

New Record of Three Marine Ciliates (Protozoa, Ciliophora) from South Korea

Atef Omar¹, Ji Hye Moon², Jae-Ho Jung^{2,*}

¹Industry Academy Cooperation Group, Gangneung-Wonju National University, Gangneung 25457, Korea

²Department of Biology, Gangneung-Wonju National University, Gangneung 25457, Korea

ABSTRACT

During a field survey of Korean marine ciliates, we collected three ciliate species from the eastern coastal waters of the Yellow Sea. Based on the observation of living and protargol and wet silver nitrate stained cells, the ciliates, belonging to the classes Spirotrichea and Oligohymenophorea, were identified as *Cardiostomatella vermiformis* (Kahl, 1928) Corliss, 1960, *Parallelostrombidium paraellipticum* Song et al., 2018, and *Pleuronema paucisaetosum* Pan et al., 2015. Both *Parallelostrombidium paraellipticum* and *Pleuronema paucisaetosum* were described only from their type localities, i.e., brackish water, suggesting that they tolerate a broad range of salinity, while *Cardiostomatella* is marine ciliate and seems to be cosmopolitan. These three species were reported for the first time in Korea. Brief descriptions, remarks to justify their identity and to compare the present isolates with similar taxa, and photomicrographs were provided for the three species.

Keywords: marine habitat, oligotrich, redescription, scuticociliates, taxonomy

INTRODUCTION

Free-living ciliates are one of the most abundant and diverse protistan groups in aquatic and terrestrial habitats, with more than 4500 described species (Finlay et al., 1996, 2004; Foissner et al., 2008; Lynn, 2008; Agatha, 2011; Foissner, 2016). During the last few decades, the number of described ciliate species dramatically increased worldwide (Foissner et al., 2002; Song et al., 2009; Foissner, 2016; Foissner and Berger, 2021). As a result, marine ciliate biodiversity has been receiving more attention, especially in the Yellow Sea (Song et al., 2009). Also, the number of ciliate species recorded in Korean marine waters is highly increasing due to the growing interest in ciliate diversity and the funds supplied by the Korean government (Jung et al., 2017; Kwon et al., 2019; Kim et al., 2020; Omar et al., 2021). In 2021, we collected several ciliate species from marine habitats. The present study briefly describes three marine ciliates, *Cardiostomatella vermiformis*, *Parallelostrombidium paraellipticum*, and *Pleuronema paucisaetosum*, from the Yellow Sea.

MATERIALS AND METHODS

The three species were collected from coastal waters from the Yellow Sea of Korea. Details about sample locality are described in the 'Material examined' section for each species. Samples were collected by gently stirring up surface water and bottom sediments and were transferred to the laboratory within a few days. All cultures were kept in Plant culture dishes at room temperature (ca. 18°C) for a few months. Also, 1–3 wheat grains were supplied for each culture to increase the growth of bacteria as food resource. The morphology of each species was studied using a stereomicroscope (Olympus SZ11, Japan), and light microscope (Olympus BX53) with differential interference contrast at magnifications of 40–1,000×, and photomicrographs were captured using a digital camera (Olympus DP74). The protargol powder was synthesized using the method of Pan et al. (2013) and Kim and Jung (2017). The protargol-impregnated specimens were prepared using the 'procedure A' method of Foissner (2014). The 'wet' silver nitrate impregnation was also conducted using the method of Foissner (2014). The differential through-focal images of the protargol-impregnated specimens were merged

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***To whom correspondence should be addressed**

Tel: 82-33-640-2312, Fax: 82-33-640-2867
E-mail: jhjung@gwnu.ac.kr

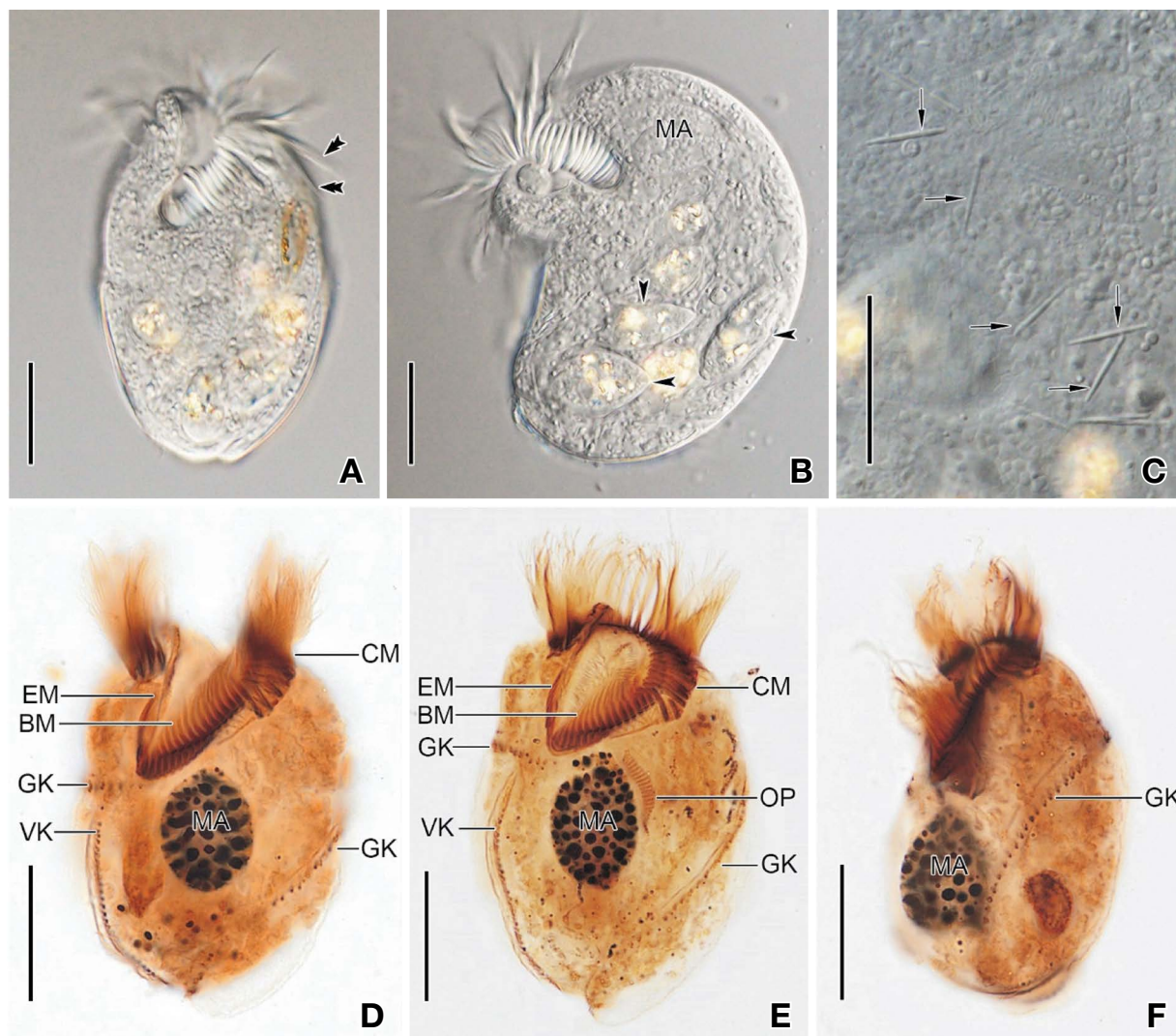


Fig. 1. *Parallelostrombidium paraellipticum* in life (A-C) and after protargol impregnation (D-F). A, Ventral view showing the body shape, the oral apparatus, the distinct anterior protrusion, and the thigmotactic membranelles (double arrowheads); B, A compressed specimen showing the macronucleus and the food vacuoles filled with flagellates and scuticociliates (arrowheads); C, Cytoplasm containing needle-shaped extrusomes (arrows); D, Ventral view showing the collar and buccal membranelles and the girdle and ventral kineties; E, A very early stage of division showing the location of the oral primordium; F, Lateral view showing the slight flattening of the cell and the girdle kinety spiraling around the cell. BM, buccal membranelles; CM, collar membranelles; EM, endoral membrane; GK, girdle kinety; MA, macronucleus; OP, oral primordium; VK, ventral kinety. Scale bars: A-F=20 μ m.

using the software of Helicon Focus 8.1.0 (HeliconSoft Ltd., Ukraine). The basic terminology and taxonomic classification mainly follow those of Lynn (2008).

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Subphylum Intramacronucleata Lynn, 1996

Class Spirotrichea Bütschli, 1889
Subclass Oligotrichia Bütschli, 1887/1889
Order Strombidiida Petz and Foissner, 1992
Family Strombidiidae Fauré-Fremiet, 1970
Genus *Parallelostrombidium* Agatha, 2004
Subgenus *Asymptokinetum* Agatha and Strüder-Kypke, 2014

¹**Parallelostrombidium paraellipticum*
Song et al., 2018 (Fig. 1)

Korean name: ¹*작은평형민소모충

Material examined. Coastal water sample (30‰) collected from a sandy beach in Janghang-eup, Seocheon-gun, Chungcheongnam-do, Republic of Korea (36°00'53.61"N, 126°39'44.14"E) on 21 Jan 2021.

Diagnosis. Size about 60 × 40 μm in vivo and 56–65 × 36–46 μm after protargol impregnation (n = 4). Body obovate, asymmetrical, dorsoventrally slightly flattened with distinct apical protrusion. Cytoplasm colorless with many food vacuoles about 20 μm across, containing heterotrophic flagellates and scuticociliates. Extrusomes about 10 μm long, needle-shaped, arrangement is difficult to recognize in vivo, in protargol-impregnated specimens they appear as faint line near girdle kinety. Macronucleus in mid-body, broadly elliptical, 18–22 × 13–14 μm in size; micronucleus not recognizable. Adoral zone occupies about one-third of body length, consisting of 23–24 collar membranelles, of which two thigmotactic, and 10–14 buccal membranelles. Girdle kinety spirals about one and half times around the body, distal end on ventral side slightly left to anterior end of ventral kinety, composed of 70–78 dikinetids. Ventral kinety parallel to posterior portion of girdle kinety consists of 33–42 dikinetids. In dividing cells, oral primordium appears de novo near mid-body of ventral side and left of body's midline.

Distribution. China (Song et al., 2018) and South Korea (present study).

Remarks. The Korean population of *Parallelostrombidium paraellipticum* is very similar to the Chinese population as described by Song et al. (2018). However, they differ in few features including the cell size after protargol impregnation (56–65 × 36–46 μm vs. 39–57 × 28–43 μm), the size of extrusomes (10 vs. 12 μm long), the number of collar and buccal membranelles (23–24 and 10–14 vs. 17–21 and 9–11, respectively), the number of dikinetids in ventral kinety (33–42 vs. 17–26), and the habitat (marine water with salinity of 30‰ vs. brackish water with salinity of 2.5‰) (Table 1). The location of the oral primordium in dividing cells is identical to that of the Chinese type population (Song et al., 2018). Considering the flattened body shape, size, and numbers of adoral membranelles and dikinetids in girdle and ventral kinety, *P. paraellipticum* is most similar to *P. ellipticum* Liu et al., 2015. However, they differ in the arrangement of extrusomes (one row vs. three rows), the distance between the anterior end of girdle kinety and ventral kinety (far vs. very close), and the horizontal (vs. oblique) girdle kinety on the dorsal side (Xu et al., 2006; Liu et al., 2015; Song et al., 2018). Also, *P. paraellipticum* is similar to *P. paratum* Xu et al., 2006 and *P. obesum* Liu et al., 2015 in the dorsoventrally flattened body and the presence of thigmotactic membranelles. However, *P. paraellipticum* can be distinguished from *P. paratum* by the

number of the collar and buccal membranelles (23–24 and 10–14 vs. 26–30 and 15–19, respectively). *Parallelostrombidium obesum* can be distinguished from *P. paraellipticum* in having a doliform (vs. obovate) body shape, more dikinetids in the girdle kinety (101–164 vs. 70–78), and the number of extrusomes rows (3 vs. 1) (Xu et al., 2006; Liu et al., 2015).

Voucher slides. One slide with protargol-impregnated specimens was deposited in the National Marine Biodiversity Institute of Korea (MABIK PR00044184).

Class Oligohymenophorea de Puytorac et al., 1974

Subclass Scuticociliatia Small, 1967

Order Philasterida Small, 1967

Family Loxocephalidae Jankowski, 1964

¹*Genus *Cardiostomatella* (Kahl, 1928) Corliss, 1960

²**Cardiostomatella vermiformis* (Kahl, 1928)

Corliss, 1960 (Figs. 2, 3)

Material examined. Coastal water sample (31.5‰, 19.8°C) collected from a sandy beach in Jung-gu, Incheon, Republic of Korea (37°27'14"N, 126°22'10"E) on 11 Oct 2021.

Diagnosis. Size 153.5–295.1 × 35.9–59.2 μm after protargol impregnation (n = 5); cell elongate, appears gradually flattened posteriorly because of the twisted body and the wrinkled cell surface. Macronucleus moniliform, composed of 4–7 nodules, each about 20 × 10 μm in size, longitudinally arranged in body's mid-line; micronuclei spherical to elliptical, 1–5 in number, usually 3.5 × 3.0 μm each, located near macronuclear nodules. Contractile vacuole subterminally without collecting canals, about 15 μm across, with an excretory pore on right side. Extrusomes filiform, densely and perpendicularly arranged underneath cortex, forming cortical seam. Free-swimming either clockwise or anticlockwise. Somatic kineties composed of 84–106 rows (excluding about 4–7 postoral kineties) with distinct suture above oral apparatus; about 10 caudal cilia. Oral opening small and indistinct at low magnification; 3 membranelles, narrow and obliquely arranged, each with three ciliary rows; paroral membrane C-shaped.

Distribution. China (Li et al., 2006; Wang et al., 2007), Denmark (Fenchel et al., 1995), England (Carey and Maeda, 1985), France (Dragesco, 2002), Germany (Kahl, 1928; Hartwig, 1973), Poland (Czapik and Jordan, 1977), Saudi Arabia (Al-Rasheid, 2001), Somalia (Ricci et al., 1982), USA (Borror, 1963), and South Korea (present study).

Remarks. The Korean population of *Cardiostomatella vermiformis* agrees very well with previous descriptions in size, general morphology, buccal apparatus, somatic ciliature, and

Korean name: ¹*작은입섬모충속, ²*다핵작은입섬모충

Table 1. Comparison of main morphological features between *Parallelostrombidium paraei* and similar congeners. Body size from life.

Characteristics	<i>P. paraei</i>	<i>P. paraei</i> (type population)	<i>P. ellipticum</i>	<i>P. paralatam</i>	<i>P. obesum</i>	<i>P. kahl</i>	<i>P. dragescoi</i>	<i>P. jankowskii</i>
Body size (µm)	~60×40	40-60×30-45	50-70×30-45	55-80×50-65	65-110×55-70	55-80×40-50	50-70×35-45	105-150×40-75
Body shape	Obovate, dorsoventrally slightly flattened	Obovate, dorsoventrally slightly flattened	Ellipsoidal or oval	Ellipsoidal, dorsoventrally slightly flattened	Doliform, dorsoventrally slightly flattened	Obconical	Obovate, dorsoventrally slightly flattened	Elongate obconical
Extrusomes size (µm)	~10	~12	~9	~10	~10	~10	~10	~16
Extrusomes, number of rows	1	1	3	1	3	1	1	1
Collar membranelles, number	23-24	17-21	18-22	26-30	24-34	47-57	18-22	24-27
Buccal membranelles, number	10-14	9-11	9-13	15-19	12-20	6-11	6-7	12-18
Girdle kinety, number of dikinetids	70-78	63-75	57-80	71-99	101-164	129-195	50-71	86-109
Ventral kinety, number of dikinetids	33-42	17-26	20-30	32-42	23-51	69-90	13-23	24-49
Distance between girdle and ventral kineties	Far	Far	Very close	Very close	Very close	Far	Close	Close
Girdle kinety, orientation on dorsal side	Horizontal	Horizontal	oblique	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal
Salinity (‰)	30.0	2.5	25.8	30.0	17.0	33.2	18.2	24.6
Reference, country	Present study, South Korea	Song et al. (2018), China	Liu et al. (2015), China	Xu et al. (2006), China	Liu et al. (2015), China	Song et al. (2018), China	Song et al. (2018), China	Song et al. (2018), China

Table 2. Comparison of main morphological features between different populations of *Cardiostomatella vermiformis*. Body size from life, the size of the Korean population was calculated from the stained cells by adding 15% preparation shrinkage.

Characteristics	South Korea	Germany (type population)	China	Saudi Arabia	USA	Denmark	Poland	France
Body size (µm)	177-340×40-67	200-350	160-460×30-120	90-510×41-117	350-450×70-117	300-500	200-300	150-600
Macronuclei, number	4-7	2-8	7-16	7-14	10	8	5-7	4-17
Micronuclei, number	1-5	5-8	1-5	5-9	?	?	?	2-11
Somatic kineties, number	84-106	?	96-130	89-109	>150	?	50	85-120
Reference	Present study	Kahl (1928)	Wang et al. (2007)	Al-Rasheid (2001)	Borror (1963)	Fenchel et al. (1995)	Czapik and Jordan (1977)	Dragesco (2002)

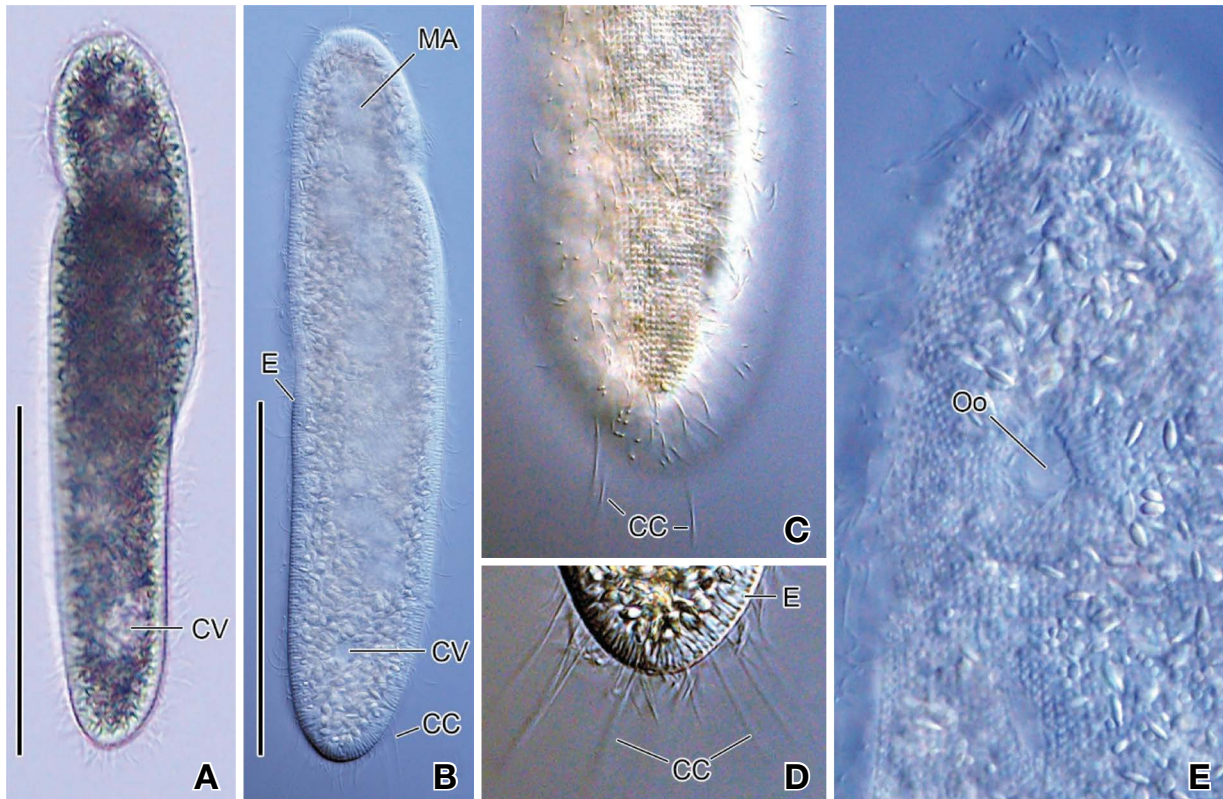


Fig. 2. *Cardiostomatella vermiformis* in life. A, B, Ventral (A) and focus through (B) view showing the body shape, the wrinkles on body surface, the subterminal contractile vacuole, the moniliform macronucleus, and the extrusomes forming seam underneath cortex; C, D, Posterior region of body showing the caudal cilia; E, Ventral view showing the small oral apparatus. CC, caudal cilia; CV, contractile vacuole; E, extrusomes; MA, macronucleus; Oo, oral opening. Scale bars: A, B= 100 μ m.

habitat (Kahl, 1928; Borror, 1963; Hartwig, 1973, 1980; Czapiak and Jordan, 1977; Ricci et al., 1982; Fenchel et al., 1995; Al-Rasheid, 2001; Dragesco, 2002; Li et al., 2006; Wang et al., 2007). However, *C. vermiformis* shows high intraspecific variations; for instance, the body length ranges from 90 μ m to 510 μ m, the number of macronuclear nodules ranges from 3 to 17, and the number of somatic kineties ranges from 50 to more than 150 (Table 2). These variations suggest that the species possibly represents species-complex and/or multiple cryptic species. Up to date, four species are assigned to the genus *Cardiostomatella*: *C. chesapeakeensis* Small and Lynn, 1985, *C. minuta* Dragesco, 1965, *C. mononucleata* Dragesco, 1963, and *C. vermiformis*. The four species differ mainly by the number of macronuclear nodules: two in *C. chesapeakeensis*, one in both *C. minuta* and *C. mononucleata*, and 3–17 in *C. vermiformis* (Dragesco, 1963, 1965; de Puytorac et al., 1981; Carey and Maeda, 1985; Small and Lynn, 1985; Wang et al., 2007). Furthermore, *C. minuta* can be easily separated from *C. mononucleata* by the smaller body size

(75 \times 25 μ m vs. 300 \times 70 μ m) (Dragesco, 1963, 1965; Carey and Maeda, 1985).

Voucher slides. Three slides were deposited in the National Marine Biodiversity Institute of Korea (protargol: MABIK PR 00044183; wet silver nitrate: MABIK PR00044185, 44186). Other slides with protargol-impregnated specimens were deposited in the Jung-lab (J.-H. Jung) in Gangneung-Wonju National University (GUC005667, 5668).

Order Pleuronematida Fauré-Fremiet in Corliss, 1956
Family Pleuronematidae Kent, 1881
Genus *Pleuronema* Dujardin, 1841

¹Pleuronema paucisaetosum* Pan et al., 2015 (Fig. 4)**

Material examined. Coastal water sample (28‰) collected from a sandy beach in Janghang-eup, Seocheon-gun, Chungcheongnam-do, Republic of Korea (36°00'54.47"N, 126°39'48.62"E) on 21 Jan 2021.

Korean name: ¹*빈모촉사모충

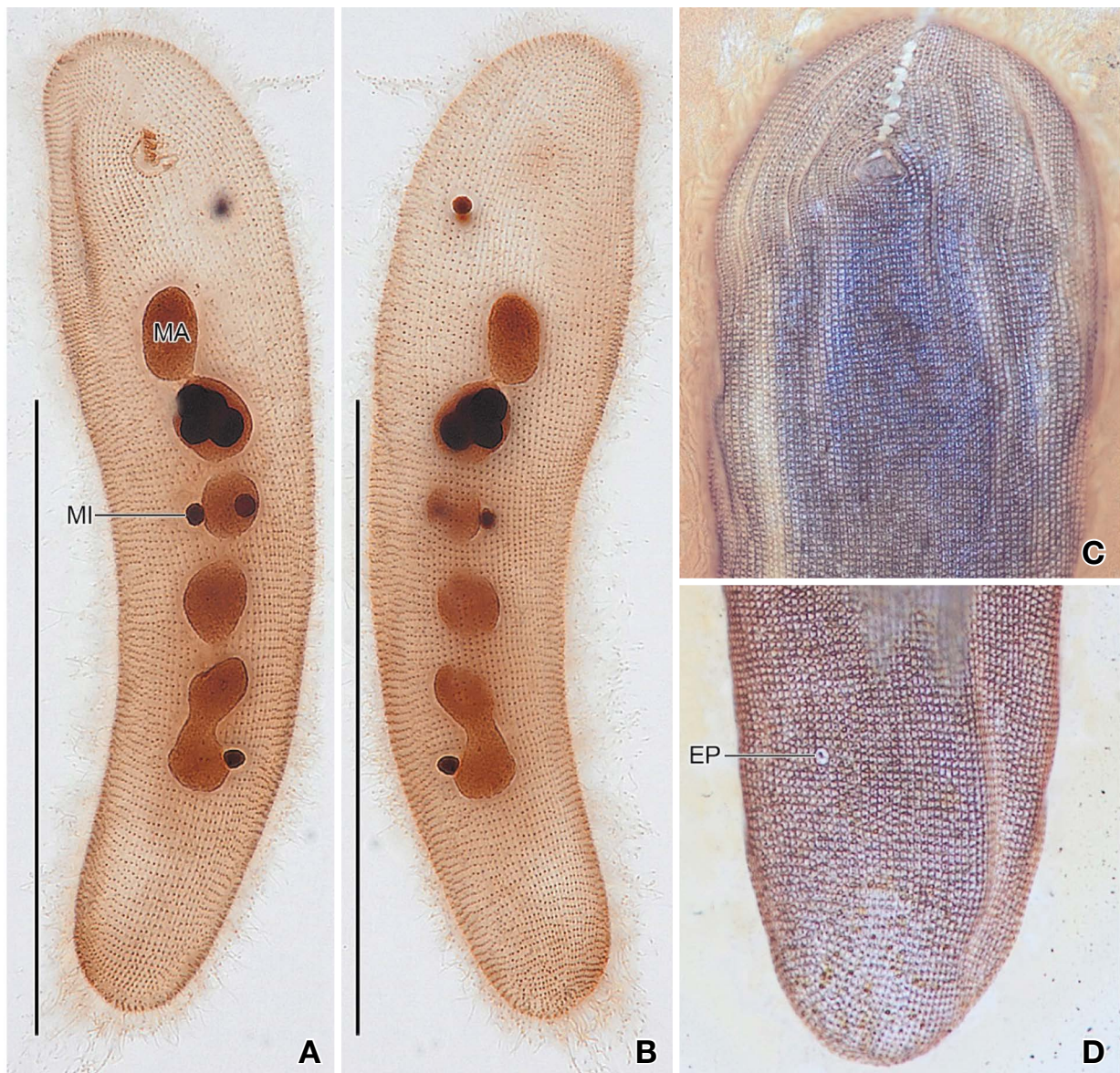


Fig. 3. *Cardiotomatella vermiformis* after protargol (A, B) and wet silver nitrate impregnation (C, D). A, B, Ventral (A) and dorsal (B) view showing the body shape, the somatic ciliature, the nuclear apparatus, and the oral ciliature; C, D, Ventral views of the anterior (C) and posterior (D) region of body showing the somatic ciliature and silverline system, the oral opening, and the excretory pore on right side of body. EP, excretory pore; MA, macronucleus; MI, micronuclei. Scale bars: A, B=100 μ m.

Diagnosis. Size 38–50 \times 18–22 μ m after protargol impregnation (n=6); cell ovoid with rounded ends, ventral side concave. Cytoplasm studded with food vacuoles filled with diatoms and flagellates. Nuclear apparatus consists of a single globular to broadly ellipsoidal macronucleus in second quarter of cell, 8–11 \times 7–10 μ m in size, one or two micronuclei near or attached to macronucleus, each about 2 μ m across. 21–24 somatic ciliary rows, each row consists of dikinetids anteriorly and monokinetids in posterior third; 4–5 preoral kineties. Buccal field very large, about

3/4 of cell length. Paroral membrane conspicuous, hook or ‘6’-shaped. M1 2-rowed, but 3-rowed at distal end. M2a more or less straight, proximal end hook-like, about 20 μ m long, two-rowed at distal and proximal end, one-rowed at middle portion; M1 : M2a length ratio about 1 : 3.5; M2b irregularly V-shaped, zigzag-pattern of basal bodies in right arm. M3 three-rowed.

Distribution. China (Pan et al., 2015), South Korea (present study).

Remarks. The Korean population of *Pleuronema paucisae-*

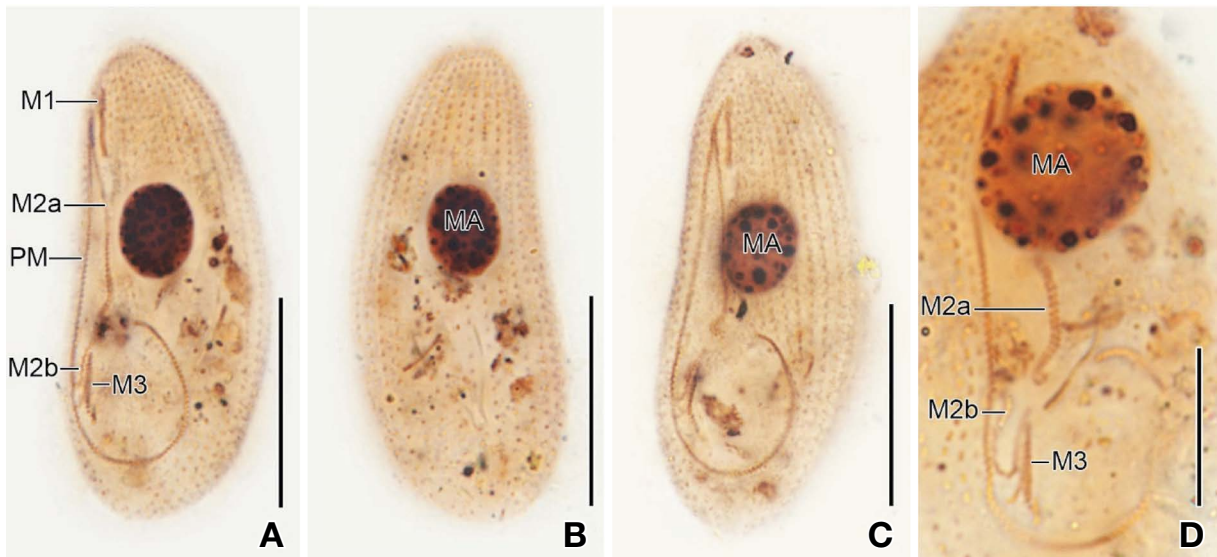


Fig. 4. *Pleuronema paucisaetosum* after protargol impregnation. A–C, Ventral (A, C) and dorsal (B) view, showing the somatic ciliary pattern, the nuclear apparatus, and the oral apparatus; D, Ventral view showing the oral structure. M1, 3, membranelles 1, 3; M2a, anterior part of membranelle 2; M2b, posterior part of membranelle 2; MA, macronucleus; PM, paroral membrane. Scale bars: A–C=20 μ m, D=10 μ m.

Table 3. Comparison of main morphological features between *Pleuronema paucisaetosum* and similar congeners. Body size from life, the size of the Korean population was calculated from the stained cells by adding 15% preparation shrinkage.

Characteristics	<i>P. paucisaetosum</i>	<i>P. paucisaetosum</i> (type population)	<i>P. coronatum</i>	<i>P. parasmalli</i>	<i>P. puytoraci</i>	<i>P. setigerum</i>
Body size (μ m)	43–57×20–25	55–85×25–55	55–170×30–85	55–85×25–35	80–100×50–60	40–50×15–20
Somatic kineties, number	21–24	21–23	27–43	26–32	28–29	14–16
Preoral kineties, number	4–5	4–5	3–7	4–6	3	3–6
Posterior end of M2a, shape	Hook-like	Hook-like	Hook-like	Hook-like	Hook-like	Curved
Macronucleus, shape	Ellipsoidal	Spherical	Spherical	Spherical	Spherical	Spherical
Micronuclei, number	1 or 2	1–5	1–3	1	?	1–4
Habitat, salinity	Marine, 28‰	Brackish, 13.4‰	Marine, ?	Freshwater	Brackish, 16.0‰	Marine, 29.0‰
Reference, country	Present study, South Korea	Pan et al. (2015), China	Wang et al. (2008), China	Liu et al. (2022), China	Pan et al. (2011), China	Pan et al. (2010), China

tosum is very similar to the Chinese type population described by Pan et al. (2015), but they differ only in the body size (38–50×18–22 μ m vs. 60–82×36–58 μ m after protargol impregnation). Furthermore, the Chinese population was described from a wetland with salinity of 13.4‰ while the Korean population is found in coastal water with salinity of 28‰, suggesting that the species tolerates a broad range of salinity (Table 3). Considering the body size and number of somatic kineties, *P. paucisaetosum* is very similar to *P. setigerum* Calkins, 1902 (38–50×18–22 μ m and 21–24 vs. 40–55×16–30 μ m and 14–22, after protargol impregnation, respectively). However, they differ mainly by the structure of M2a (posterior end hook-like and middle portion one-rowed vs. ring-like posterior

end and middle portion in zigzag pattern) (Pan et al., 2010, 2015). *Pleuronema paucisaetosum* is also similar to *P. coronatum* Kent, 1881 and *P. puytoraci* Grolière and Detcheva, 1974 in the structure of M2a but it has smaller body size and fewer number of somatic ciliary rows (Wang et al., 2008; Pan et al., 2011). Also, *P. paucisaetosum* is similar to *P. parasmalli* Liu et al., 2022 in the body size and the oral structure but it differs in the fewer number of somatic kineties (21–24 vs. 26–32), the higher number of micronuclei (1 or 2 vs. only 1), and the habitat (marine vs. freshwater) (Liu et al., 2022).

Voucher slides. One slide with protargol-impregnated specimens was deposited in the National Marine Biodiversity Institute of Korea (MABIK PR00044182).

ORCID

Atef Omar: <https://orcid.org/0000-0002-7759-632X>

Ji Hye Moon: <https://orcid.org/0000-0001-9889-0096>

Jae-Ho Jung: <https://orcid.org/0000-0001-5497-8678>

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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