to the raw samples to enrich the growth of bacteria, which were then used as a food source for ciliates.

For species identification, living cells and protargol-impregnated specimens were observed as described by Foissner (2014), and protargol was synthesized according to the protocol of Pan *et al.* (2013). For details of the protargol impregnation procedure, refer to Foissner (2014). In brief, we fixed ciliates using Bouin's fluid or ethanol, and sorted the fixed cells via the centrifugation of 15-mL conical tubes (3,000 rpm for 1 min; Beckman GS-15R, CA) or using a watch glass. A stereomicroscope (Olympus SZ11, Japan) and a light microscope with differential interference contrast (Olympus BX53, Japan) were used to observe the ciliates at a magnification range of 18× to 1,000×. Microphotographs were taken using digital cameras (Sony α7R, Japan; Nikon DS-Ri2, Japan), and the sequential through-focal images of stained specimens were merged using Helicon Focus software (HeliconSoft Ltd, Ukraine). General terminology and classification follows Lynn (2008).

RESULTS AND DISCUSSION

The 13 species were assigned to four classes, seven orders, 10 families, and 12 genera (for details, see below) (Figs. 1, 2):

Phylum Ciliophora Doflein, 1901
 Class Colpodea Small and Lynn, 1981
 Order Colpodida de Puytorac *et al.*, 1974
 Family Hausmanniellidae Foissner, 1987
 Genus *Bresslauides* Blatterer and Foissner, 1988

1. *Bresslauides discoideus* (Kahl, 1931) Foissner, 1993 (Fig. 1A)

Diagnosis. Body size (stained) approximate 250 μm in diameter; body shape roughly spherical, flattened; lip-like vestibular bottom. Macronuclear nodule broadly spherical. Right oral polykinetid thin and inconspicuous.

Remarks. This is the first report of the genus *Bresslauides* in Korea. Of the colpodean ciliates recorded in Korea, *B. discoideus* exhibited the largest body size. This species ingested rotifers (multicellular eukaryotes) and testate amoebae.

Voucher slides. Two slides, including protargol-impregnated specimens (JM), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107185, NIBRPR0000107186).

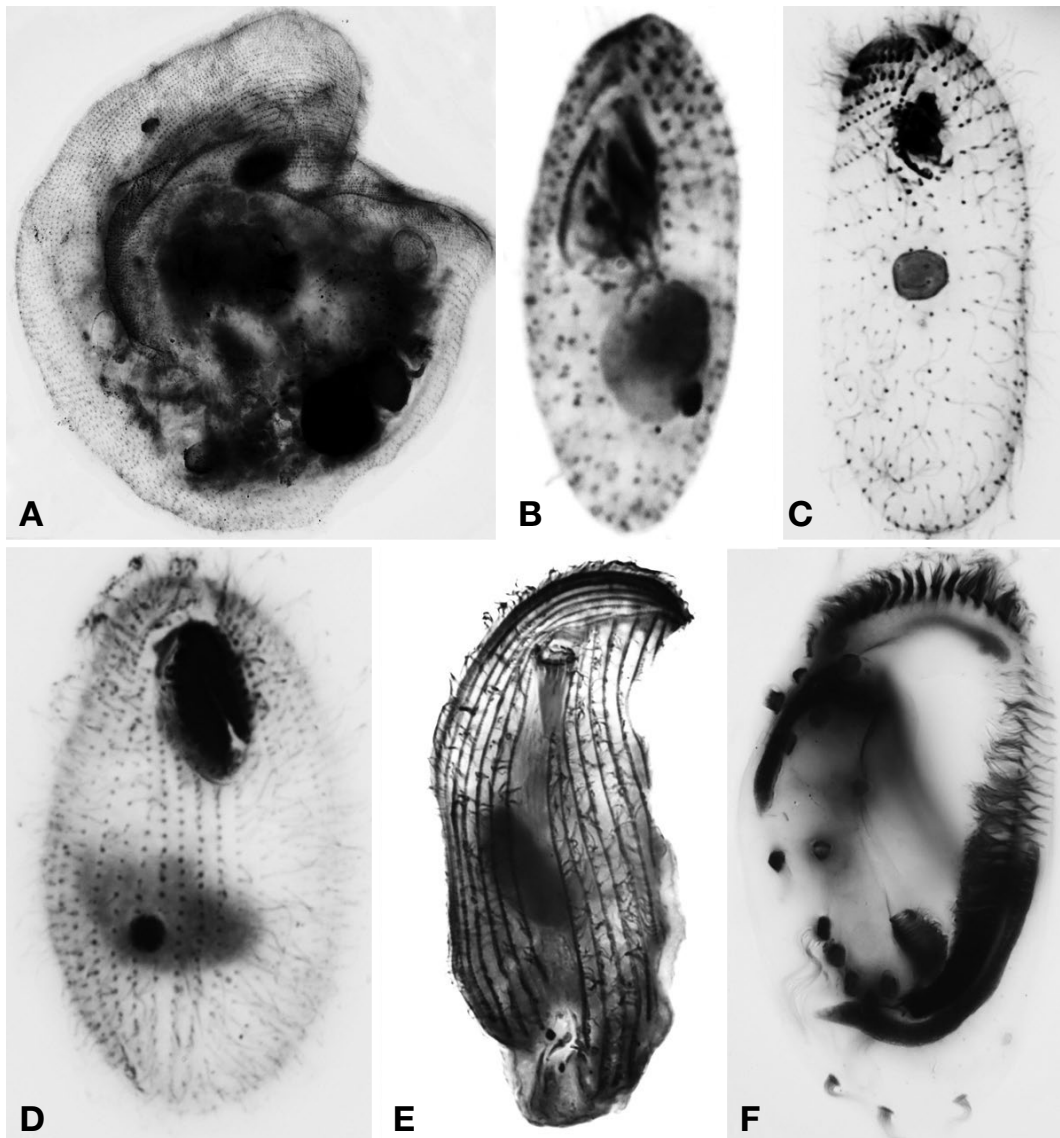


Fig. 1. Protargol-stained specimens. A. Right lateral view of *Bresslauides discoideus*, 250 μm in diameter; B. Ventral view of *Sathrophilus muscorum*, 25 μm in length; C. Ventral view of *Dextotricha granulosa*, 50 μm in length; D. Ventral view of *Glaucoma reniforme*, 40 μm in length; E. Ventral view of *Trithigmostoma bavariensis*, 125 μm in length; F. Ventral view of *Euplotes woodruffi*, 100 μm in length.

Class Oligohymenophorea de Puytorac *et al.*, 1974
 Order Philasterida Small, 1967
 Family Cinetochilidae Perty, 1852
 Genus *Sathrophilus* Corliss, 1960

2. *Sathrophilus muscorum* (Kahl, 1931) Corliss, 1960 (Fig. 1B)

Diagnosis. Body size approximate $25 \times 10 \mu\text{m}$ in protargol preparations; body ellipsoidal. Nuclear apparatus composed of an ellipsoidal macronuclear nodule with an ellipsoidal micronucleus, located below mid-body. Contractile vacuole at posterior body end. Cytoplasm colourless. Oral apparatus composed of 3 membranelles, with 1 basal body patch between membranelles 2 and 3, and a slightly curved paroral membrane. Typically 15 somatic ciliary rows with a caudal cilium.

Remarks. This is the first record of the genus *Sathrophilus* in Korea. The Korean population was rare in the soil samples, and the stained specimens were only obtained following the centrifugation of conical tubes containing the fixed cells.

Voucher slides. Two slides, including protargol-impregnated specimens (HG), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107203, NIBRPR0000107204).

Family Loxocephalidae Jankowski, 1964
 Genus *Dexiotricha* Stokes, 1885

3. *Dexiotricha granulosa* (Kent, 1881) Foissner, Berger and Kohmann, 1994 (Fig. 1C)

Diagnosis. Body $40\text{--}60 \times 20\text{--}25 \mu\text{m}$ in protargol preparations; ellipsoidal shape; rigid. Macronuclear nodule spherical to slightly ellipsoidal, positioned at mid-body; 1 micronucleus near macronuclear nodule, spherical. Contractile vacuole at mid-body; excretory pore conspicuous in protargol preparations. Cytoplasm colourless with conspicuous and dense ring structures. 1 caudal cilium, ca. $20 \mu\text{m}$ in length; about 33 somatic kineties.

Remarks. This is the first record of the genus *Dexiotricha* in Korea. The species has conspicuous spherical granules in the cytoplasm, which were easily recognizable during *in vivo* observation. Specimens were easily cultured in mineral water with rice grains. *Aponotohymena australis* and *Paramecium* sp. co-occurred in the cultures.

Voucher slides. Two slides, including protargol-impregnated specimens (GP), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107189, NIBRPR0000107190).

Order Tetrahymenida Fauré -Fremiet in Corliss, 1956

Family Glaucoidae Corliss, 1971
 Genus *Glaucoma* Ehrenberg, 1830

4. *Glaucoma reniforme* Schewiakoff, 1892 (Fig. 1D)

Diagnosis. Body size approximate $40 \times 20 \mu\text{m}$ in protargol preparations; body kidney-shaped. Macronuclear nodule ellipsoidal. Contractile vacuole at posterior body end. Cytoplasm colourless. Typically 26 ciliary rows; caudal cilium lacking.

Remarks. This is the first record of the genus *Glaucoma* in Korea. The species has similar morphological attributes to *G. scintillans*, and they can be distinguished based on the position of a gap in the somatic kinety above the oral apparatus (Foissner *et al.*, 1994).

Voucher slides. Two slides, including protargol-impregnated specimens (GP), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107193, NIBRPR0000107194).

Class Phyllopharyngea de Puytorac *et al.*, 1974
 Order Chlamyodontida Deroux, 1976
 Family Chilodonellidae Deroux, 1970
 Genus *Trithigmotoma* Jankowski, 1967

5. *Trithigmotoma bavariensis* (Kahl, 1931) Foissner, 1987 (Fig. 1E)

Diagnosis. Body size approximate $125 \times 25 \mu\text{m}$ in protargol preparations; body shape broadly ellipsoidal, anterior body end curved leftward, flattened dorsoventrally; flexible. 1 macronuclear nodule at centre of body. Cytoplasm colourless. Movement, without any peculiarities, sliding on soil surface. Conspicuous longitudinal gap between postoral ciliary rows.

Remarks. This is the first record of the genus *Trithigmotoma* in Korea. *Trithigmotoma bavariensis* has a conspicuous gap between postoral ciliary rows in the body centre compared to congeners. For instance, *T. cucullulus* lacks the gap. In addition, the innermost right ciliary row of *T. bavariensis* is shorter than the other rows.

Voucher slides. Two slides, including protargol-impregnated specimens (DC4), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107207, NIBRPR0000107208).

Class Spirotrichea Bütschli, 1889
 Order Euplotida Small and Lynn, 1985
 Family Euplotidae Ehrenberg, 1838
 Genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831

6. *Euplotes woodruffi* Gaw, 1939 (Fig. 1F)

Diagnosis. Body size approximate $100 \times 60 \mu\text{m}$ in protargol preparations; body ellipsoidal, flattened dorsoventrally, both body ends rounded; rigid. T-shaped macronucleus with 1 micronucleus. Cytoplasm colourless. Movement, without any peculiarities, crawling on surface. 9 frontoventral and 5 transverse cirri; 2 marginal and 2 caudal cirri arranged in a row along posterior body margin.

Remarks. *Euplotes woodruffi* has similar morphological attributes to its congener *E. parawoodruffi*. However, Dai *et al.* (2013) suggested *E. parawoodruffi* as a junior synonym of *E. woodruffi*. Kwon *et al.* (2007) reported a Korean population of *E. parawoodruffi*. Interestingly, the SSU rRNA gene similarity matrix of Dai *et al.* (2013) showed relatively higher intraspecific variation (max. 0.8%) than congeneric interspecific variation, which suggested the presence of sibling species within the *E. woodruffi* - *E. parawoodruffi* group. Park *et al.* (2010) reported interspecific variation of less than 1.0% in *Euplotes*, so we refrained from synonymizing the two species in this report.

Voucher slides. Two slides, including protargol-impregnated specimens (GS), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107191, NIBRPR0000107192).

Order Sporadotrichida Fauré-Fremiet, 1961
Family Oxytrichidae Ehrenberg, 1830
Genus *Tachysoma* Stokes, 1887

7. *Tachysoma humicola humicola* Gellért, 1957 (Fig. 2A)

Diagnosis. Body size approximate $30 \times 15 \mu\text{m}$ in protargol preparations; body shape ellipsoidal, flattened dorsoventrally, both body ends rounded. Nuclear apparatus composed of 2 macronuclear nodules with a single micronucleus at left mid-body; micronucleus located between macronuclear nodules. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri composed of 18 cirri on average, as is typical in oxytrichids.

Remarks. *Tachysoma humicola* consists of two subspecies, *Tachysoma. humicola humicola* and *T. humicola longisetum*, which could be distinguished by the length of the posterior-most dorsal cilia ($2\text{--}3 \mu\text{m}$ in *T. humicola humicola* vs. approximate $10 \mu\text{m}$ in *T. humicola longisetum*; Berger, 1999). Of the *Tachysoma* species, Shin and Kim (1993a) reported *T. pellionellum* in Korea.

Voucher slides. Two slides, including protargol-impregnated specimens (TB6), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107205, NIBRPR0000107206).

Family Trachelostylidae Small and Lynn, 1985
Genus *Gonostomum* Sterki, 1878

8. *Gonostomum albicarpathicum* Vd'ačný and Tirjaková, 2006 (Fig. 2B)

Diagnosis. Body size approximate $65 \times 20 \mu\text{m}$ in protargol preparations; body shape elongated ellipsoidal, flattened dorsoventrally, both body ends rounded with slightly narrow anterior end; flexible. Nuclear apparatus composed of 6 macronuclear nodules and several micronuclei, arranged in a longitudinal row. Cortical granules lacking. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri pattern, on average, composed of 3 frontal, 2 frontoterminal, 1 buccal, 7 frontoventral, and 4 pretransverse ventral and transverse cirri; 3 dorsal kineties and 2 caudal cirri; paroral membrane composed of 10 kinetids.

Remarks. *Gonostomum albicarpathicum* can be easily distinguished from congeners by the number of macronuclear nodules (more than two vs. two in the others; Berger, 2011). Three *Gonostomum* species have been reported in Korea (Shin and Kim, 1995; Kim and Shin, 2006; Kim *et al.*, 2016): *G. affine*, *G. algicola*, and *G. strenuum*.

Voucher slides. Two slides, including protargol-impregnated specimens (HG), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107195, NIBRPR0000107196).

Order Stichotrichida Fauré-Fremiet, 1961
Family Pseudourostylidae Jankowski, 1979
Genus *Pseudourostyla* Borror, 1972

9. *Pseudourostyla dimorpha* Foissner, 2016 (Fig. 2D)

Diagnosis. Body size approximate $180 \times 30 \mu\text{m}$ in protargol preparations; body shape elongated ellipsoidal, flattened dorsoventrally, both body ends rounded; flexible. Nuclear apparatus composed of ca. 100 macronuclear nodules and several micronuclei throughout cytoplasm. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri pattern, on average, composed of 9 cirri forming bicorona, 1 buccal, 2 frontoterminal cirri, 8 cirral pairs, and 8 transverse cirri; 7 left and 5 right marginal rows.

Remarks. *Pseudourostyla dimorpha* includes two morphological types, broad and slender (Foissner, 2016). In the moss-covered soil sample, we found only the slender type, which corresponded well with the original description.

Voucher slides. Two slides, including protargol-impregnated specimens (HG), were deposited at the National Institute of Biological Resources in Korea (NIBR

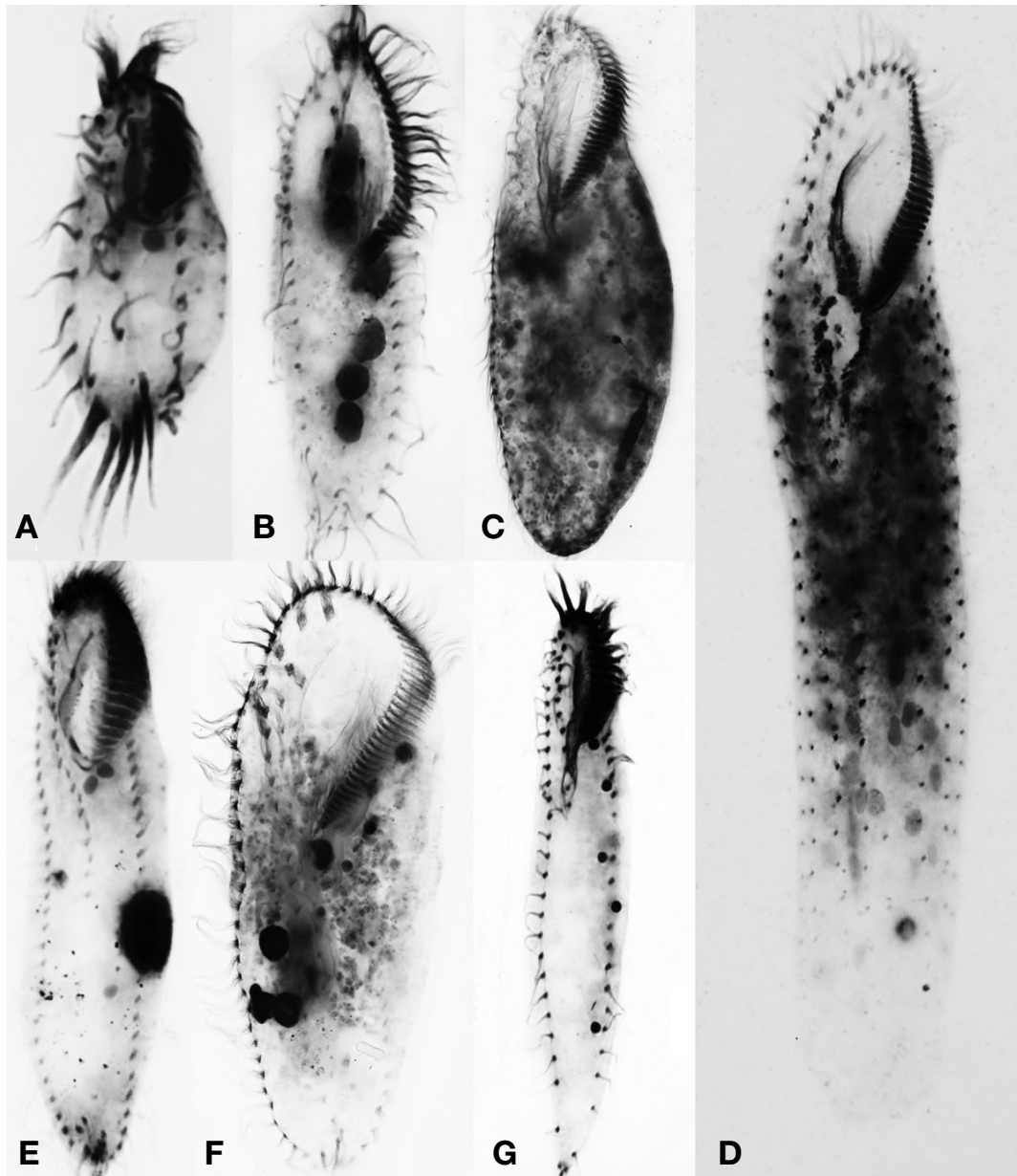


Fig. 2. Protargol-stained specimens. A. Ventral view of *Tachysoma humicola humicola*, 30 μm in length; B. Ventral view of *Gonostomum albicarpaticum*, 65 μm in length; C. Ventral view of *Anteholosticha antecirrata*, 140 μm in length; D. Ventral view of *Pseudourostyla dimorpha*, 180 μm in length; E. Ventral view of *Anteholosticha sphagni*, 65 μm in length; F. Ventral view of *Caudiholosticha stueberi*, 185 μm in length; G. Ventral view of *Paragastrostyla terricola*, 80 μm in length.

PR0000107201, NIBRPR0000107202).

Family Urostylidae Bütschli, 1889
Genus *Anteholosticha* Berger, 2003

10. *Anteholosticha antecirrata* Berger, 2006 (Fig. 2C)

Diagnosis. Body size 120-145 \times 45-65 μm in protargol preparations; 250 \times 75 μm *in vivo*; body shape elongated ellipsoidal, flattened dorsoventrally, both body ends

rounded; flexible. Nuclear apparatus composed of more than 100 macronuclear nodules with several spherical micronuclei throughout cytoplasm. Contractile vacuole anterior of left mid-body. Cortical granules arranged in longitudinal rows; yellow-greenish. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri pattern, on average, composed of 3 frontal cirri, 2 or 3 buccal cirri, 2 frontoterminal cirri, 2 pretransverse ventral cirri, 9-11 transverse cirri, 1 left

and 1 right marginal row; transverse cirri anteriorly displaced; 3 dorsal kineties.

Remarks. The genus *Anteholosticha* is a species-rich taxon with more than 40 reported species (Berger, 2006). As in the species-group name, the etymology refers to the anteriorly displaced transverse cirri that barely project beyond the rear body end (Berger, 2006).

Voucher slides. Two slides, including protargol-impregnated specimens (TB9), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107181, NIBRPR0000107182).

11. *Anteholosticha sphagni* (Grolière, 1975) Berger, 2003 (Fig. 2E)

Diagnosis. Body size approximate $65 \times 15 \mu\text{m}$ in protargol preparations; body shape elongated ellipsoidal, flattened dorsoventrally, both body ends rounded; flexible. Nuclear apparatus composed of ca. 10 macronuclear nodules with several micronuclei at left mid-body. Cortical granules lacking. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri pattern, on average, composed of 3 frontal cirri, 1 buccal cirrus, 2 frontoterminal cirri, ca. 9 midventral cirral pairs, 2 pretransverse ventral cirri, 5 transverse cirri, 1 left and 1 right marginal row; 4 dorsal kineties.

Remarks. The species rarely occurred in our samples, and only two specimens were examined. Further investigation is necessary to provide morphometric data for the Korean population. Of the *Anteholosticha* species, *A. distyla* has a similar morphology to *A. sphagni*, and it mainly differs from the latter based on the number of transverse cirri (typically two and five in *A. distyla* and *A. sphagni*, respectively) (Berger, 2006).

Voucher slides. Two slides, including protargol-impregnated specimens (TB6), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107183, NIBRPR0000107184).

Genus *Caudiholosticha* Berger, 2003

12. *Caudiholosticha stueberi* (Foissner, 1987) Berger, 2003 (Fig. 2F)

Diagnosis. Body size $170\text{--}200 \times 70\text{--}80 \mu\text{m}$ in protargol preparations, approximate $250 \mu\text{m}$ *in vivo*; body shape elongated ellipsoidal, flattened dorsoventrally, both body ends rounded; flexible. Nuclear apparatus composed of 2 macronuclear nodules with several micronuclei at left mid-body. Cortical granules lacking. Cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri pattern, on average, composed of 3 frontal cirri, 1 buccal cirrus, 2 frontoterminal cirri, 17 midventral cirral pairs, 3 transverse cirri, 1 left and 1

right marginal row; 6 dorsal kineties; 3 caudal cirri.

Remarks. Berger (2003) transferred *Holosticha* species with caudal cirri to the new genus *Caudiholosticha*. The species can be distinguished from congeners based on the presence of two macronuclear nodules, body size, and terrestrial habitat (Berger, 2006). Of the *Caudiholosticha* species, only one species was recorded in Korea (Shin and Kim, 1993b): *C. sylvatica*.

Voucher slides. Two slides, including protargol-impregnated specimens (HG), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107187, NIBRPR0000107188).

Genus *Paragastrostyla* Hemberger, 1985

13. *Paragastrostyla terricola* (Foissner, 1988) Berger, 2006 (Fig. 2G)

Diagnosis. Body size approximate $80 \times 15 \mu\text{m}$ in protargol preparations; body shape ellipsoidal with narrow posterior body end, flattened dorsoventrally. Nuclear apparatus composed of approximate 15 macronuclear nodules with several spherical micronuclei at left mid-body. Cortical granules present; cytoplasm colourless. Movement, without any peculiarities, crawling on soil surface. Cirri composed of 3 frontal, 3 frontoterminal cirri, 3 midventral cirral pairs, and a longitudinal row composed of 6 cirri. 1 left and 1 right cirral row. 2 dorsal kineties; caudal cirri present.

Remarks. This is the first record of the genus *Paragastrostyla* in Korea. *Paragastrostyla* includes two species, which can be separated based on the cortical granules (presence in *P. terricola* vs. absence in *P. lanceolata*; Berger, 2006).

Voucher slides. Two slides, including protargol-impregnated specimens (TB1), were deposited at the National Institute of Biological Resources in Korea (NIBR PR0000107197, NIBRPR0000107198).

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