

Unrecorded species of Korean protozoans discovered through the project of ‘Discovery of Korean Indigenous Species’ II

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Through the ‘Discovery of Korean Indigenous Species’ project hosted by the National Institute of Biological Resources (NIBR), new or unrecorded species of various protozoans inhabiting the Korean Peninsula have been discovered. Samples were collected from marine, freshwater, and soil habitats in South Korea and all species were identified and reviewed by experts working on the respective taxonomic group. This study includes 17 unrecorded Korean protozoan species discovered through this project, which belong to four phyla: Amoebozoa Lühe, 1913, Cercozoa Cavalier-Smith, 1998, Euglenozoa Cavalier-Smith, 1981, and Ciliophora Doflein, 1901. Among them, three families (Rhogostomidae Dumack *et al.*, 2017, Parauronematidae Small and Lynn, 1985, and Cyclidiidae Ehrenberg, 1838) and three genera (*Rhogostoma* Belar, 1921, *Parauronema* Thompson, 1967, and *Cyclidium* Müller, 1773) were reported for the first time in Korea. Unlike the previous paper of 2017, this study provides a comprehensive taxonomic account of each species (e.g., species name, collection site, synonyms, specimen vouchers, diagnoses, and figures). Additionally, all the species were assigned Korean names.

Keywords: Amoebozoa, biodiversity, Cercozoa, Ciliophora, Euglenozoa

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INTRODUCTION

Through the ‘Discovery of Korean Indigenous Species’ project hosted by the National Institute of Biological Resources (NIBR), new or unrecorded species of various protozoan species inhabiting the Korean Peninsula have been discovered. Specifically, according to the report from the NIBR, 749 species of new or unrecorded protozoan species were discovered from 2006 to 2021 (NIBR, 2021).

Of the 749 species, only 622 (83%) have been published and officially registered on the ‘National Species List of Korea’. As unpublished species cannot be registered on the ‘National Species List of Korea’, the registration of the remaining 127 unpublished species is still pending.

Some of the unrecorded protozoan species from Korea discovered through the ‘Discovery of Korean Indigenous Species’ project were published in 2017 (Park *et al.*, 2017) in a simple and short checklist format with only species names and collection sites. In this study, additional taxo-

nomic information, including synonyms, specimen vouchers, diagnoses, and figures, is provided as well as species name and collection site. This information can be used for future re-verification.

MATERIALS AND METHODS

Unrecorded Korean protozoan species were discovered through the ‘Discovery of Korean Indigenous Species’ project (2006–2021).

Amoebozoa specimens were collected from freshwater habitats such as streams, ponds, and paddy ditches. Freshwater samples contained water plants and bottom layers. In the laboratory, testate amoebae were isolated under an inverted microscope, CKX53 (Olympus, Tokyo, Japan), the selected specimens were transferred on hole slides, and then slides were mounted with Canada balsam media. Slide specimens were observed under a research microscope, BX53 (Olympus).

Cercozoan and euglenozoan specimens were collected from intertidal marine sediments and freshwater sediments. The sediment materials were covered with lens tissue and then coverslips were placed on the lens tissue. After 12 hours, the coverslips were removed, and samples were observed with a Leica DMR microscope (Leica, Wetzlar, Germany) equipped with a Zeiss Axiocam HR digital camera and its associated software (Axiovision 4.6).

Ciliophora specimens were collected from freshwater and soil habitats. The soil samples were placed in Petri dishes, mixed with mineral water, and then incubated at room temperature (Foissner *et al.*, 2002). Raw cultures were microscopically observed *in vivo* (Leica DM2500, Wetzlar, Germany) from $\times 50$ to $\times 1000$ magnification. Cell staining was performed to use Procedure A method described by Foissner (2014).

Detailed collecting sites including GPS were provided and specimens were deposited at the National Institute of Biological Resources (NIBR), Korea.

RESULTS

Seventeen unrecorded species (belonging to 4 phyla, 5 classes, 7 orders, 9 families, and 10 genera) were reported. Amoebozoa had the highest number of species (10), followed by Ciliophora (4), Cercozoa (2), and Euglenozoa

(1). Korean names were assigned to all species.

LIST OF SPECIES

Phylum Amoebozoa Lühe, 1913
Class Tubulinea Smirnov *et al.*, 2005
Order Arcellinida Kent, 1880 유각변형충목
Family Centropyxidae Jung, 1942 베레모벌레과
Genus *Centropyxis* Stein, 1859 베레모벌레속

1. *Centropyxis aerophila sphagnicola*

Deflandre, 1929

물이끼꼬마베레모벌레 (신칭) (Fig. 1A)

Centropyxis aerophila sphagnicola Deflandre, 1929.

Material examined. Ganghwa-gun (37°45'36"N, 126°23'08"E), Incheon, South Korea; 27 September 2019.

Deposition. NIBRPR0000110247.

World distribution. Cosmopolitan.

Diagnosis. Shell medium-sized, ovoid; abdomen rounded; mouth semicircle, lower margin rounded. Shell length 50 μm ; mouth width 30 μm , length 20 μm .

2. *Centropyxis compressa* van Oye, 1948

눌린베레모벌레 (신칭) (Fig. 1B)

Centropyxis compressa van Oye, 1948.

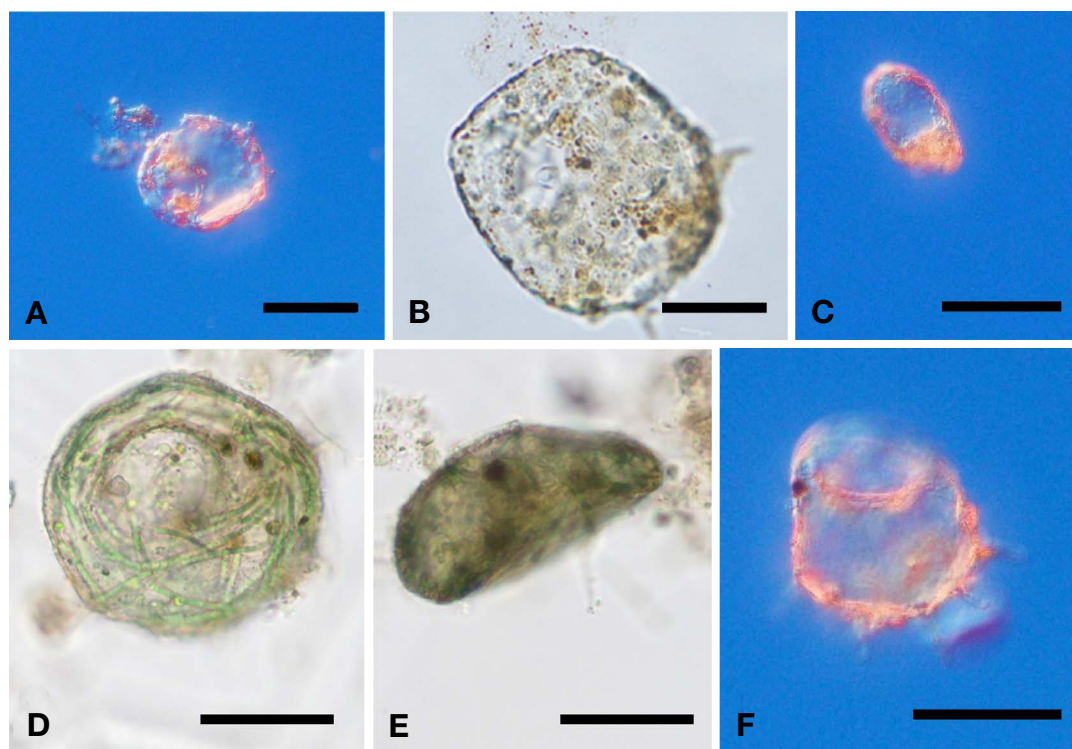


Fig. 1. Photomicrographs of Amoebozoa (A–F). A, *Centropyxis aerophila sphagnicola*; B, *C. compressa*; C, *C. elongata*; D, apertural view of *C. cassis*; E, lateral view of *C. cassis*; F, *C. notonyx*. Scale bars = 50 μm .

Material examined. Ganghwa-gun (37°41'54.20"N, 126°25'52.46"E), Incheon, South Korea; 17 September 2019.

Deposition. NIBRPR0000110854.

World distribution. Cosmopolitan.

Diagnosis. Shell large, oval, width subequal its length, dorsoventrally strongly flattened; short spines located along the edge of the shell. Mouth irregularly rounded, slightly eccentric. Shell length 120 µm, width 130 µm, height 30 µm; mouth width 50 µm, length 30 µm.

3. *Centropyxis elongata* (Penard, 1890)

Thomas, 1959

길쭉베레모벌레 (신칭) (Fig. 1C)

Diffflugia constricta var. *elongata* Penard, 1890.

Material examined. Ganghwa-gun (37°37'22"N, 126°30'05"E), Incheon, South Korea; 14 April 2019.

Deposition. NIBRPR0000110241.

World distribution. Cosmopolitan.

Diagnosis. Shell elliptic in ventral view, elongated with almost parallel or slightly tapering lateral sides towards the mouth; posterior region slightly inflated laterally; mouth circular or semicircular, strongly biased towards the anterior margin, occupying a third of the entire length of the shell. Shell length 50 µm, width 30 µm; mouth width 15 µm.

4. *Centropyxis laevigata* Penard, 1890

매끈베레모벌레 (신칭) (Fig. 1D, E)

Centropyxis laevigata Penard, 1890.

Material examined. Ganghwa-gun (37°48'22"N, 126°24'09"E), Incheon, South Korea; 27 September 2019.

Deposition. NIBRPR0000110855.

World distribution. Cosmopolitan.

Diagnosis. Shell large, almost round dorsally, hemispherical laterally or slightly less than hemisphere; mouth slightly eccentric, deepened inside the shell. Shell diameter 61–135 µm, height 30 µm; mouth diameter 33 µm.

5. *Centropyxis notonyx* Jung, 1942

작은타원베레모벌레 (신칭) (Fig. 1F)

Centropyxis notonyx Jung, 1942.

Material examined. Ganghwa-gun (37°37'22"N, 126°30'05"E), Incheon, South Korea; 14 April 2019.

Deposition. NIBRPR0000110242.

World distribution. Cosmopolitan.

Diagnosis. Shell medium, broadly elliptical in apertural view, relatively high, slightly shorter than long, tapering near the mouth in lateral view; mouth wide elliptical. Shell length 65 µm, width 58 µm, height 50 µm; mouth width 30 µm, length 17 µm.

Genus *Diffflugia* Leclerc, 1815 꽃병벌레속

6. *Diffflugia elegans angustata* (Penard, 1890)

Deflandre, 1926

작은잘록허리원통벌레 (신칭) (Fig. 2A)

Diffflugia elegans Penard, 1890.

Material examined. Yangju-si (37°43'28.12"N, 126°58'35.44"E), Gyeonggi-do, South Korea; 15 May 2018.

Deposition. NIBRPR0000110248.

World distribution. Cosmopolitan.

Diagnosis. Shell relatively large, cylindrical, ending in a thick spike on fundus; surface uneven; mouth rounded. Shell length 90 µm, width 40 µm; mouth diameter 25 µm.

7. *Diffflugia levanderi* Playfair, 1918

매끈꽃병벌레 (신칭) (Fig. 2B)

Diffflugia levanderi Playfair, 1918.

Material examined. Ganghwa-gun (37°45'36"N, 126°23'08"E), Incheon, South Korea; 27 September 2019.

Deposition. NIBRPR0000110243.

World distribution. Cosmopolitan.

Diagnosis. Shell relatively large, ovoid, strongly tapering to the mouth, made of small grains of sand; surface smooth; mouth rounded. Shell length 85 µm, width 55 µm; mouth diameter 32 µm.

8. *Diffflugia pecac* Ogden, 1984

작은손가락벌레 (신칭) (Fig. 2C, D)

Diffflugia pecac Ogden, 1984.

Material examined. Jeju-si (33°28'29"N, 126°35'50"E), Jeju-do, South Korea; 25 June 2021.

Deposition. NIBRPR000011061.

World distribution. Cosmopolitan.

Diagnosis. Shell medium, elongated; aboral region rounded; compressed along the entire length; covered with flattened mineral grains forming a smooth surface; mouth oval. Shell length 60 µm, width 30 µm; mouth width 18 µm.

9. *Diffflugia viscidula* Penard, 1902

방망이꽃병벌레 (신칭) (Fig. 2E)

Diffflugia viscidula Penard, 1902.

Material examined. Paju-si (37°43'49.97"N, 126°50'05.78"E), Gyeonggi-do, South Korea; 19 June 2018.

Deposition. NIBRPR0000107213.

World distribution. Cosmopolitan.

Diagnosis. Shell large, elongated ovoid; mouth rounded, surrounded by fine sand grains. Shell length 180 µm, width 120 µm; mouth diameter 65 µm.

Family Nebelidae Taranek, 1882 병벌레과
Genus *Nebela* (Leidy, 1874) 병벌레속

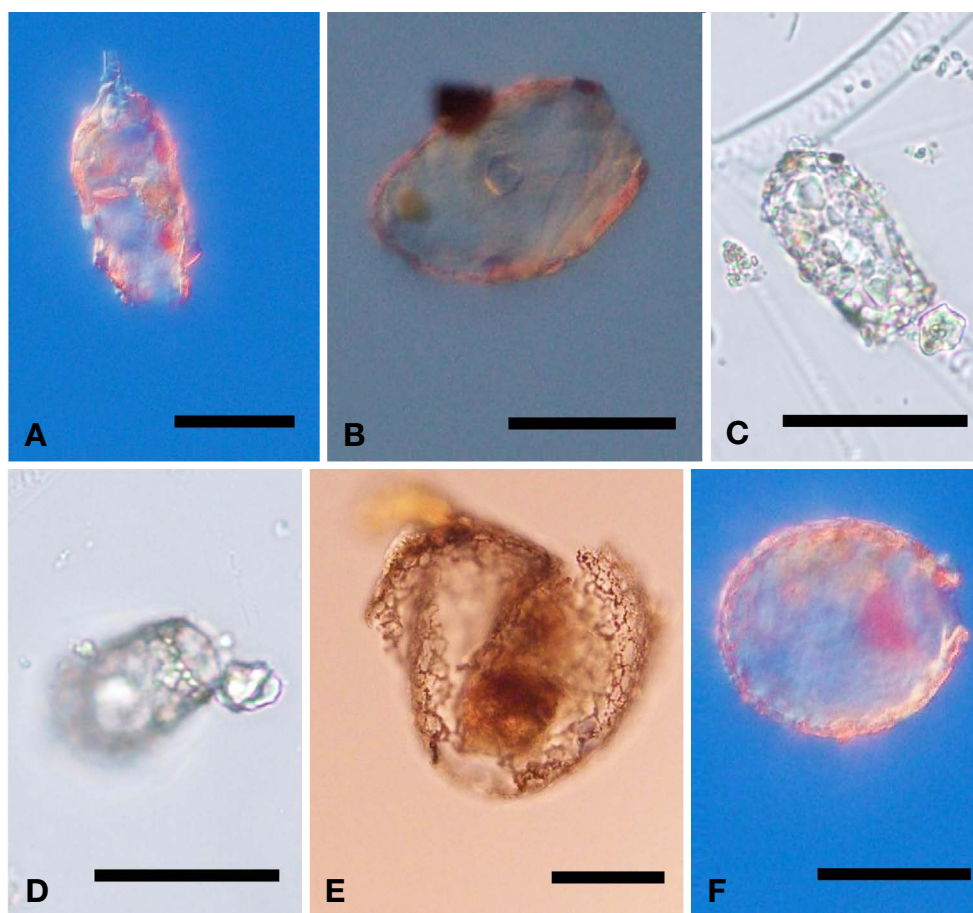


Fig. 2. Photomicrographs of Amoebozoa (A–F). A, *Diffugia elegans angustata*; B, *D. levanderi*; C, lateral view of *D. pecac*; D, apertural view of *D. pecac*; E, *D. viscidula*; F, *Nebela tincta*. Scale bars = 50 μ m.

10. *Nebela tincta* (Leidy, 1879) Awerintzew, 1906

황색병벌레 (신칭) (Fig. 2F)

Hyalosphenia tincta Leidy, 1879.

Material examined. Ganghwa-gun (37°37'22"N, 126°30'05"E), Incheon, South Korea; 14 April 2019.

Deposition. NIBRPR0000110244.

World distribution. Cosmopolitan.

Diagnosis. Shell relatively large, ovoid, laterally compressed; mouth elliptical with two additional pores on the sides. Shell length 85 μ m, width 65 μ m; mouth width 25 μ m.

Phylum Cercozoa Cavalier-Smith, 1998
Class Imbricatea Cavalier-Smith, 2003
Order Euglyphida Wallich, 1864 붕어벌레목
Family Euglyphidae Wallich, 1864 붕어벌레과
Genus Euglypha Dujardin, 1840 붕어벌레속

11. *Euglypha tuberculata* Dujardin, 1841

흑붕어벌레 (신칭) (Fig. 3A)

Euglypha tuberculata Dujardin, 1841.

Material examined. Goyang-si (37°41'49.42"N, 126°54'01.08"E), Gyeonggi-do, South Korea; 15 May 2015.

Deposition. NIBRPR0000109495.

World distribution. Cosmopolitan.

Diagnosis. Shell medium, elongated ovoid in lateral view, circular in cross section; mouth round, surrounded by 1–2 rows of idiosomes. Shell length 80 μ m, width 36 μ m; mouth diameter 12 μ m.

Class Thecofilosea Cavalier-Smith, 2003, emend. Cavalier-Smith, 2011
Order Cryomonadida Cavalier-Smith, 1993
Family Rhogostomidae Dumack *et al.*, 2017
원반아메바과 (신칭)
Genus *Rhogostoma* Belar, 1921 원반아메바과 (신칭)

12. *Rhogostoma minus* Belar, 1921

원반아메바 (신칭) (Fig. 3B, C)

Rhogostoma minus Belar, 1921.

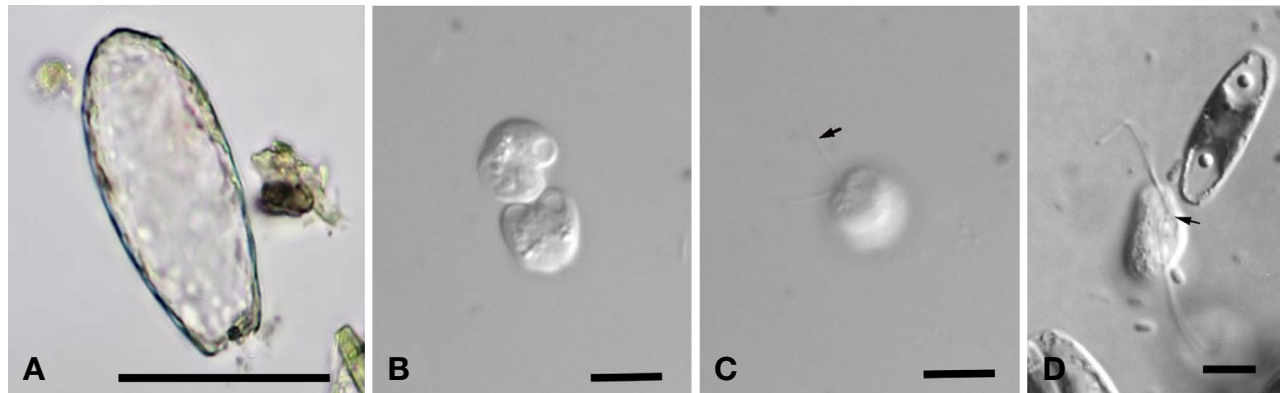


Fig. 3. Photomicrographs of Cercozoa (A–C) and Euglenozoa (D). A, *Euglypha tuberculata*; B, general appearance of *Rhogostoma minus*; C, pseudopodia of *R. minus* (arrow); D, *Parabodo caudatus*, general appearance of cell, showing ventral view. Note flagellar insertion (arrow). Scale bars: A = 50 μm ; B–D = 10 μm .

Material examined. Uichang-gu (35°14'47.82"N, 128°37'07.84"E), Changwon-si, South Korea; 25 April 2021.

Deposition. NIBRPR0000111050.

World distribution. Czech Republic and South Korea.

Diagnosis. Cell broader diameter seen from dorsal end is 6.5–8.5 μm . Cleft-like aperture is beneath the cell hard to see. Pseudopodia emerge through the cleft, can extend many times the cell length and pull the cell along the substratum. The cells pull over leading pseudopodia as trailing pseudopodia retract, progress smooth. Refractive granules; several small vesicles; large lateral contractile vacuole; nucleus usually central. Form groups of several cells.

Phylum Euglenozoa Cavalier-Smith, 1981 유글레나문
Class Kinetoplasta Honigberg, 1963 운동핵편모충강
Order Bodonea Hollande, 1952 보도충목 (신칭)
Family Parabonidae Cavaier-Smith, 2016 겹보도충과
Genus *Parabodo* Leach, 1814 겹파라보도충속

13. *Parabodo caudatus* (Stein, 1878)

Moreira *et al.*, 2004

파라보도충 (Fig. 3D)

Bodo caudatus (Duajrdin, 1841) Stein, 1878.

Material examined. Gimhae-si (35°15'24.7"N, 128°52'05.6"E), Gyeongsangnam-do, South Korea; 12 May 2013.

Deposition. N.A.

World distribution. Austria and South Korea.

Diagnosis. Cells are usually ovate, compressed and are 11–16 μm long with a conspicuous apical mouth. The cells are very flexible but not amoeboid. Two flagella emerge from a subapical pocket; the anterior flagellum is recurved so that it is directed towards the rear and beats with a paddling motion. The acronematic posterior flagellum appears to attach to the cell body or to lie in a groove when

the cells are not moving. Move by skidding or swimming.

Phylum Ciliophora Doflein, 1901 섬모충문
Class Spirotrichea Butschli, 1889 선모강
Subclass Stichotrichia Small & Lynn, 1985
열하모충아강
Order Sporadotrichida Faure-Fremiet, 1961
산포하모목
Family Oxytrichidae Ehrenberg, 1830 침모하모충과
Genus *Monomicrocaryon* Foissner, 2016

14. *Monomicrocaryon saprobia* (Kahl, 1932)

Foissner, 2016

단소핵하모충 (신칭) (Fig. 4A, B)

Oxytricha saprobia Kahl, 1932.

Material examined. Jung-gu (37°22'15.91"N, 126°26'2.48"E), Incheon, South Korea; 31 December 2018.

Deposition. NIBRPR0000110235.

Distribution. Austria, China, Germany, Hungary, USA, and South Korea.

Diagnosis. Cell size 85–95 \times 33–45 μm in protargol preparation; elliptical body shape; micronuclei between two macronuclear nodules; 22–23 adoral zone of membranelles; paroral and endoral optically intersect in an *Oxytricha*-pattern; 18 frontal-ventral-transverse cirri; one left (14 cirri) and one right (16–18 cirri) marginal cirral row; two pretransvers cirri; five transverse cirri; six dorsal kineties; three caudal cirri.

Class Oligohymenophorea de Puytorac *et al.*, 1974
Order Philasterida Small, 1967
Family Parauronematidae Small and Lynn, 1985
유사꼬리섬모충과 (신칭)
Genus *Parauronema* Thompson, 1967
유사꼬리섬모충속 (신칭)

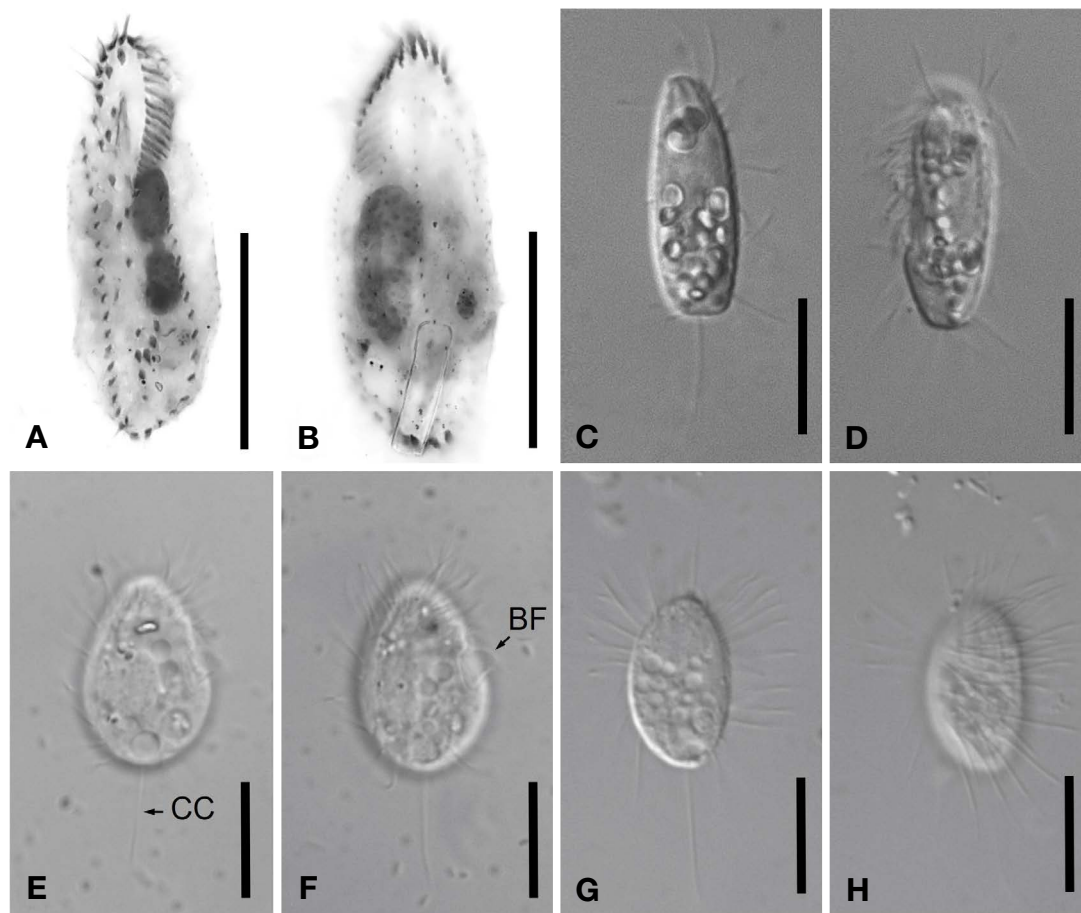


Fig. 4. Photomicrographs of Ciliophora (A-H). A, B, ventral (A) and dorsal (B) view of *Monomicrocaryon saprobia* after protargol impregnation; C, D, general appearance of *Parauronema virginianum*. E, F, general appearance of *Uronema nigricans*; G, H, general appearance of *Cyclidium varibonneti*; BF, buccal field; CC, caudal cilium. Scale bars: A, B = 50 μ m; C-H = 10 μ m.

15. *Parauronema virginianum* Thompson, 1967

버진유사꼬리섬모충 (신칭) (Fig. 4C, D)

Parauronema virginianum Thompson, 1967.

Material examined. Chungcheongnam-do (salinity 30 PSU, 36°59'16.4"N, 126°18'47.7"E), South Korea; 23 February 2016.

Deposition. NIBRPR0000107972.

World distribution. USA (Virginia), Northern China, and South Korea.

Diagnosis. Cells are 23–30 μ m long and elongate with a rounded posterior end and a bluntly pointed anterior end. The area of the buccal cavity is somewhat flattened. The buccal cavity occupies the central part of the anterior ventral surface and contains a tetrahymenal buccal apparatus. The undulating membrane occupies a position along the right margin of the buccal cavity and is about 7 μ m long. Single contractile vacuole.

Family Uronematidae Thompson, 1964

Genus *Uronema* Dujardin, 1841

16. *Uronema nigricans* (Müller, 1786) Florentin, 1901

니그리꼬리섬모충 (신칭) (Fig. 4E, F)

Cyclidium nigricans Müller, 1786.

Material examined. Seo-gu (37°32'33.12"N, 126°37'11.12"E), Incheon, South Korea; 13 January 2020.

Deposition. NIBRPR0000111047.

World distribution. Cosmopolitan.

Diagnosis. Cells are 20–35 μ m long with a truncated apical plate; pellicle thin and inconspicuously notched, with ridges located longitudinally along ciliary rows. There are 10–15 somatic kineties; somatic kinety 1 usually shortened, posterior end extending at about 3/4 to 4/5 of the cell. The oral apparatus is typical of the genus. The membranelle 1 is clearly separated from other membranelles.

Order Pleuronematida Fauré-Fremiet in Corliss, 1956
 Family Cyclidiidae Ehrenberg, 1838
 회전꼬리섬모충과 (신칭)
 Genus *Cyclidium* Müller, 1773
 회전꼬리섬모충속 (신칭)

17. *Cyclidium varibonneti* Song, 2000

배리보회전꼬리섬모충 (신칭) (Fig. 4G, H)
Cyclidium varibonneti Song, 2000.

Material examined. Changwon-si (35°09'47.93"N, 128°34'43.96"E), South Korea; 24 April 2021.

Deposition. NIBRPR0000111053.

World distribution. China and South Korea.

Diagnosis. Cells are 19–27 µm long, oval in ventral view and not flattened. Food granules of equal size are often observed. Distinct pellicle ridges are present between somatic kineties. Somatic cilia are slightly longer than extended paroral cilia, about 10 µm long. Caudal cilium is about 20 µm long. Eleven or 12 somatic kineties. Oral apparatus as described originally; scutica possibly composed of three dikinetids arranged closely beneath posterior end of paroral membrane.

DISCUSSION

Among the taxa discovered in this study, three families (Rhogostomidae Dumack *et al.*, 2017, Parauronematidae Small and Lynn, 1985, and Cyclidiidae Ehrenberg, 1838) and three genera (*Rhogostoma* Belar, 1921, *Parauronema* Thompson, 1967, and *Cyclidium* Müller, 1773) were reported for the first time in Korea. The discovery of new taxa demonstrates the need for future Korean protozoan research. In addition, there is no doubt that the 'Discovery of Korean Indigenous Species' project will provide a great deal of information and help follow-up studies on the discovered species.

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