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Unrecorded species of Korean protozoans discovered through the project of 'Discovery of Korean Indigenous Species' III

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In this paper, we present unrecorded protozoans of Korea that were discovered, through the 'Discovery of Korean Indigenous Species' project hosted by the 'National Institute of Biological Resources (NIBR)'. A taxonomic account is provided for each identified species, offering comprehensive information such as species name, Korean name, collection site, synonyms, specimen vouchers, diagnoses, and figures. This study introduces 13 previously unrecorded Korean protozoan species that are classified into three phyla: Amoebozoa, Cercozoa, and Ciliophora. Notably, the cercozoan family Chlamydophryidae was recorded in Korea for the first time, together with the discovery of three previously unreported genera: *Diaphoropodon* within Cercozoa, and *Metauroleptus* and *Hemicycliostyla* within Ciliophora.

Keywords: Amoebozoa, biodiversity, Cercozoa, Ciliophora

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Introduction

The 'Discovery of Korean Indigenous Species' project, initiated by the Ministry of Environment in 2006, is an ongoing project that was transferred to the 'National Institute of Biological Resources (NIBR)', under the Ministry of Environment. The main objective of this project was to discover unrecognized species on the Korean Peninsula and include them in "the National Species List of Korea". However, it is necessary to verify new or unrecorded species to include them in this list. Therefore, publishing scientific papers play a significant role in the formalization of records for this national project.

This study provides additional taxonomic information, including synonyms, specimen vouchers, diagnoses, and figures of unrecorded indigenous protozoan species discovered during the 'Discovery of Korean Indigenous Species' project from 2016 to 2022. The format of this paper refers to the previously published paper by Chae *et al*., 2022, and this information can be used for future reconfirmation.

Materials and Methods

Amoebozoan and cercozoan specimens were collected from freshwater habitats, such as rivers, lakes, and rice field channels. The freshwater samples consisted of aquatic plants and sediment layers. Testate amoebae were isolated in the laboratory using an inverted microscope, CKX53 (Olympus, Tokyo, Japan). Selected specimens were transferred onto cavity slides and mounted with Canada balsam. The slide specimens were observed under a BX53 light microscope (Olympus).

Ciliophoran specimens were collected from marine and soil habitats. The soil samples were placed in petri dishes, mixed with mineral water, and incubated at room temperature (20°C) (Foissner *et al*., 2002). Raw cultures were observed microscopically in vivo (Leica DM2500; Wetzlar, Germany) at ×50 to ×1000 magnification. Cell staining was performed using the protargol staining method as described by Foissner (2014).

Detailed information in the collection sites is provided in the material examined and the specimens were deposited at the NIBR, Korea.

Results

Thirteen unrecorded species (belonging to three phyla, three classes, four orders, six families, and eight genera) were identified. Amoebozoa had the highest number of species (eight), followed by Ciliophora (four) and Cercozoa (one). Korean names were assigned to all species.

List of Species

Phylum Amoebozoa Lühe, 1913 아메바문
Class Testacealobosea De Saedeleer, 1934
유각엽상근족충강
Order Arcellinida Kent, 1880 유각변형충목
Family Centropyxidae Jung, 1942 베레모벌레과
Genus *Centropyxis* Stein, 1859 베레모벌레속

1. *Centropyxis aculeata minima* van Oye, 1956
(Fig. 1A) 작은뿔베레모벌레 (신칭)
Centropyxis aculeata minima van Oye, 1956

Material examined. Ganghwa-gun (37°37′22″N, 126°30′05″E), Incheon, South Korea; 14 April 2019.
Deposition. NIBRPR0000110246.
World distribution. Cosmopolitan.
Diagnosis. Shell oval in apertural view; aperture strongly eccentric; spines relatively long, located only on the fundus. Shell length, 80–90 μm, height 50 μm, mouth diameter 40 μm.
Remarks. The shape and size of this specimen are consistent with previous descriptions.

2. *Centropyxis cassis spinifera* (Playfair, 1918) Deflandre, 1929 (Fig. 1B) 가시건포도베레모벌레 (신칭)
Difflugia cassis spinifera Playfair, 1918

Material examined. Paju-si (37°41′50.75″N, 126°41′57.82″E), Incheon, South Korea; 2 May 2022.
Deposition. NIBRPR0000111161.
World distribution. Cosmopolitan.
Diagnosis. Shell medium in size, broadly elliptical in apertural view, rounded at the ends; slightly less than a hemisphere in lateral view, flattened above the mouth. Shell length 57 μm, width 58 μm; mouth diameter 28 μm.
Remarks. This specimen is slightly smaller than previous descriptions.

Genus *Cyclopyxis* Deflandre, 1929 둥근벌레속

3. *Cyclopyxis aplanata* (Penard, 1911) Deflandre, 1929 (Fig. 1C) 납작둥근벌레 (신칭)
Centropyxis arcelloides Penard, 1911

Material examined. Ganghwa-gun (37°45′36″N, 126°23′08″E), Incheon, South Korea; 27 September 2019.
Deposition. NIBRPR0000110245.
World distribution. Cosmopolitan.
Diagnosis. Shell large, flattened, margin between dorsal

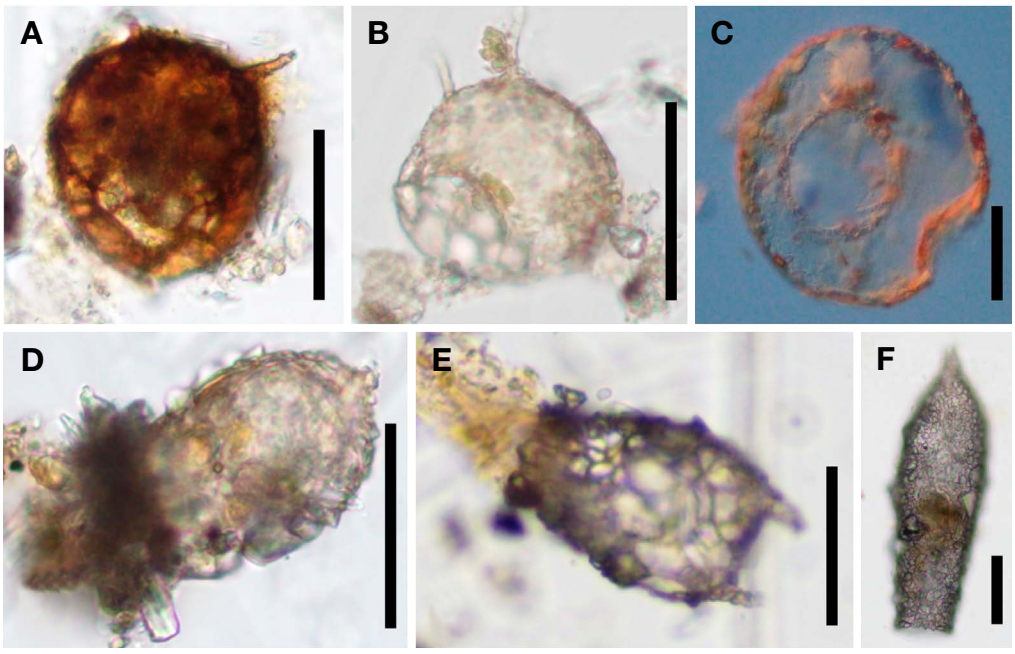


Fig. 1. Photomicrographs of Amoebozoa (A–F). A, *Centropyxis aculeata minima*; B, *C. cassis spinifera*; C, *C. aplanata*; D, *Difflugia amphoralis*; E, *D. bicruris*; F, *D. curvicaulis*. Scale bars: 50 μm.

and ventral sides rounded, dorsal side arcuately curved. The mouth is central, rounded, the contour slightly irregular. Shell diameter 148 μm; mouth diameter 48 μm.
Remarks. Shell diameter of this specimen is shorter than previous descriptions.

Family Difflugiidae Wallich, 1864 꽃병벌레과
Genus *Difflugia* Leclerc, 1815 꽃병벌레속

4. *Difflugia amphoralis* Cash & Hopkinson, 1909 (Fig. 1D) 입가돌기꽃병벌레 (신칭)
Difflugia amphoralis Cash & Hopkinson, 1909

Material examined. Jeju-si (33°28′29″N, 126°35′50″E), Jeju-do, South Korea; 25 June 2021.
Deposition. NIBRPR0000111162.
World distribution. Cosmopolitan.
Diagnosis. Shell transparent, short pear shaped, base of fundus pointed; mouth round, surrounded by a rim of sand grains. Shell length 80 μm, width 49 μm; mouth diameter 24 μm.
Remarks. This specimen is slightly smaller than previous descriptions.

5. *Difflugia bicruris* Gauthier-Lièvre et Thomas, 1958 (Fig. 1E) 두뿔꽃병벌레 (신칭)
Difflugia bicruris Gauthier-Lièvre et Thomas, 1958

Material examined. Pocheon-si (37°47′38.45″N, 127°09′53.07″E), Gyeonggi-do, South Korea; 30 April 2021.
Deposition. NIBRPR0000111163.
World distribution. Cosmopolitan.
Diagnosis. Shell elongate ovoid, in cross section circular, with almost parallel sides; mouth truncated, round. Fundus with two tubular horns, long, straight, and sharply pointed, separated from each other by a U-shaped space. Covered with small mineral grains. Shell length 105 μm, width 58 μm; mouth diameter 35 μm; horn length 20 μm.
Remarks. This specimen is slightly smaller than previous descriptions.

6. *Difflugia curvicaulis* Penard, 1899 (Fig. 1F)
굽은꼬리원통벌레 (신칭)
Difflugia curvicaulis Penard, 1899

Material examined. Deokyang-gu (37°41′49.42″N, 126°54′01.08″E), Goyang-si, South Korea; 15 May 2018.
Deposition. NIBRPR0000109499.
World distribution. Cosmopolitan.
Diagnosis. Shell tubular, narrowing from the fundus to the aperture, usually transparent, colorless, or brown, with a distinct aboral horn curved; shell usually with smooth outline, composed of small and medium sized pieces of flat quartz and fragments of diatoms. Mouth circular, surrounded by regular arrangement of small particles. Shell length 180 μm; mouth diameter 48 μm.
Remarks. The shape and size of this specimen are consistent with previous descriptions.

7. *Difflugia scalpellum* Penard, 1899 (Fig. 2A)
총알꽃병벌레 (신칭)
Difflugia scalpellum Penard, 1899

Material examined. Paju-si (37°41′50.75″N, 126°41′57.82″E), Gyeonggi-do, South Korea; 2 May 2022.
Deposition. NIBRPR0000111164.
World distribution. Cosmopolitan.
Diagnosis. Shell large, colorless, almost transparent especially in the aboral region, elongated tubular; lateral sides gradually widen from the mouth to the middle of the shell, then narrow to the pontied fundus base; covered with small mineral grains. Mouth rounded. Shell length 178 μm, width 69 μm; mouth diameter 35 μm.
Remarks. Shell length of this specimen is shorter than previous descriptions.

Family Phryganellidae Jung, 1942 큰입둥근벌레과
Genus *Phryganella* Penard, 1902 큰입둥근벌레속 (신칭)

8. *Phryganella nidulus* Penard, 1902 (Fig. 2B)
넙적큰입둥근벌레

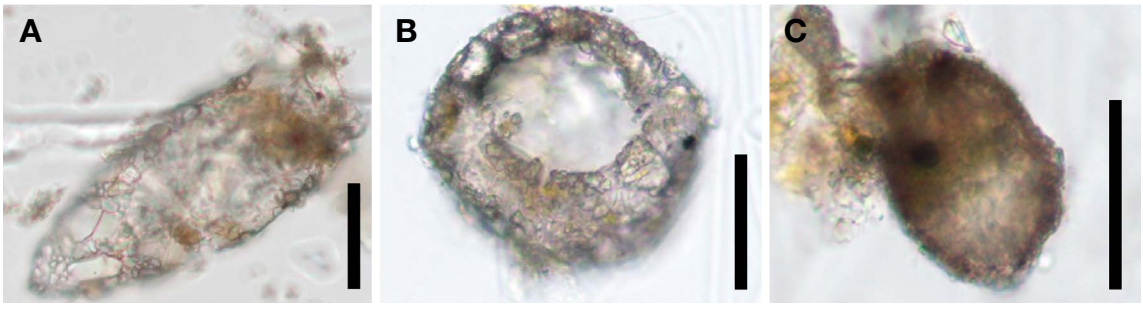


Fig. 2. Photomicrographs of Amoebozoa (A, B) and Cercozoa (C). A, *Difflugia scalpellum*; B, *Phryganella nidulus*; C, *Diaphoropodon mobile*. Scale bars: 50 μm.

Phryganella nidulus Penard, 1902

Material examined. Goheung-gun (37°47′38.45″N, 127° 09′53.07″E), Jeollanam-do, South Korea; 19 August 2021.
Deposition. NIBRPR0000111165.
World distribution. Cosmopolitan.
Diagnosis. Shell large, usually of rough contour, brown, opaque, circular, and hemispherical in lateral view, usually composed of a mixture of diatom frustules and quartz particles; one or more large particles contributes to the irregular outline of the shell and aperture; aperture large and slightly invaginated; pseudopodia slender, but often accompanied by broad lobes of protoplasm extending radially in all directions. Shell length 110 μm; mouth diameter 60 μm.
Remarks. Mouth diameter of this specimen is shorter than previous descriptions.

Phylum Cercozoa Cavalier-Smith, 1998
Class Thecofilosea Cavalier-Smith, 2003, emend. Cavalier-Smith, 2011
Order Tectofilosida Cavalier-Smith, 2003
Family Chlamydophryidae de Saedeleer, 1934
Genus *Diaphoropodon* Archer, 1869

9. *Diaphoropodon mobile* Archer, 1869 (Fig. 2C)
불투명계란벌레 (신칭)
Diaphoropodon mobile Archer, 1869

Material examined. Pocheon-si (37°47′38.45″N, 127°09′ 53.07″E), Gyeonggi-do, South Korea; 30 April 2021.
Deposition. NIBRPR0000111166.
World distribution. Cosmopolitan.
Diagnosis. Shell ovoid or pear shaped, sometimes irregular, membranous, flexible, and composed of organic material, covered with many small particles; color normally brown up to black; mouth flexible, varying in shape, more or less circular, usually hard to detect. Shell length 70 μm, width 48 μm.
Remarks. The shape and size of this specimen are consistent with previous descriptions.

Phylum Ciliophora Doflein, 1901 섬모충문
Class Spirotrichea Butschli, 1889 선모강
Subclass Hypotrichia Stein, 1859 하모충아강
Order Stichotrichida Fauré-Fremiet, 1961 열하모충목
Genus *Metauroleptus* Foissner *et al*., 2008
변화긴꼬리하모충속

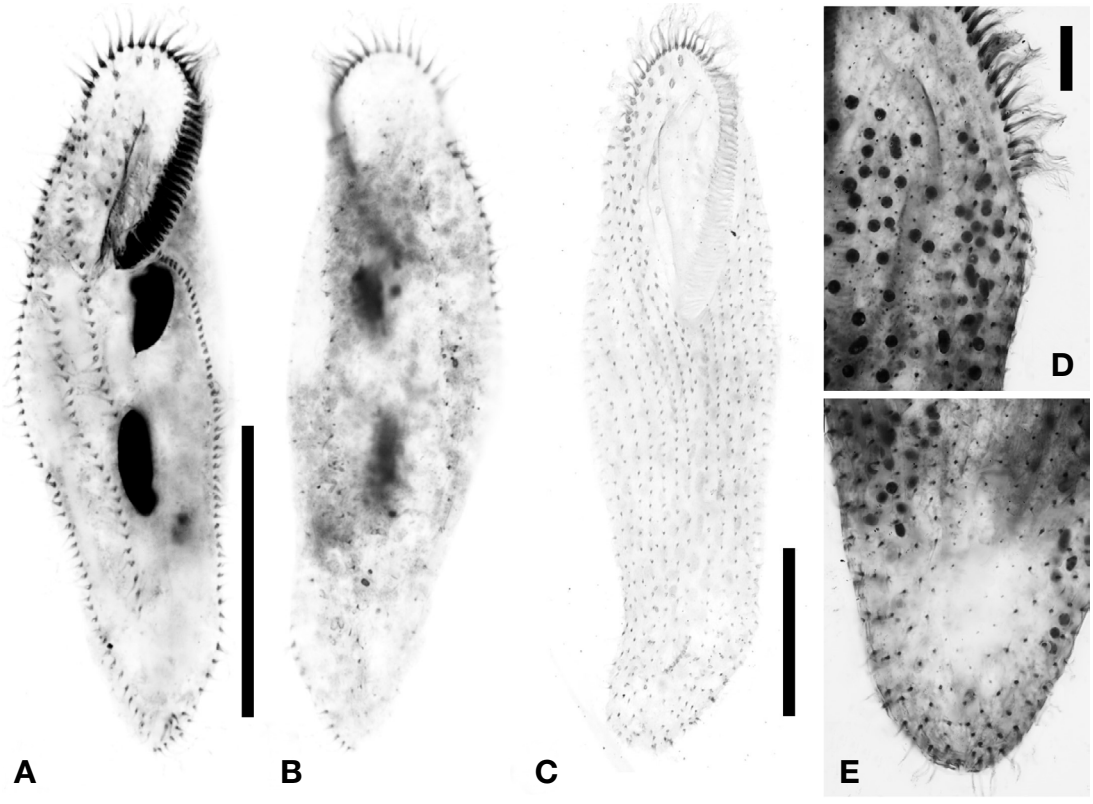


Fig. 3. Photomicrographs of Ciliophora (A–D). A, B, ventral (A) and dorsal (B) view of *Metauroleptus arabicus* after protargol impregnation; C, ventral view of *Hemicycliostyla franzi* after protargol impregnation; D, E, anterior (D) and posterior (E) dorsal view of *H. franzi* after protargol impregnation. Scale bars: 50 μm (A, D), 10 μm (C).

10. *Metauroleptus arabicus* Foissner *et al*., 2008 (Figs. 3A, B) 아라비아하모충(신칭)
Metauroleptus arabicus Foissner *et al*., 2008

Material examined. Jeju-si (33°24′15.2″N, 126°20′59.6″E), Jeju-do, South Korea; 1 July 2020.
Deposition. NIBRPR0000111140.
Distribution. Saudi Arabia, and South Korea.
Diagnosis. Cell size 130−170 × 40−60 μm in vivo; body slender to elliptical shape; 37−40 adoral zone of membranelles; 2 macronuclear nodules with 2 to 3 micronuclei; 4 frontal cirri; 4 frontoventral rows; 4−6 buccal cirri; 1 left (45−54 cirri) and 1 right (45−55 cirri) marginal cirral row; 2−3 transverse cirri; 3 dorsal kineties; 3−6 caudal cirri.
Remarks. The Korean population of *Metauroleptus arabicus* corresponds to the Saudi Arabian population in terms of body size, four frontal cirri, four frontoventral rows and three dorsal kineties (Foissner *et al*., 2008).

Order Urostylida Jankowski, 1979 미주하모목
Family Hemicycliostylidae Lyu *et al*., 2018
Genus *Hemicycliostyla* Stokes, 1886
반원하모충(신칭)

11. *Hemicycliostyla franzi* (Foissner, 1987) Paiva *et al*., 2012 (Figs. 3C, D) 녹색반원하모충(신칭)
Pseudourostyla franzi Foissner, 1987

Material examined. Goesan-gun (36°48′38.21″N, 127°57′37.11″E), Chungcheongbuk-do, South Korea; 1 September 2015.
Deposition. NIBRPR0000107159, NIBRPR0000107160.
Distribution. China, Costa Rica, Australia, Kenya, Namibia, and South Korea.
Diagnosis. Cell size 160−230 μm × 40−55 μm in vivo; body highly flexible with elliptical shape. Exactly, 232−323 macronuclear nodules with more than 10 micronuclei. Contractile vacuole at left mid-body. Cortical granules arranged around cirri and dorsal bristles. Exactly, 60−75 adoral membranelles, bicoronal frontal cirri rows, 3 or 4 buccal cirrus and 1−3 parabuccal cirri present, invariably 2 frontoterminal cirri, 7−11 transverse cirri present. midventral cirral pairs present. Exactly, 7 or 8 left and 7 or 8 marginal cirral rows. Invariable 4 bipolar dorsal kineties, caudal cirri lacking.
Remarks. *Hemicycliostyla sphagni* differs from *H. franzi* in the following features: body color (green vs. brown) and transverse cirri (lacking vs. invariably present) (Paiva

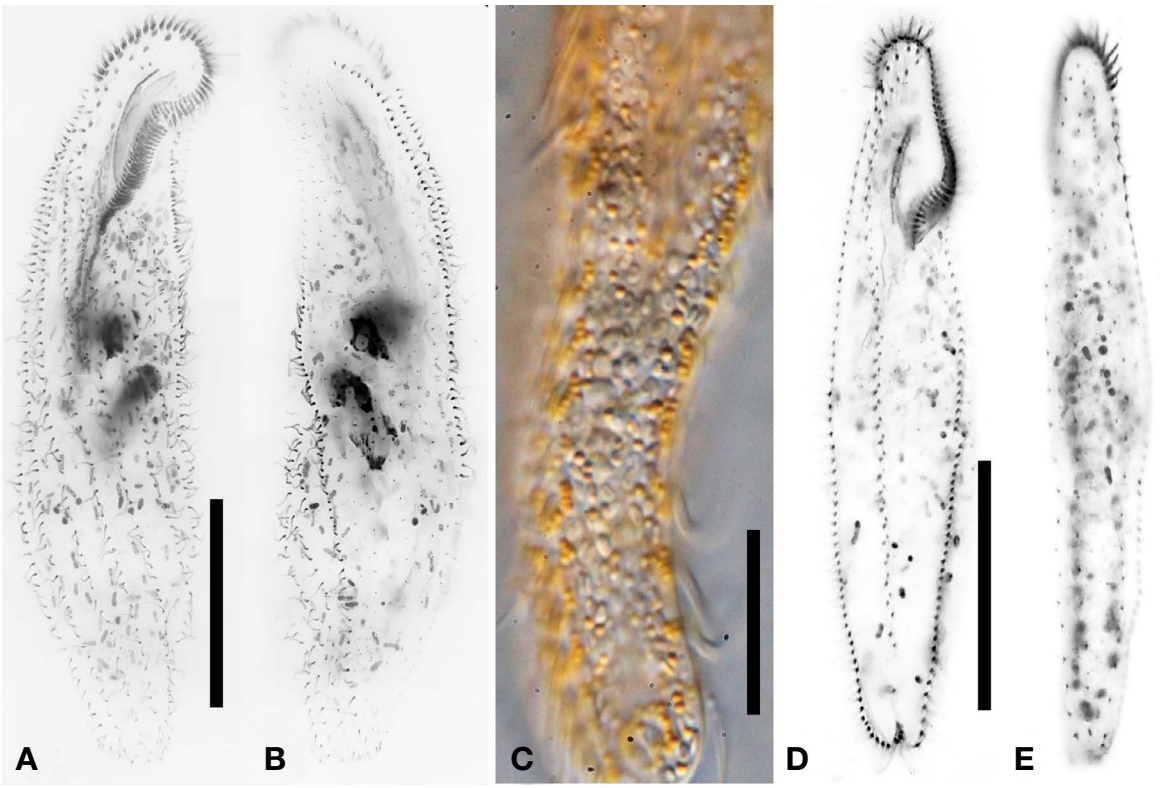


Fig. 4. Photomicrographs of Ciliophora (A−D). A, B, ventral (A) and dorsal (B) view of *Hemicycliostyla sphagni* after protargol impregnation; C, ventral view of *Pseudokeronopsis songi* from life; D, E, ventral (D) and dorsal (E) view of *P. songi* after protargol impregnation. Scale bars: 50 μm (A, D), 10 μm (C).

et al., 2012).

12. *Hemicycliostyla sphagni* Stokes, 1886 (Figs. 4A, B)
갈색반원하모충 (신칭)
Hemicycliostyla sphagni Stokes, 1886

Material examined. Jeju-si (33°24′15.2″N, 126°20′59.6″E), Jeju-do, South Korea; 25 October 2018.
Deposition. NIBRPR0000110229.
Distribution. Austria, Costa Rica, and South Korea.
Diagnosis. Cell size 230−340 × 50−90 μm from after protargol impregnation; body flexible with slender to elliptical shape; 67−76 adoral zone of membranelles; scattered many macronuclear nodules; 15−23 frontal cirri in bicorona; midventral complex composed of 2 midventral cirral rows; 4 or 5 left and 3 or 4 right marginal rows; transverse cirri lacking; 6 dorsal kineties; 5 or 6 caudal cirri.
Remarks. *Hemicycliostyla sphagni* should be compared with two congeners, *H. lacustris* and *H. marina*. *Hemicycliostyla sphagni* differs from *H. lacustris* based on body length (230−340 μm vs. about 190 μm) and number of adoral zone membranelles (67−76 vs. 50−60) (Gellért and Tamás, 1958). *Hemicycliostyla sphagni* can be distinguished from *H. marina* based on its habitat (terrestrial vs. marine) (Kahl, 1932).

Family Pseudokeronopsidae Borror & Wicklow, 1983
유사각모과
Genus *Pseudokeronopsis* Borror & Wicklow, 1983
위각모충속

13. *Pseudokeronopsis songi* Li *et al*., 2017 (Figs. 4C, D)
송위각모충 (신칭)
Pseudokeronopsis songi Li *et al*., 2017

Material examined. Seogwipo-si (33°14′9.21″N, 126°35′55.338″E), Jeju-do, South Korea; 24 August 2017, by seawater (salinity 38‰).
Deposition. NIBRPR0000110237.
Distribution. China and South Korea.
Diagnosis. Cell size 117−204 × 21−50 μm in protargol preparation; elongated elliptical body shaped, slim body; one contractile vacuole at about posterior left body; two types of cortical granule, yellowish cortical granule around cirri, blood cell shaped colorless granule; 35−56 adoral zone of membranelles; scattered numerus macronuclear nodules; 4−7 anterior frontal cirri, 4−7 posterior frontal cirri, bicorona; 2 frontoterminal cirri; 1 buccal cirrus; 24−40 midventral pairs; 3−6 transverse cirri; 1 left (36−60) and 1 right (42−68) marginal cirral row; 3 or 4 dorsal kineties; caudal cirri lacking.
Remarks. The Korean population of *Pseudokeronopsis songi* corresponds to the Chinese population in body size, two types of cortical granules, five transverse cirri, and three dorsal kineties (Li *et al*., 2017). *Pseudokeronopsis songi* differs from *P. pararubra* in the number of transverse cirri (3−6 vs. 6−12) and dorsal kineties (3 or 4 vs. 4−8) (Li *et al*., 2017). *Pseudokeronopsis songi* differs from *P. carnea* in the number of dorsal kineties (3 or 4 vs. 5−8) (Li *et al*., 2017). *Pseudokeronopsis songi* differs from *P. rubra* in the number of transverse cirri (3−6 vs. 6−9) and dorsal kineties (3 or 4 vs. 5−8) (Wirnsberger *et al*., 1987).

Discussion

Among the taxa discovered in this study, one family (Chlamydophryidae) and three genera (*Diaphoropodon*, *Metauroleptus*, and *Hemicycliostyla*) were reported for the first time in South Korea.

Acknowledgements

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