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New record of marine scuticociliates and euplotids (Protozoa, Ciliophora) from coastal waters of South Korea

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During a field survey of marine ciliate diversity in South Korea, four ciliate species were newly recorded as follows: *Falcicyclidium citriforme*, *Homalogastra setosa*, *Euplotes orientalis*, and *Euplotes shini*. The identification of these species was based on morphological examination through observations of both living cells and silver-impregnated specimens. A brief description, remarks, and photomicrographs are provided for each species.

Keywords: infraciliature, morphology, redescription, silver impregnation, taxonomy

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Introduction

Scuticociliates are a species-rich group of ciliates commonly found in coastal habitats (Carey, 1992; Lynn, 2008; Jung, 2021). According to the National Institute of Biological Resources (2023), thirty species of scuticociliates have been reported in Korea. Some species are known to cause severe diseases, such as scuticociliatosis in wild and mariculture fish populations (Noga, 2010). Although scuticociliates are facultative parasites capable of living independently, they may become parasitic under certain environmental conditions (Thompson Jr. and Moewus, 1964).

The genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831, is one of the most species-rich genera in the phylum Ciliophora, with approximately 160 species. Euplotids are benthic ciliates found in terrestrial, freshwater, and marine environments worldwide (Curds, 1975; Song *et al*., 2009; Foissner, 2016; Živaljić *et al*., 2020; Abraham *et al*., 2021; Do *et al*., 2024). Despite their cosmopolitan distribution, only 23 species have been reported in Korea, according to the National Institute of Biological Resources. Despite the prevalence and diversity of the scuticociliates and euplotids, our understanding of their diversity in South Korea remains limited.

In this study, we provide brief descriptions, remarks, photomicrographs, and locality information for four newly recorded ciliates, two scuticociliate and two euplotid species from South Korea.

Materials and Methods

Sample collection and identification

The four species were collected from coastal waters, with detailed locality information provided in the "Material examined" section for each species. Water samples were transferred to the laboratory and incubated in Petri dishes, with mealworms or sterile wheat grains added as food sources. All four species were examined using a stereomicroscope (Olympus SZ11, Tokyo, Japan) and light microscopes (Olympus BX53), under bright-field and differential interference contrast (DIC) at magnifications ranging from 50× to 1000×.

The protargol impregnation 'Procedure A' of Foissner (2014) was performed using synthesized protargol and an acetone developer to reveal infraciliature and nuclear apparatus. Protargol powder was synthesized according to the methods described by Pan *et al*. (2013) and Kim and Jung (2017). Additionally, the Chatton-Lwoff silver nitrate impregnation technique was used to reveal the infraciliature and silverline system (Corliss, 1953). Terminology and classification follow Curds (1975) and Lynn (2008).

Results and Discussion

Class Oligohymenophorea de Puytorac *et al*., 1974
Subclass Scuticociliatia Small, 1967
Order Philasterida Small, 1967
Family Cyclidiidae Ehrenberg, 1838

Genus *Falcicyclidium* Fan *et al*., 2011

1. *Falcicyclidium citriforme* Fan *et al*., 2017

Material examined. Marine water (salinity 33.7‰, temperature 27.9°C) collected from Anin Beach, Gangdong-myeon, Gangneung-si, Gangwon-do, Korea (37°44′2″N, 128°59′26″E) on August 03, 2021.

Diagnosis. Body size 30–35 × 15–20 μm in vivo, on average 30 × 20 μm after protargol impregnation (n = 5) (Fig. 1A, B). Body shape ordinary to broadly oval (Fig. 1A–C). One globular macronucleus, 6 μm across on average after protargol impregnation, located slightly above the body center; nucleoli globular to ellipsoidal and evenly distributed; micronucleus lacking or indistinguishable (Fig. 1B). Contractile vacuole terminal, 4–5 μm across in vivo at end of diastole (Fig. 1A). Cytoplasm with food vacuoles, crystals, and lipid (Fig. 1A). Somatic and caudal cilia 6–10 μm and 14–18 μm long in vivo, respectively; invariably ten bipolar somatic kineties; kineties 1 and 10 with 24–27 and 22–26 mono/dikinetids, respectively (Fig. 1A–C). Oral apparatus occupies about 66% of body length after silver nitrate impregnation, composed of three adoral membranelles and paroral membrane; paroral membrane about 16 μm long (Fig. 1A–C). Silverline consists of primary meridians, which connect the somatic kinetids of each kinety, and transverse connectives without secondary meridians (Fig. 1C).

Distribution. China (Fan *et al*., 2017) and Korea (present study).

Remarks. The Korean population of *Falcicyclidium citriforme* is similar to the Chinese type population described by Fan *et al*. (2017). However, the Korean population has a slightly smaller body length compared to the type population (30–35 μm vs. 40–60 μm in vivo; Fan *et al*., 2017). In comparison to other species in the genus, *F. atractodes* Fan *et al*., 2011 resembles *F. citriforme* but can be easily distinguished by its body shape, with *F. atractodes* having spiny anterior and posterior ends, while *F. citriforme* lacks these spines (Fan *et al*., 2017). Additionally, *Falcicyclidium fangi* Fan *et al*., 2011 differs from *F. citriforme* in the ratio of the buccal field to body length (0.75 vs. 0.54; Fan *et al*., 2011; 2017).

Compared to other similar genera, *Cyclidium plouneouri* Dragesco, 1963 resembles *F. citriforme* but can be easily distinguished by a lower number of somatic kineties (14–16 vs. 10) and according to Wilbert, 1986 also by a lower number of somatic kineties (11 or 12 vs. 10). Additionally, *Cyclidium borrori* Borror, 1965 differs from *F. citriforme* by a lower number of somatic kineties (12 or 13 vs. 10).

Voucher slides. Two slide with protargol-impregnated specimens (MABIK PR00045077, MABIK PR00045078) and one slide with silver nitrate-impregnated specimens (MABIK PR00045079) were deposited at the National Marine Biodiversity Institute of Korea.

Family Uronematidae Thompson, 1964
Genus *Homalogastra* Kahl, 1926

2. *Homalogastra setosa* Kahl, 1926

Material examined. Marine water (salinity 35‰) collect-

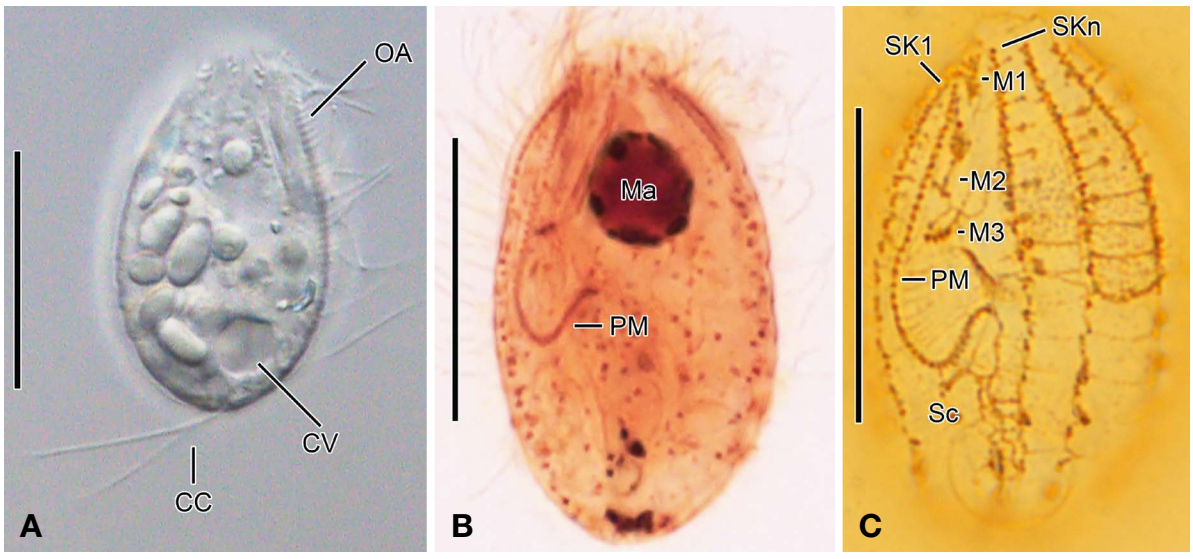

Fig. 1. *Falcicyclidium citriforme* from life (A), after protargol (B) and Chatton-Lwoff silver nitrate (C) impregnation. (A) Ventral view of a representative specimen showing the body shape, oral apparatus, contractile vacuole, somatic and caudal cilia. (B, C) Ventral views showing the infraciliatures and nuclear apparatus. CC, caudal cilia; CV, contractile vacuole; M1–3, membranelles 1–3; Ma, macronucleus; OA, oral apparatus; PM, paroral membrane; SK1 and n, somatic kineties 1 and n; Sc, scutica. Scale bars: 20 μm.

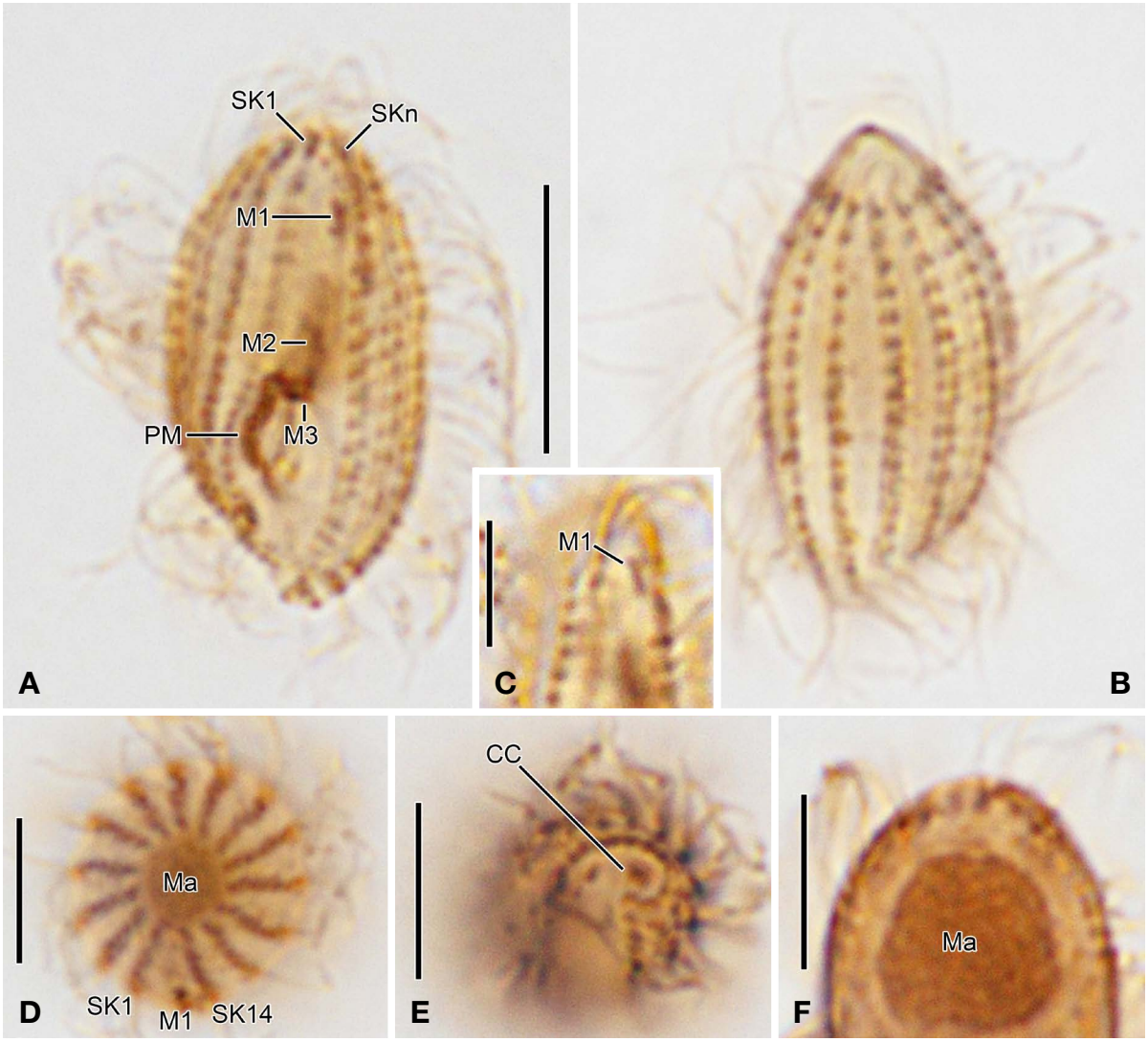

Fig. 2. *Homalogastra setosa* after protargol impregnation. (A, B) Ventral (A) and dorsal (B) view of the same specimen. The ventral view shows the body shape, cilia, and oral apparatus, while the dorsal view displays the somatic kineties and the barren apical plate. (C) Ventral view showing the single rowed membranelle 1. (D, E) Apical views of the anterior (D) and posterior (E) end of the cell, showing the somatic kineties, membranelle 1, macronucleus and caudal basal bodies. (F) Macronucleus in anterior portion of cell. CC, caudal cilium; M1−3, membranelles 1−3; Ma, macronucleus; PM, paroral membrane; SK1, 14 and n, somatic kineties 1, 14 and n. Scale bars: 10 μm (A), 3 μm (C), and 5 μm (D−F).

ed from Udo-myeon, Jeju-si, Jeju-do, Korea (33°30′51″N, 126°56′50″E) on May 29, 2024.

Diagnosis. Body size 12.6−15.3 × 7.4−8.3 μm after protargol impregnation (n = 11) (Fig. 2A, B). Body spindle-shaped, both ends pointed, posterior end slightly asymmetrical (Fig. 2A, B). One globular macronucleus size 4.0−6.1 × 3.7−5.1 μm after protargol impregnation, located slightly above the body center (Fig. 2F). 13−15 bipolar somatic kineties (Fig. 2A, B); anterior apical plate conical and distinctly baren (Fig. 2B, D); one caudal cilium (Fig. 2E). Oral apparatus occupies about 74% of body length after protargol impregnation, composed of three adoral membranelles and paroral membrane; membranelle 1 with one basal bodies row (Fig. 2C); paroral membrane on average 5 μm long after protargol impregnation (Fig. 2A).

Distribution. Africa (Buitkamp, 1977), Australia (Pomp and Wilbert, 1988), Austria (Foissner *et al*., 1982), Azerbaijan (Alekperov, 2005), China (Liu *et al*., 2020), Germany (Kahl, 1926), and Korea (present study).

Remarks. The Korean population resembles the type population described by Foissner *et al*. (1982), but differs slightly in its smaller body length after protargol impregnation (12.6−15.3 μm vs. 20−25 μm), a higher number of somatic kineties (13−15 vs. 12), and a pointed anterior end (vs. truncated).

Homalogastra setosa and *H. parasetosa* Liu *et al*., 2020

closely resemble each other but can be distinguished by the number of ciliary rows in membranelle 1 (1 vs. 2). When Liu *et al*. (2020) established *H. parasetosa*, they considered populations of *H. setosa* with a double-row membranelle 1 as junior synonyms of *H. parasetosa*.

Voucher slides. One slide with protargol-impregnated specimens (MABIK PR00045076) were deposited at the National Marine Biodiversity Institute of Korea.

Class Spirotrichea Bütschli, 1889
Subclass Euplotia Jankowski, 1979
Order Euplotida Small and Lynn, 1985
Family Euplotidae Ehrenberg, 1838
Genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831

3. *Euplotes orientalis* Jiang *et al*., 2010

Material examined. Marine water (salinity 34‰) collected from Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′47″N, 126°57′59″E) on May 29, 2024.

Diagnosis. Body size 32.1–34.3 × 15.5–17.9 μm after silver nitrate impregnation (n = 5), shape rotund; 21–23 adoral membranelles; 10 frontoventral (including 2 non-ciliated reduced cirri), 5 transverse, 2 caudal and 2 marginal cirri (Fig. 3A). 7 dorsal kineties with 6 or 7 dikinetids in the middle one; dorsal argyrome pattern of double-*patella*-I type (Fig. 3B).

Distribution. China (Jiang *et al*., 2010) and Korea (present study).

Remarks. The Korean population corresponds well with the Chinese type population described by Jiang *et al*. (2010) in several characteristics: body size after silver impregnation (32.1–34.3 μm vs. 32–42 μm); number of adoral membranelles (21–23 vs. 18–25), frontoventral cirri (8 vs. 8), caudal cirri (2 vs. 2), marginal cirri (2 vs. 2), dorsal kineties (7 vs. 6 or 7), dikinetids in the mid-dorsal row (6 or 7 vs. 6–8); dorsal argyrome pattern (double-*patella*-I); and habitat (marine).

Among the approximately 160 congeners, the presence of a reduced cirrus is a unique feature, with only eight species exhibiting this trait (Do *et al*., 2024). Of these species, *Euplotes raikovi* Agamaliev, 1966 is similar to *E. orientalis* but can be distinguished by the number of frontoventral cirri (7 vs. 8) and reduced cirri (1 vs. 2). *Euplotes pseudoraikovi* Alekperov, 2005 also resembles *E. orientalis*, but differs in the number of frontoventral cirri (8 vs. 6).

Voucher slides. Four slides with silver nitrate-impregnated specimens (MABIK PR00045072–PR00045075) were deposited at the National Marine Biodiversity Institute of Korea.

4. *Euplotes shini* Lian *et al*., 2020

Material examined. Marine water (salinity 29‰) collected from Buk-myeon, Uljin-gun, Gyeongsangbuk-do, Korea

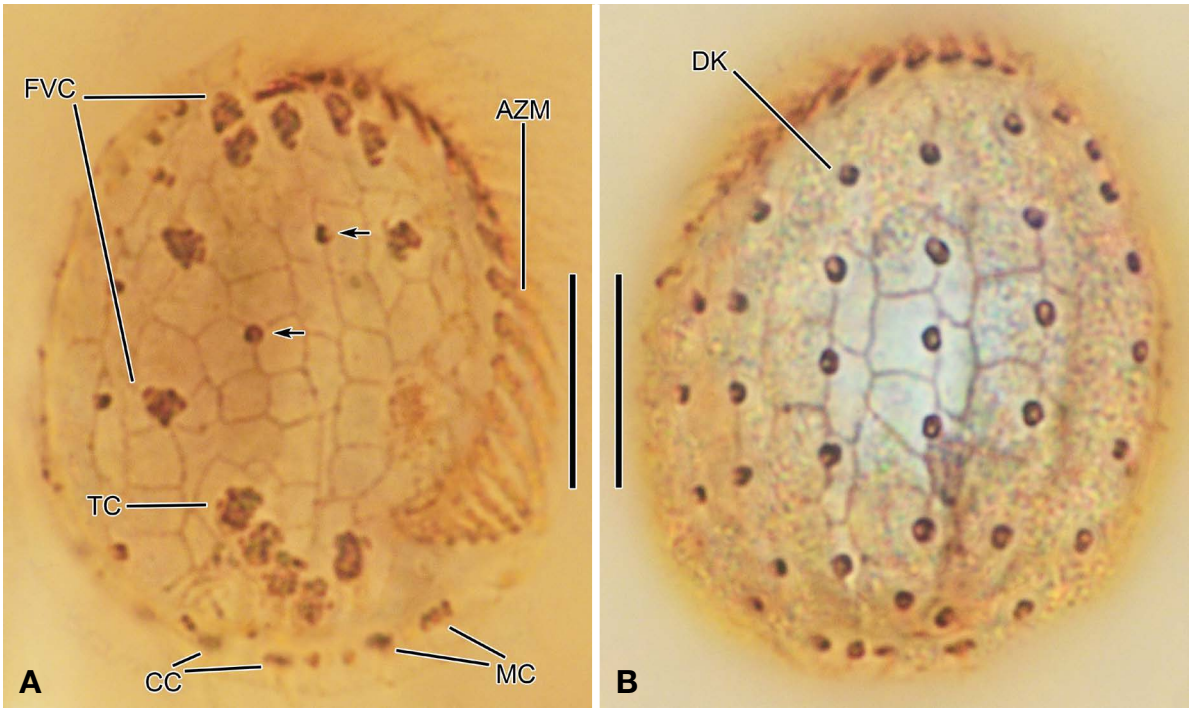

Fig. 3. *Euplotes orientalis* from Chatton-Lwoff silver nitrate impregnation. (A) Ventral view showing the body shape, ventral cirri, and oral apparatus. Arrows denote the reduced cirri. (B) Dorsal view showing the dorsal kineties and argyrome pattern. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri; TC, transverse cirri. Scale bars: 10 μm.

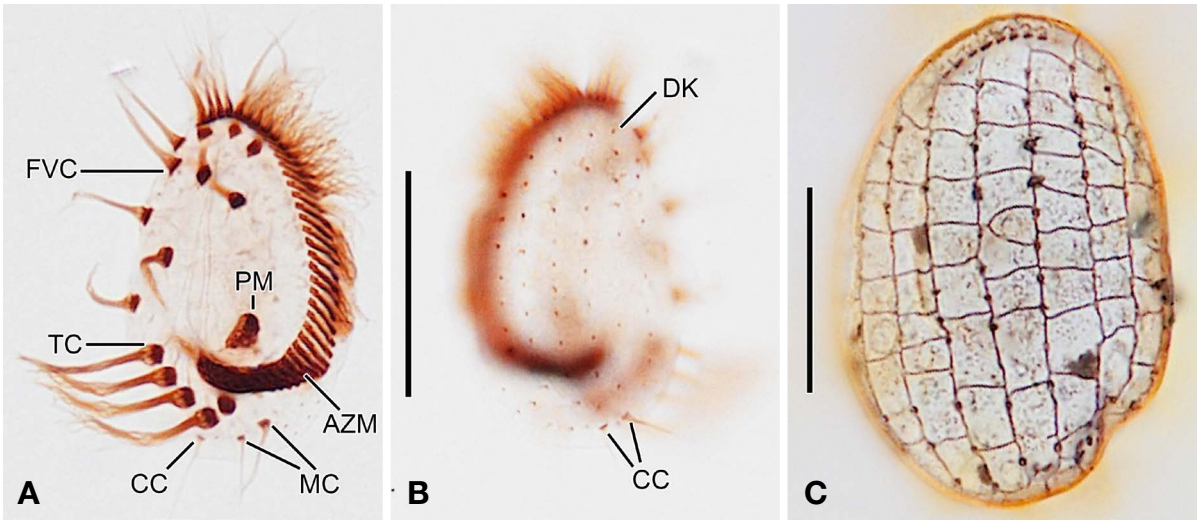

Fig. 4. *Euplotes shini* after protargol (A, B) and Chatton-Lwoff silver nitrate impregnation (C). (A, B) Ventral (A) and dorsal (B) view of the same specimen, showing body shape, ventral cirri, oral apparatus, and dorsal kineties. (C) Dorsal view showing the dorsal kineties and argyrome pattern. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri, PM, paroral membrane; TC, transverse cirri. Scale bars: 20 μm.

(37°32′5″N, 130°51′21″E) on May 23, 2023.

Diagnosis. Body size 43.8–55.0 × 20.7–29.1 μm after protargol impregnation (n = 11). Body shape broadly oval; macronucleus C-shaped, with a single small spherical micronucleus attached to it; 45–48 adoral membranelles; 10 frontoventral, 5 transverse, 2 caudal and 1 or 2 marginal cirri (Fig. 4A). 8 or 9 dorsal kineties with 11 or 12 dikinetids in the middle one; dorsal argyrome pattern of single-*vannus* type (Fig. 4B, C).

Distribution. China (Lian, 2020) and Korea (present study).

Remarks. The Korean population corresponds well with the Chinese type population described by Lian *et al.* (2020) in several aspects: buccal field length (72% vs. 75% on average); number of adoral membranelles (45–48 vs. 37–46), frontoventral cirri (10 vs. 10), caudal cirri (2 vs. 2 or 3), marginal cirri (1 or 2 vs. 2), dorsal kineties (8 or 9 vs. 9), dikinetids in the mid-dorsal row (11 or 12 vs. 10–14); dorsal argyrome pattern (single-*vannus*), and habitat (marine).

Compared to other species in the genus, *Euplotes minuta* Yocum, 1930 resembles *E. shini*, but the two species can be differentiated by the size of their marginal cirri, i.e., *E. minuta* has marginal cirri of approximately the same size (Park *et al.*, 2010), whereas *E. shini* has marginal cirri of different sizes. As noted by Lian *et al.* (2020), *E. minuta* is presumed to represent a species complex.

Voucher slides. Two slides with protargol-impregnated specimens (MABIK PR00045070, MABIK PR00045071) and one slide with silver nitrate-impregnated specimens (MABIK PR00045069) were deposited at the National Marine Biodiversity Institute of Korea.

Conflicts of Interest

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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