

## New record of five anaerobic ciliate species from South Korea

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During a field survey to report unrecorded Korean ciliates, we collected five anaerobic ciliate species in two samples from freshwater and marine environments. These species belong to the classes Armophorea and Plagiopylea. The morphology of these species was studied based on live observations, protargol impregnation, and scanning electron microscope. *Brachonella contorta* is characterized by a size of 80–100 × 40–60 μm *in vivo*, a bullet-shaped body, and about 60 oral polykinetids making 360° spiral around long axis. *Brachonella pulchra* is characterized by a size of 80–110 × 50–70 μm *in vivo* and adoral zone composed of about 40 oral polykinetids with minimal spiralization. *Metopus setosus* is characterized by a size of 45–70 × 20–30 μm *in vivo*, about 22 ciliary rows, very long caudal cilia, and 20 oral polykinetids. *Plagiopyla nasuta* is a freshwater species characterized by a size of 80–120 × 50–60 μm *in vivo*, striated band present, straight extrusomes, and about 70 somatic ciliary rows. The marine *Plagiopyla frontata* is characterized by a size of 80–120 × 40–60 μm *in vivo*, an oval to ellipsoid body shape, 50–60 somatic ciliary rows, and curved extrusomes.

Keywords: Anaerobe, Metopidae, Plagiopylidae, redescription, taxonomy

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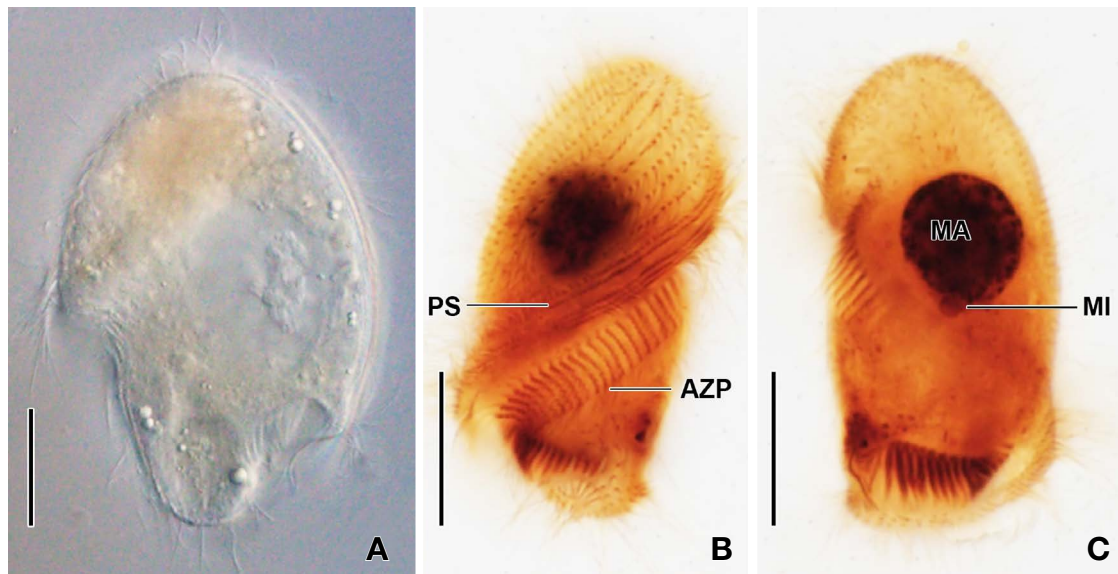
### INTRODUCTION

Anaerobic habitats harbor many protists, especially ciliates of the classes Armophorea Lynn, 2004 and Plagiopylea Small and Lynn, 1985 (Jankowski, 1964a; 1964b; Esteban *et al.*, 1995; Lynn, 2008; Bourland *et al.*, 2017a). Anaerobic ciliates are characterized by possessing hydrogenosomes and endosymbiotic prokaryotes (Müller, 1988; Biagini *et al.*, 1997; Hackstein *et al.*, 1999). Several outstanding studies dealing with anaerobic ciliates have been published in the recent few years (Bourland and Wendell, 2014; Foissner, 2016a; 2016b; Bourland *et al.*, 2017a; 2017b; 2018a; 2018b; 2020; 2022; Li *et al.*, 2017; Omar *et al.*, 2017; Vd'ačný and Foissner, 2017a; 2017b; Fernandes *et al.*, 2018; Nitla *et al.*, 2018; Vd'ačný *et al.*, 2019). Despite the large number of known anaerobic ciliate species, only two metopids (*Metopus es* (Müller, 1776) Lauterborn, 1916 and *M. hasei* Sondheim, 1929) and one plagiopylid (*Trimyema koreanum*) species were described from Korean habitats (Cho *et al.*, 2008; He and Choi, 2015; Omar and Jung, 2021). In this study, we provide a brief description of three armophorids and two plagiopylid ciliates found in two anaerobic samples each collec-

ted from freshwater and marine habitats. The freshwater anaerobic sample was species-rich and besides the four species reported here, several other unidentified anaerobic caenomorphids, metopids, and odontostomatids were found in the preparations suggesting a higher diversity of the anaerobic ciliates.

### MATERIALS AND METHODS

The ciliate species, *Brachonella contorta*, *B. pulchra*, *Metopus setosus*, and *Plagiopyla nasuta*, were found in sulfidic freshwater sediments in Sangju-si, South Korea. *Plagiopyla frontata* was found in sulfidic marine sediments (salinity 26.8‰) in Gangneung-si, South Korea. The samples (water and sediment) were collected and immediately transferred to the laboratory. The sample containers were tightly sealed and kept at room temperature for about one month until the emission of strong sulfidic odor and the appearance of anaerobic ciliate species. The living morphology was observed using a stereomicroscope (Olympus SZ11, Japan), an optical microscope (Olympus BX53, Japan) at low (×40–200) to high (×400–1,000)



**Fig. 1.** *Brachonella contorta* in life (A) and after protargol impregnation (B, C). A. Ventral view showing the body shape, the adoral zone of polykinetids, which extends to posterior body end and spirals 360° around long body axis, and the long preoral dome. B, C. Ventral and dorsal view showing the somatic and oral ciliature, the perizonal ciliary stripe, and the nuclear apparatus. AZP, adoral zone of polykinetids; MA, macronucleus; MI, micronucleus; PS, Perizonal ciliary stripe. Scale bars 20 µm.

magnifications, and photomicrographs were captured using a digital camera (Olympus DP74, Japan). Protargol powder was synthesized using the method of Pan *et al.* (2013) and Kim and Jung (2017). The permanent slides were prepared using protargol impregnation method of the ‘procedure A’ of Foissner (2014). Cells were fixed for 10 min in 10% (v/v) aqueous formalin. Scanning electron microscopy technique was conducted following the procedure of Foissner (2014) and Moon *et al.* (2017). The differential through-focal images of the protargol-impregnated specimens were merged using the software of Helicon Focus 6.8.0 (Helicon Soft Ltd.). The basic terminology and taxonomic classification follow Lynn (2008). The specific terminology of each taxon follows the previous studies.

## RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901  
 Subphylum Intramacronucleata Lynn, 1996  
 Class Armophorea Lynn, 2004  
 Order Metopida Jankowski, 1980  
 Family Metopidae Kahl, 1927  
 Genus *Brachonella* Jankowski, 1964

### 1. *Brachonella contorta* (Levander, 1894) Jankowski, 1964 (Fig. 1)

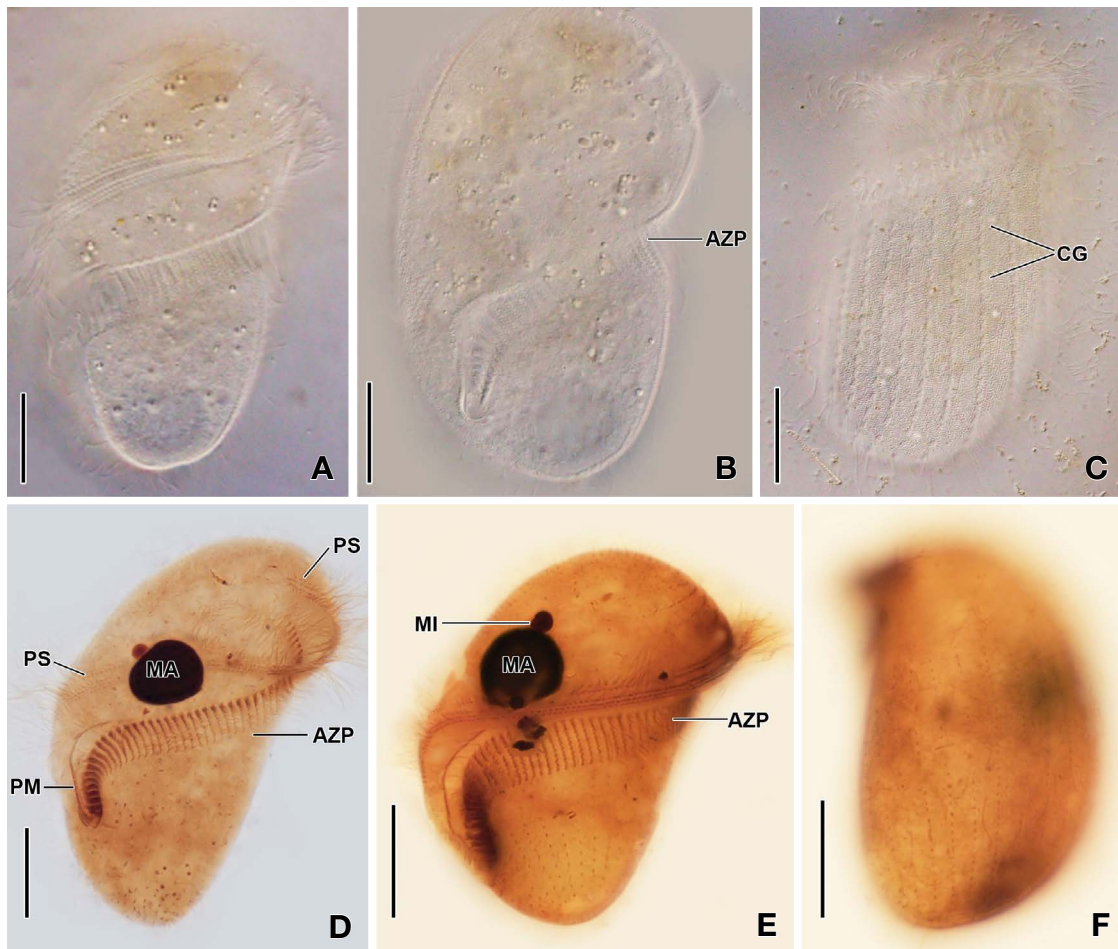
**Material examined.** Freshwater sample collected from Bunhwangji, Nakdong-myeon, Sangju-si, Gyeongsang-

buk-do, Republic of Korea (36°23'50.4"N, 128°15'47.7"E) on 26 April 2021.

**Diagnosis.** Size 80–100 × 40–60 µm *in vivo* and 60–80 × 35–50 µm after protargol impregnation (n = 7); body bullet-shaped, preoral dome very large, conical, postoral part short and bluntly truncate; cortex covered with minute cortical granules arranged in rows between somatic kineties; cytoplasmic granules yellowish to brownish, aggregate at anterior end of ventral side of preoral dome; cytostome displaced in posterior end of body at end of adoral zone; one globular macronucleus and one micronucleus in anterior half of body; 40–50 dikinetidal ciliary rows, 20 of which are dome kineties, caudal cilia slightly elongated; perizonal ciliary stripe consists of 5 rows, rows 1–3 close together and separated from rows 4 and 5, rows 1–3 not arranged in false kineties; adoral zone makes 360° spiral around long axis, comprises ~60 polykinetids on average.

**World distribution.** Cosmopolitan (Levander, 1894; Kahl, 1932; Schulze, 1959; Jankowski, 1964a; Dragesco and Dragesco-Kernéis, 1986; Bourland *et al.*, 2017a).

**Remarks.** The Korean population of *Brachonella contorta* agrees to the most recent description of Bourland *et al.* (2017a) from USA in almost all features. *Brachonella contorta* also agrees with other descriptions, however, the species shows high morphological variability (Levander, 1894; Kahl, 1932; Schulze, 1959; Jankowski, 1964a; Dragesco and Dragesco-Kernéis, 1986; Bourland *et al.*, 2017a). According to Bourland *et al.* (2022), six species are included to the genus *Brachonella* so far: *B. comma* Bourland *et al.*, 2022; *B. contorta* (Levander, 1894) Jankow-



**Fig. 2.** *Brachonella pulchra* in life (A–C) and after protargol impregnation (D–F). A–C. Ventral (A, B) and dorsal (C) view showing the body shape, the adoral zone of polykinetids, the cortical granules, and the large preoral dome. D–F. Ventral (D, E) and dorsal (F) view showing the somatic and oral ciliature, the perizonal ciliary stripe, and the nuclear apparatus. AZP, adoral zone of polykinetids; CG, cortical granules; MA, macronucleus; MI, micronucleus; PM, paroral membrane; PS, perizonal ciliary stripe. Scale bars 20  $\mu\text{m}$ .

ski, 1964a; *B. cydonia* (Kahl, 1927) Jankowski, 1964b; *B. lemani* (Dragesco, 1960) Jankowski, 1964a; *B. elongata* Jankowski, 1964b; and *B. pulchra* (Kahl, 1927) Bourland *et al.*, 2018a. However, only *B. comma*, *B. contorta*, and *B. pulchra* are morphologically well characterized. *Brachonella contorta* could be easily separated from *B. comma* by the bullet-shaped (vs. elongate) body shape, the straight (vs. ventrally curved) body axis, and the number of somatic kineties (>34 vs. 20–25). Also, it could be easily separated from *B. pulchra* by the shape of the preoral dome (conical vs. broadly rounded). Furthermore, it is the only species within the genus *Brachonella* with a prominent anterior apical suture, the cytostome in posterior end of body, and it has the higher degree (360°) of adoral zone spiralization (Jankowski, 1964a; 1964b; Bourland *et al.*, 2017a; 2018a; 2022).

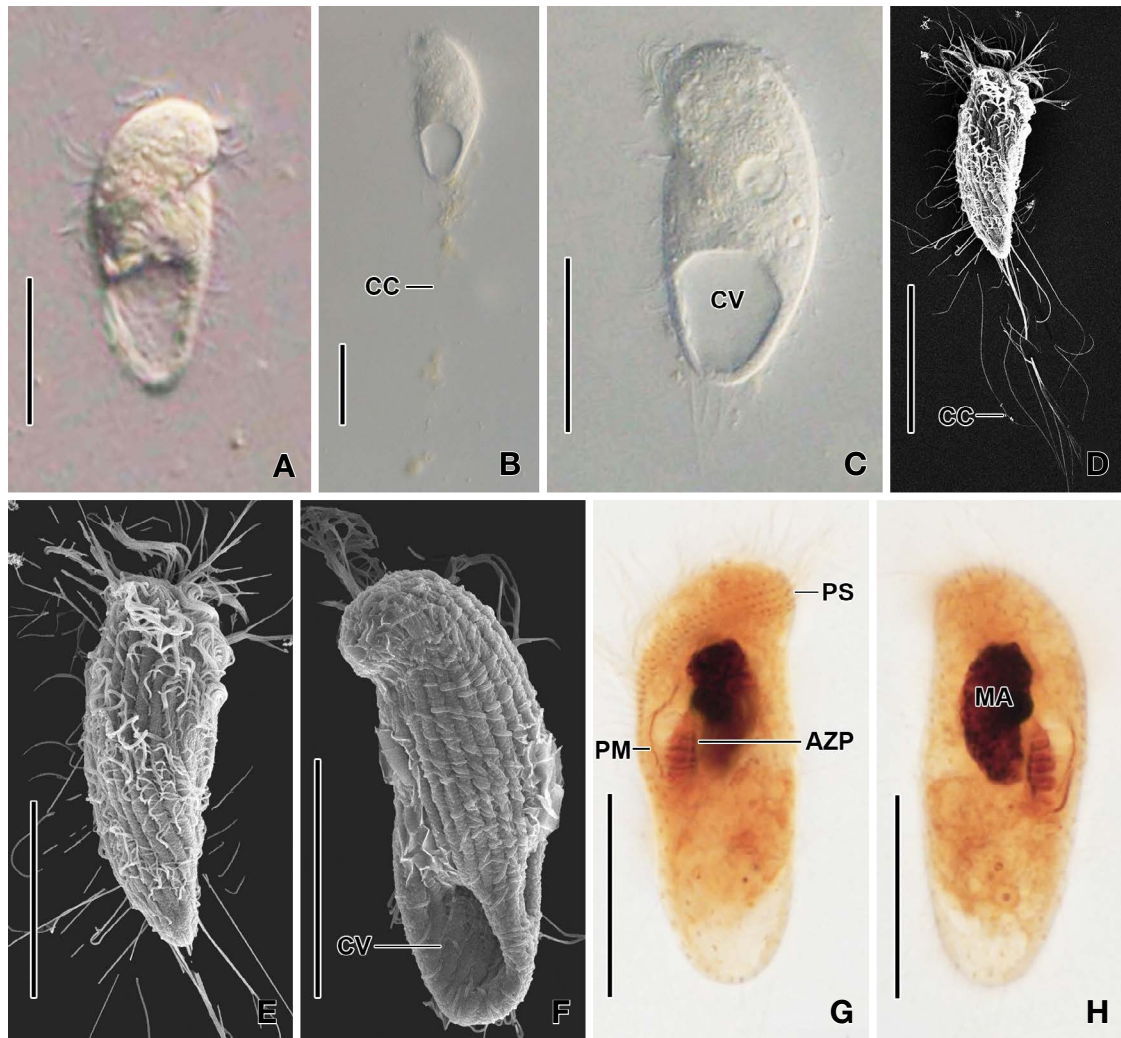
**Voucher slide.** One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111066).

## 2. *Brachonella pulchra* (Kahl, 1927) Bourland *et al.*, 2018 (Fig. 2)

**Material examined.** Freshwater sample collected from Bunhwangji, Nakdong-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea (36°23'50.4"N, 128°15'47.7"E) on 26 April 2021.

**Diagnosis.** Size *in vivo* 80–110  $\times$  50–70  $\mu\text{m}$  and 60–90  $\times$  40–55  $\mu\text{m}$  after protargol impregnation (n = 7); body shape broadly ellipsoidal to obovate, distinctly twisted anteriorly and slightly flattened dorsoventrally; preoral dome huge, ventral side distinctly convex; cytoplasmic granules aggregate at anterior end of ventral side of preoral dome; one globular macronucleus and one micronucleus in anterior half of body; cortex covered with minute cortical granules; 30–40 ciliary rows, caudal cilia slightly elongated; perizonal stripe consists of 5 rows not arranged in false kineties, kineties 1–3 closely spaced, separated from rows 4 and 5 by a gap; adoral zone comprises ~40 polykinetids on





**Fig. 3.** *Metopus setosus* in life (A–C), in the scanning electron microscope (D–F), and after protargol impregnation (G, H). A–C. Ventral (A) and ventrolateral (B, C) views showing the torsion of the body, the adoral zone of membranelles, the very long caudal cilia, and the large, terminal contractile vacuole. D–F. Dorsal (D, E) and ventrolateral (F) view, showing the body shape, the somatic ciliature, and the very long caudal cilia. G, H. Ventral and dorsal view showing the somatic and oral ciliature, the perizonal ciliary stripe, and the nuclear apparatus. AZP, adoral zone of polykinetids; CC, caudal cilia; CV, contractile vacuole; MA, macronucleus; PM, paroral membrane; PS, perizonal ciliary stripe. Scale bars 30  $\mu\text{m}$  (A–D) and 20  $\mu\text{m}$  (E–H).

average, only slightly spirals onto dorsal side anteriorly.

**World distribution.** Czech Republic, Finland, Germany, and South Korea (Kahl, 1927; Jankowski, 1964b; Bourland *et al.*, 2018a).

**Remarks.** The Korean population of *Brachonella pulchra* agrees to the most recent description of Bourland *et al.* (2018a) in all features. *Brachonella pulchra* could be easily separated from *B. contorta* (see above). Also, it differs from *B. comma* by the body shape (broadly ellipsoidal vs. elongate, comma-shaped) and the number of somatic kineties (30–40 vs. 20–25) (Bourland *et al.*, 2018a; 2022).

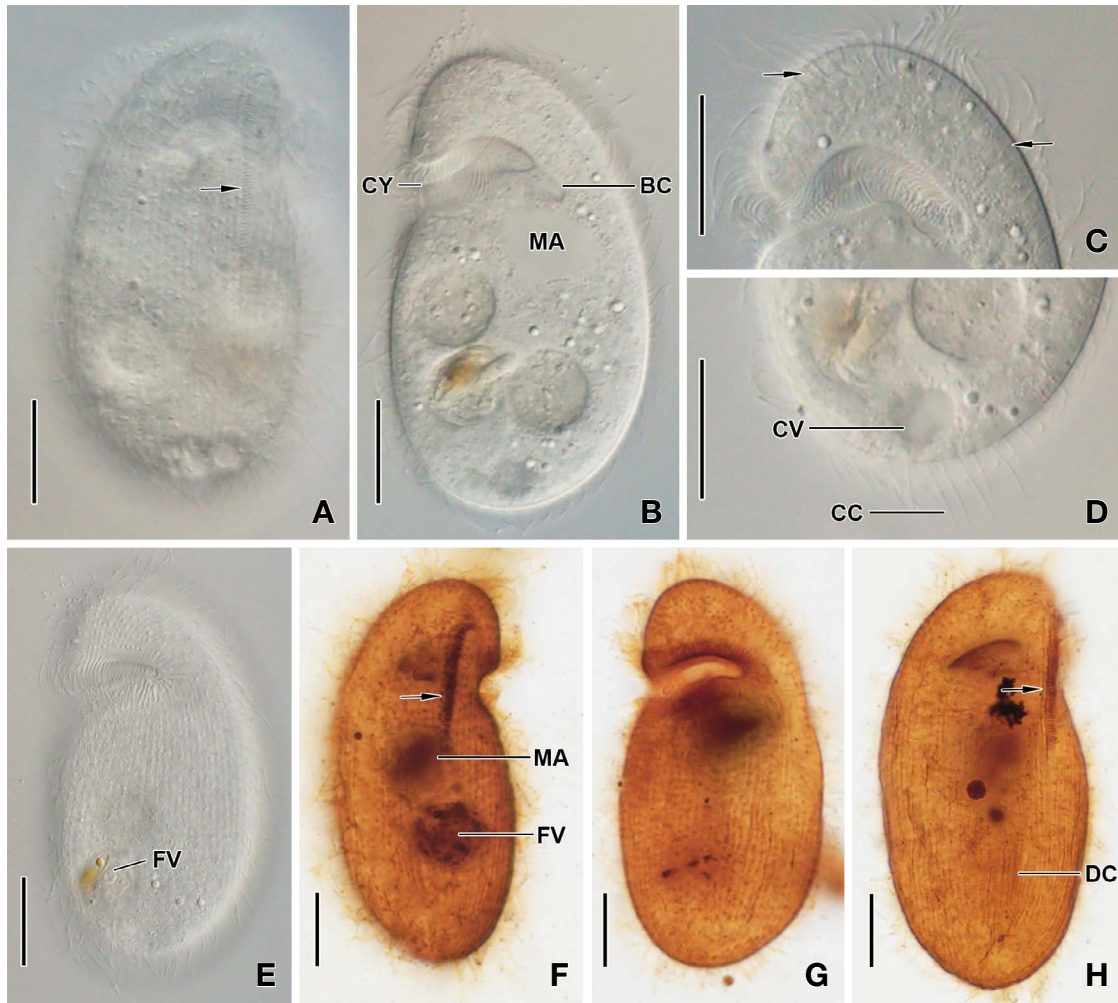
**Voucher slide.** One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111062).

Genus *Metopus* Claparède and Lachmann, 1858

### 3. *Metopus setosus* Kahl, 1927 (Fig. 3)

**Material examined.** Freshwater sample collected from Bunhwangji, Nakdong-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea (36°23'50.4"N, 128°15'47.7"E) on 26 April 2021.

**Diagnosis.** Size 45–70  $\times$  20–30  $\mu\text{m}$  *in vivo* and 35–50  $\times$  12–16  $\mu\text{m}$  after protargol impregnation (n = 6); body obovate, slightly obconical, distinctly twisted anteriorly, left side convex and right side concave; preoral dome distinctly convex, slightly overhangs on left side, extends to half body length; one ellipsoidal macronucleus about 14  $\times$  7



**Fig. 4.** *Plagiopyla frontata* in life (A–E) and after protargol impregnation (F–H). A, B, E. Dorsal (A) and ventral (B, E) views showing the body shape, the striated band (arrow), and the oral apparatus. C. Ventral view of the oral region showing the oral tube curving upwards in the middle and the curved extrusomes (arrows). D. The caudal cilia and the terminal contractile vacuole. F–H. Dorsal (F, H) and ventral (G) view, showing the somatic ciliature, the striated band (arrows), and the dense ciliary rows left of cytoproct. BC, buccal cavity; CC, caudal cilia; CV, contractile vacuole; CY, cytostome; DC, dense ciliary rows; FV, food vacuole; MA, macronucleus. Scale bars 30  $\mu\text{m}$  (A, B, E) and 20  $\mu\text{m}$  (C, D, F–H).

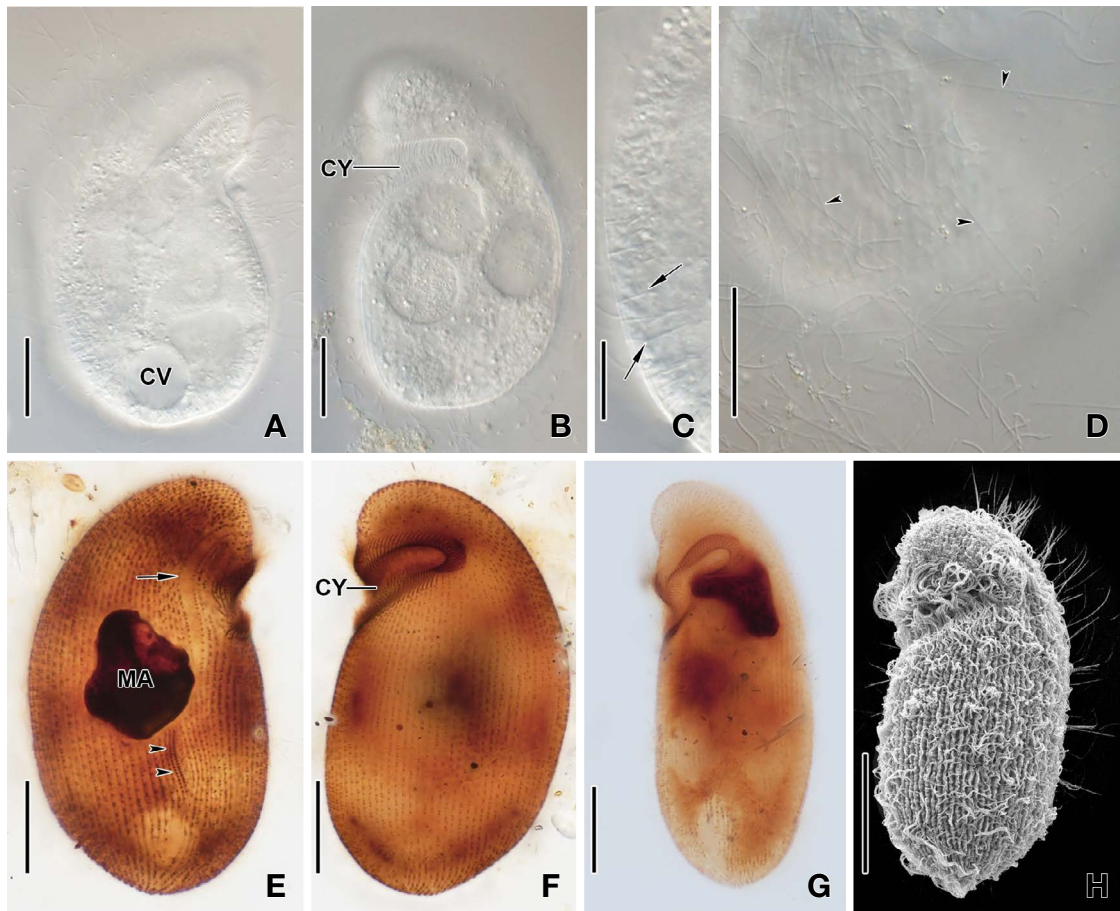
$\mu\text{m}$  in size after protargol impregnation and one micronucleus about 3  $\mu\text{m}$  across anterior to mid-body; contractile vacuole very large, in posterior body end; cortex flexible, furrowed along ciliary rows, cortical granules arranged in rows between ciliary rows; cytoplasm colorless, few to many lipid droplets and food vacuoles contain bacteria; on average 22 dikinetidal ciliary rows, somatic cilia 10–12  $\mu\text{m}$  long, caudal cilia as long as or longer than body; perizonal stripe consists of 5 rows, rows 1–3 very close together and their dikinetids arranged in false kineties; adoral zone comprises 20 membranelles on average.

**World distribution.** Australia, China, Germany, and South Korea (Kahl, 1932; Wang and Nie, 1935; Foissner, 1980; Esteban *et al.*, 1995; Foissner *et al.*, 2002; Vd'ačný and Foissner, 2017a).

**Remarks.** The Korean population of *Metopus setosus* agrees to the most recent description of Vd'ačný and Foissner (2017a) and to a Chinese population studied by Wang and Nie (1935) in most features. *Metopus setosus* could be confused with the small species with long caudal cilia such as *M. minor*, *M. recurvatus* and *M. setifer*. *Metopus setosus* differs from *M. minor* by the larger body size (54–60 vs. 30–40  $\mu\text{m}$ ) and the higher number of ciliary rows (22 vs. 10). *Metopus recurvatus* was considered a junior synonym of *M. minor* by Foissner (1980). *Metopus setifer* is also similar to *M. setosus* in the body size, but they differ mainly in the body shape and the ciliary rows number (Kahl, 1932; Foissner, 1980; Esteban *et al.*, 1995; Foissner *et al.*, 2002; Vd'ačný and Foissner, 2017a).

**Voucher slide.** One slide with protargol-impregnated





**Fig. 5.** *Plagiopyla nasuta* in life (A–D), after protargol impregnation (E–G), and in scanning electron microscope (H). A, B. dorsal and ventral view showing the body shape, the shape of the oral opening and the striated band on dorsal side. C, D. Resting (arrows) and extruded (arrowheads) extrusomes. E–G. dorsal (E), Ventral (F), and ventrolateral (G) view, showing the body shape, the shape and position of the oral opening, the striated band (arrow), and the dense ciliary rows left of cytoproct (arrowheads). H. ventral view showing the body shape and the dense somatic ciliation. CV, contractile vacuole; CY, cytostome; MA, macronucleus. Scale bars 20  $\mu\text{m}$  (A, B, D–H) and 10  $\mu\text{m}$  (C).

specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111064).

Class Plagiopylea Small and Lynn, 1985  
 Order Plagiopylida Jankowski, 1978  
 Family Plagiopylidae Schewiakoff, 1896  
 Genus *Plagiopyla* Stein, 1860

#### 4. *Plagiopyla frontata* Kahl, 1931 (Fig. 4)

**Material examined.** Marine water sample collected from Anin Beach, Gangdong-myeon, Gangneung-si, Gangwon-do, Republic of Korea (37°44'4"N, 128°59'25"E) on 14 June 2021.

**Diagnosis.** Size *in vivo* 80–120  $\times$  40–60  $\mu\text{m}$  and about 100  $\times$  55  $\mu\text{m}$  after protargol impregnation (n = 5); body oval to ellipsoidal, dorsoventrally flattened, anterior and posterior end rounded; 50–60 somatic ciliary rows; about 10 caudal cilia along left posterior margin; buccal open-

ing slit-like, at right ventral side extending transversely to left and curving upwards in mid-region; frontal region (between anterior cell margin and upper oral lip) about 1/6 of body length; striated band on dorsal side commences at level of oral opening and forms a hook-like structure and ends near mid-body; one globular to elliptical macronucleus slightly anterior to mid-body, one micronucleus attached to macronucleus; cytoproct on posterior third of dorsal side, 1–3 dense ciliary rows left of cytoproct slit; extrusomes curved, 3–4  $\mu\text{m}$  long *in vivo*; contractile vacuole terminal.

**World distribution.** Germany, Denmark, Australia, and South Korea (Kahl, 1931; Fenchel, 1968; Fenchel *et al.*, 1995; Esteban *et al.*, 2000; Nitla *et al.*, 2018).

**Remarks.** *Plagiopyla frontata* was studied a few times, but a complete characterization of the species is lacking (Kahl, 1931; Fenchel, 1968; Fenchel *et al.*, 1995; Esteban *et al.*, 2000; Nitla *et al.*, 2018). The Korean population of *P. frontata* agrees very well with previous descriptions,

especially that of the type population (Kahl, 1931). Esteban *et al.* (2000), who studied *P. frontata* from Australia, also mentioned that their population is almost identical to the type population. Within the marine *Plagiopyla* species, *P. frontata* is most similar to *P. ovata*, which was described recently from marine water in China (Li *et al.*, 2021). However, they can be separated by the body shape (oval to ellipsoidal vs. obovate), the shape of extrusomes (curved vs. straight), the length of the frontal region anterior to upper lip (1/6 vs. 1/4 of body length), and the shape of buccal cavity tube (straight vs. curved upwards in its mid-region). The curved extrusomes were also found in the freshwater species, *P. megastoma* (Smith, 1898) Kahl, 1931, *P. ramani* Nitla *et al.*, 2018, and *P. narasimhamurtii* Nitla *et al.*, 2018 (Nitla *et al.*, 2018).

**Voucher slide.** One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111070).

### 5. *Plagiopyla nasuta* Stein, 1860 (Fig. 5)

**Material examined.** Freshwater sample collected from Bunhwangji, Nakdong-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea (36°23'50.4"N, 128°15'47.7"E) on 26 April 2021.

**Diagnosis.** Size about 80–120 × 50–60 µm *in vivo*, and about 100 × 50 µm after protargol impregnation (n = 7); body ovate, dorsoventrally flattened; striated band commences above oral region curves forming hook-like structure and extends posteriorly to mid-body on left side; a single globular macronucleus in mid-body and a spherical micronucleus attached to it; straight extrusomes about 6 µm long and about 20 µm when exploded; contractile vacuole in posterior body portion; one or two dense ciliary rows on dorsal side left of cytoproct; about 70 somatic ciliary rows, ventral kineties terminate near buccal opening; buccal opening slit like on ventral side and buccal cavity tube-like extends transversely to left.

**World distribution.** Cosmopolitan (Roux, 1899; Penard, 1922; Wetzel, 1928; Kahl, 1931; Jankowski, 1964b; Mahajan and Nair, 1971; Bick, 1972; Dragesco, 1972; Agamaliyev, 1978; Dragesco and Dragesco-Kerneis, 1986; Sola *et al.*, 1988; Foissner *et al.*, 1995; Şenler and Yildiz, 2004).

**Remarks.** The Korean population of *Plagiopyla nasuta* agrees with the most recent description from India (Nitla *et al.*, 2018). However, this species was described several times (Penard, 1922; Jankowski, 1964b; Dragesco, 1972; Dragesco and Dragesco-Kerneis, 1986; Sola *et al.*, 1988; Foissner *et al.*, 1995) and some of these descriptions do not agree with the original type population, for example, *P. nasuta* var. *wetzeli* from Kahl (1931), which was elevated to *P. wetzeli* by (Jankowski, 2007) and considered as *P. nasuta* again by Nitla *et al.* (2018). *Plagiopyla nasuta* and *P. simplex* Wetzel, 1928 differ from other free-living

freshwater *Plagiopyla* species by the presence of one type of straight extrusomes and they differ from each other by the body size (80–120 vs. 135–150 µm) and the presence (vs. absence) of the striated band (Wetzel, 1928).

**Voucher slide.** One slide with protargol-impregnated specimens was deposited at the Nakdonggang National Institute of Biological Resources (NNIBRPR21236).

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