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New record of five *Euplotes* species (Protozoa, Ciliophora) collected from South Korea

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Five ciliate species of *Euplotes* were isolated from fresh and coastal water during a sampling survey to identify unrecorded ciliates in South Korea. Their morphology was investigated using live observation, protargol and "wet" silver nitrate staining methods. Brief descriptions and microphotographs of each species and a comparison with related species are provided. *Euplotes focardii* is characterized by an average size of 65 × 47 μm after protargol impregnation, 6 dorsal and 3 ventral ridges and dorsal argyrome pattern of double-*eurystomus* type. *Euplotes nobilii* shows an average size of 34 × 20 μm after protargol staining, 6 dorsal and 3 ventral ridges and dorsal argyrome pattern of double-*patella* type. *Euplotes octocarinatus*, the only freshwater species described in the present study, is characterized by an average size of 66 × 46 μm after protargol impregnation, 6 dorsal and 3 ventral ridges and dorsal argyrome pattern of double-*patella* type. *Euplotes petzi* has an average size of 43 × 30 μm after protargol staining, a macronucleus hook-shaped and dorsal argyrome pattern in double-*patella* type. *Euplotes raikovi* is characterized by an average size of 40 × 24 μm after protargol staining, 6 dorsal and 3 ventral ridges and dorsal argyrome pattern of double-*patella* type.

Keywords: biodiversity, biogeography, infraciliature, morphology, protargol impregnation, redescription, silver nitrate impregnation, taxonomy

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Introduction

The genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831 is one of the most species-rich groups, including about 150 species, in the phylum Ciliophora. Euplotids are benthic inhabitants of terrestrial, freshwater, and saline water environments worldwide (Curds, 1975; Song *et al*., 2009; Foissner, 2016; Živaljić *et al*., 2020; Abraham *et al*., 2021). Despite their cosmopolitan distribution in a wide variety of environments, their diversity in Korea has been insufficiently investigated. To date, only 15 euplotid species have been reported in Korea (Jung *et al*., 2017; Moon *et al*., 2017; Park *et al*., 2017; Kim and Lee, 2019). The aim of this study is not only to enhance the existing knowledge of euplotids in Korean, but also to broaden the taxon sampling within the genus. Through a detailed analysis of morphological characteristics, we have identified five previously unreported euplotids. Each species is accompanied by a short yet comprehensive description and micrographs showing their main diagnostic features.

Materials and Methods

Five water samples were collected from four coastal and one freshwater environments. The information about the sampling sites and dates is provided in the 'Material examined' section for each species. The samples were transferred to the laboratory and incubated in Petri dishes. One or several cells were isolated, and 2−3 sterilized rice grains were supplied into both pure and raw cultures to promote bacterial growth. For species identification, cells were fixed using Bouin's fluid (Coats and Heinbokel, 1982), and the infraciliature was revealed by protargol ('Procedure A') and 'wet' silver nitrate methods (Foissner, 2014). Living and impregnated specimens were studied using a stereomicroscope (Olympus SZ11, Japan) and an optical microscope (Olympus BX53) at magnifications of

40−1000×. Terminology and classification are according to Curds (1975) and Lynn (2008).

Results and Discussion

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

1. *Euplotes focardii* Valbonesi and Luporini, 1990 (Fig. 1)

Material examined. Marine water (salinity 34.9‰, temperature 21°C) collected from Seobudu, Geonib-dong, Jeju-si, Jeju-do, Korea (33°31′2″N, 126°32′3″E) on February 20, 2020.

Diagnosis. Body size 53.9−72.7×41.7−58.8 μm (on average 65.1×47.1 μm) after protargol impregnation, shape ellipsoidal, 6 dorsal and 3 ventral ridges (shorter ventral ridges between frontoventral cirri are not included); macronucleus C-shaped, with a single small spherical micronucleus attached to it; 49−56 adoral membranelles; 10 frontoventral, 5 transverse, 2 caudal and 2 marginal cirri; 9 or 10 dorsal kineties, of which the middle kinety composed of about 15−20 dikinetids; dorsal argyrome pattern of double-*eurystomus* type.

Distribution. Antarctica (Valbonesi and Luporini, 1990b) and Korea (present study).

Remarks. The Korean population resembles the type population except for the dorsal (distinct vs. indistinct) and ventral ridges (3 vs. 2) (Valbonesi and Luporini, 1990b). These populations were collected from marine environments and the other morphometric data correspond each other. The identification of *E. focardii* is rather complex due to the strong overlapping of morphometric data among several *Euplotes* species (e.g., body size, adoral membranelles, cirri, dorsal kineties). *Euplotes focardii* morphologically resembles *E. neapolitanus* Wichterman, 1964, *E. platystoma* Dragesco and Dragesco-Kernéis, 1986 and *E. shanghaiensis* Song *et al*., 1998. *Euplotes neapolitanus* differs from *E. focardii* by the body size (130−150×70−75 μm vs. 38−110×30−92 μm), the shape of anterior body end (truncated vs. rounded) and the arrangement of marginal and caudal cirri (evenly distributed vs. a distinct gap between marginal and caudal cirri) (Curds, 1975; Liu *et al*., 2020). *Euplotes shanghaiensis* can be distinguished from *E. focardii* by the body shape (D-shaped vs. ellipsoidal), the number of dorsal kineties (12 or 13 vs. 9 or 10) and the habitat (freshwater vs. marine) (Song *et al*., 1998).

Based on the original descriptions, *E. platystoma* differs from *E. focardii* in the number of dorsal kineties (11 vs. 10), the arrangement of marginal and caudal cirri (evenly distributed vs. a distinct gap between marginal and caudal cirri), and the habitat (brackish water vs. marine) (Dragesco and Dragesco-Kernéis, 1986; Valbonesi and Luporini, 1990b). However, recent descriptions blur the boundary between these two species. For instance, the number of dorsal kineties varies among *E. platystoma* populations (11 in the type and Shenzhen populations, 13 in Shanghai population, and 10 or 11 in Huizhou population). Regarding the habitat, the type population was collected from brackish water, while Lian *et al*. (2018) sampled two populations from freshwater and brackish water (6‰), respectively. In addition, while Dragesco and Dragesco-Kernéis (1986) did not include any information about the dorsal ridges in the type population, Lian *et al*. (2018) and Yan *et al*. (2018) described the ridges as indistinct or absent, respectively. Interestingly, according to Lian *et al*. (2018),

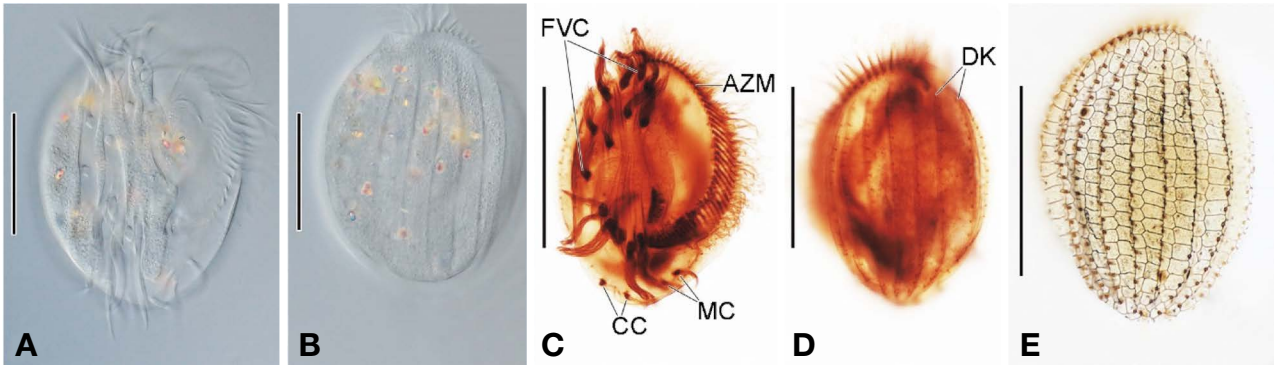

Fig. 1. *Euplotes focardii* in vivo (A) and after protargol (B, C) and "wet" silver nitrate impregnation (D). A−D. Ventral (A, C) and dorsal (B, D) views of two specimens showing the infraciliature and the three ventral and six dorsal ridges. E. Dorsal argyrome in double-*eurystomus* pattern. AZM, adoral zone of membranelles; CC; caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri. Scale bars: 20 μm.

the small subunit ribosomal RNA gene sequences of the Shanghai population (13 dorsal kineties, brackish water) and Shenzhen population (11 dorsal kineties, freshwater) are identical. Considering the gap between marginal and caudal cirri, it varies among populations (evenly distributed in the Huizhou population vs. a distinct gap in Shanghai and Shenzhen populations) (Lian *et al*., 2018; Yan *et al*., 2018). In conclusion, when considering the features analyzed in both the original and recent redescriptions of *E. platystoma* and *E. focardii*, the main differences between these two species lie in their habitat preferences (fresh to brackish water vs. marine) and genetic markers (as depicted in the tree by Liu *et al*., 2020, they are clearly distinct from each other).

Voucher slides. One slide with protargol-impregnated specimens (NNIBRPR25655) and one slide with wet silver nitrate-impregnated specimens (NNIBRPR25656) were deposited at the Nakdonggang National Institute of Biological Resources, Sangju, Republic of Korea.

2. *Euplotes nobilii* Valbonesi and Luporini, 1990 (Fig. 2)

Material examined. Marine water (salinity 21‰) collected from Gyeonpo Lake, Jeo-dong, Gangneung-si, Gangwon-do, Korea (37°48′6″N, 128°54′14″E) on July 23, 2020.

Diagnosis. Body size about 29.7–41.7 × 14.9–26.4 μm (on average 33.8 × 20.0 μm) after protargol impregnation, body shape sharp oval, left margin slightly more convex than right margin, 6 dorsal and 3 ventral ridges; one macronucleus C-shaped with one spherical micronucleus attached; 19–29 adoral membranelles; 10 frontoventral, 5 transverse, 2 caudal and 1 marginal cirrus; 7 or 8 dorsal kineties, of which the middle kinety composed of 6–9 dikinetids; dorsal argyrome pattern of double-*patella* type.

Distribution. Probably cosmopolitan (Greenland, Tierra del Fuego, Terra Nova, Antarctic Ocean and Korea; Valbonesi and Luporini, 1990a; Di Giuseppe *et al*., 2013).

Remarks. The Korean population corresponds well with the type population (Valbonesi and Luporini, 1990a), although one outlier specimen from the Korean population showed a slightly higher number of adoral membranelles (i.e., 29 adoral membranelles) than the type population (19–23 vs. 18–22).

Compared to other species in the genus, *E. rariseta* Curds *et al*., 1974 highly resembles *E. nobilii*, but it differs from the latter by the number of dikinetids in the middle kinety (maximum 6 vs. 6–9) (Curds *et al*., 1974; Valbonesi and Luporini, 1990a). However, the description of the morphometric features of the dorsal kinety of *E. rariseta* has experienced some variation since it was originally described, to include variability among populations, i.e., (number of dorsal kineties/number of dikinetids on mid-kinety) 5/maximum 6 (Wilbert and Kahan, 1981), 6/5–7 (Kim and Lee, 2019), 7/7 (Dallai *et al*., 1987), 7/5–7 (Song and Packroff, 1997; Ma *et al*., 2007), 7/8–10 (Valbonesi and Luporini, 1990a). The Antarctic population reported by Valbonesi and Luporini (1990a) might be new to science, as already mentioned by the authors, because it has more adoral membranelles than the others: 28–30 vs. 23 (as seen in the original line drawings of the type population in Curds *et al*., 1974), 22 ± 2 (Dallai *et al*., 1987), 17–21 (Song and Packroff, 1997), 17–22 (Ma *et*

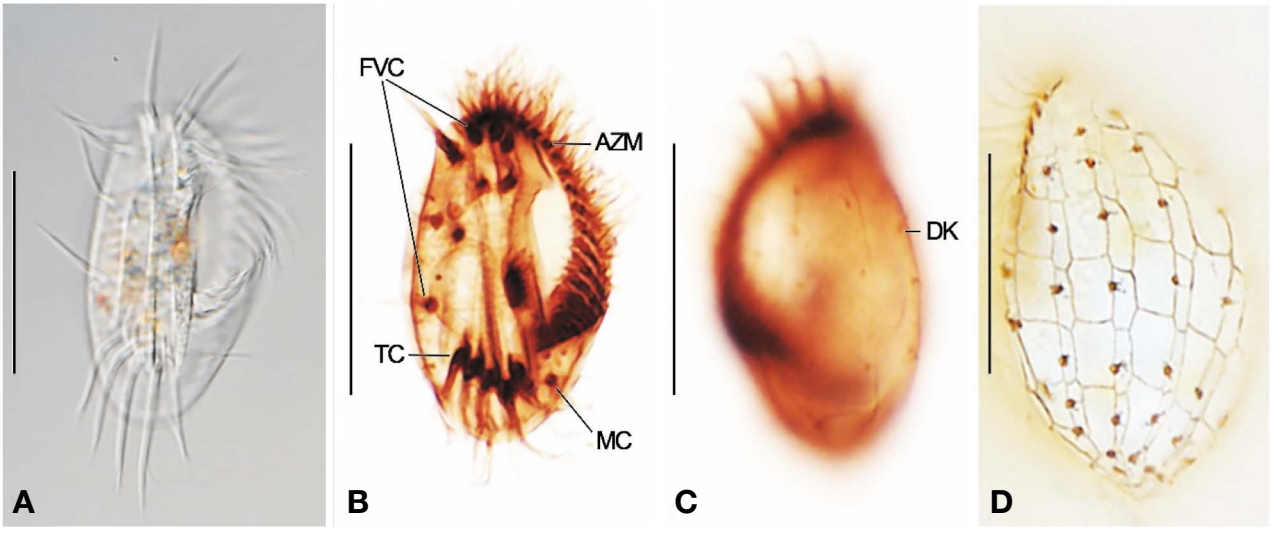

Fig. 2. *Euplotes nobilii* in vivo (A) and after protargol (B, C) and "wet" silver nitrate impregnation (D). A–C. Ventral (A, B) and dorsal (C) views showing the infraciliature. D. Dorsal view showing the argyrome pattern of double-*patella* type. AZM, adoral zone of membranelles; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri; TC, transverse cirri. Scale bars: 30 μm (A), 20 μm (B–D).

al., 2007). Unfortunately, phylogenetic analyses based on small subunit ribosomal DNA (SSU rDNA), do not provide enough resolution to discriminate among species of *Euplotes*. Thus, *E. nobilii* and *E. rariseta* (GenBank accession numbers KC599234 and AF492706, respectively) cluster together as almost similar species according to the SSU rDNA phylogeny (Lian *et al*., 2021). In contrast to the limited resolution provided by the SSU rDNA gene to identify cryptic species, mitochondrial cytochrome *c* oxidase subunit I gene (*CO1*) has been proved as a valuable barcode to distinguish among cryptic species with identical SSU rDNA regions (Quintela-Alonso *et al*., 2013), because it has a distinct 'barcode gap' between maximum intra-specific and minimum inter-specific divergences (Park *et al*., 2019).

Voucher slides. One slide with protargol-impregnated specimens (NNIBRPR25657) and one slide with wet silver nitrate-impregnated specimens (NNIBRPR25658) were deposited at the Nakdonggang National Institute of Biological Resources.

3. *Euplotes octocarinatus* Carter, 1972 (Fig. 3)

Material examined. Freshwater collected from ecological reservoir, Gyeongpo-dong, Gangneung-si, Gangwon-do, Korea (37°46′57″N, 128°52′60″E) on July 23, 2020.

Diagnosis. Body size 60.3–79.3 × 32.0–49.9 μm (on average 66.5 × 46.4 μm) after protargol impregnation, shape oval to ellipsoidal, 6 dorsal and 3 ventral ridges; one macronucleus C-shaped with a single small spherical micronucleus attached; 33–36 adoral membranelles; 9 frontoventral, 5 transverse, 2 caudal and 2 marginal cirri; invariably 8 dorsal kineties, of which the middle kinety composed of about 14–19 dikinetids; dorsal argyrome pattern in double-*patella* type.

Distribution. Worldwide (Carter, 1972; Méndez-Sánchez *et al*., 2020).

Remarks. The characteristics of the Korean population correspond well with the type (Carter, 1972) and Mexican populations (Méndez-Sánchez *et al*., 2020).

Some species with silverline system of double-*patella* type in *Euplotes* (i.e., *E. apsheronicus* Agamaliev, 1966, *E. patella* (Müller, 1773) Ehrenberg, 1838, *E. zenkewitchi* Curds, 1975 and *E. elegans* Kahl, 1932) also resemble *E. octocarinatus*. However, *E. apsheronicus* differs from *E. octocarinatus* in the number of dorsal kineties (9 vs. 8) and the habitat (marine vs. freshwater) (Curds, 1975). *Euplotes patella* can be distinguished from *E. octocarinatus* by the body size (90–120 × 55–75 vs. 60–79 × 32–50) and the number of dorsal kineties (9 vs. 8) (Foissner *et al*., 1991). *Euplotes zenkewitchi* differs from *E. octocarinatus* in the body size (80 × 50 vs. 60–79 × 32–50), the number of adoral membranelles (50–55 vs. 33–36), the number of dorsal kineties (10 vs. 8), and the habitat (marine vs. freshwater) (Curds, 1975). *Euplotes elegans* differs from *E. octocarinatus* in the body size (65–118 × 33–63 vs. 60–79 × 30–50), the number of adoral membranelles (47–64 vs. 33–36), the number of dorsal kineties (9–10 vs. 8), and the habitat (marine vs. freshwater) (Schwarz *et al*., 2007).

Voucher slides. One slide with protargol-impregnated specimens (NNIBRPR25659) and one slide with wet silver nitrate-impregnated specimens (NNIBRPR25660) were deposited at the Nakdonggang National Institute of Biological Resources.

4. *Euplotes petzi* Wilbert and Song, 2008 (Fig. 4)

Material examined. Marine water (salinity 28‰) collected from West Sea, Janghang-eup, Seocheon-gun, Chungcheongnam-do, Korea (36°00′55″N, 126°39′49″E) on January 21, 2021.

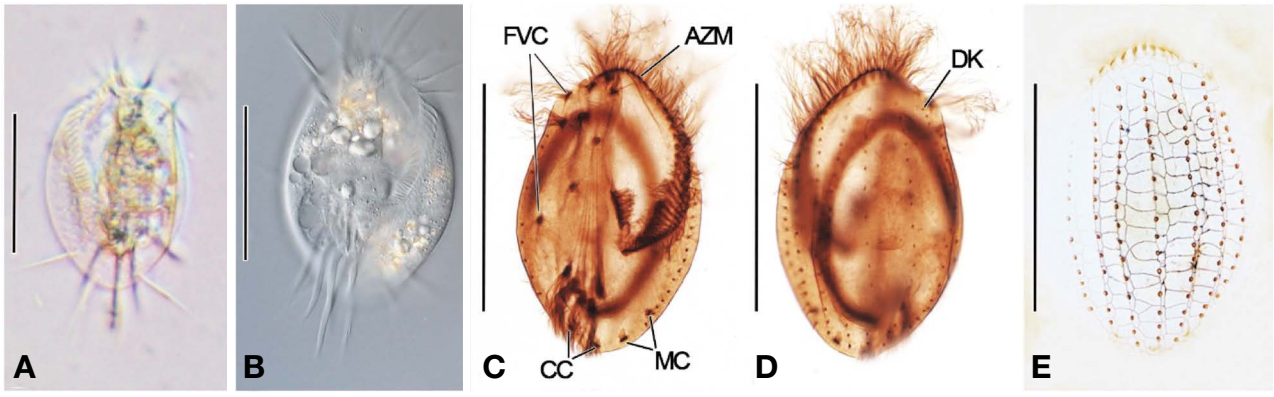

Fig. 3. *Euplotes octocarinatus* in vivo (A, B) and after protargol (C, D) and "wet" silver nitrate impregnation (E). A–D. Ventral (A, C) and dorsal (B, D) views of different specimens showing the infraciliature and nuclear apparatus. E. Dorsal argyrome of double-*patella* type. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri. Scale bars: 50 μm.

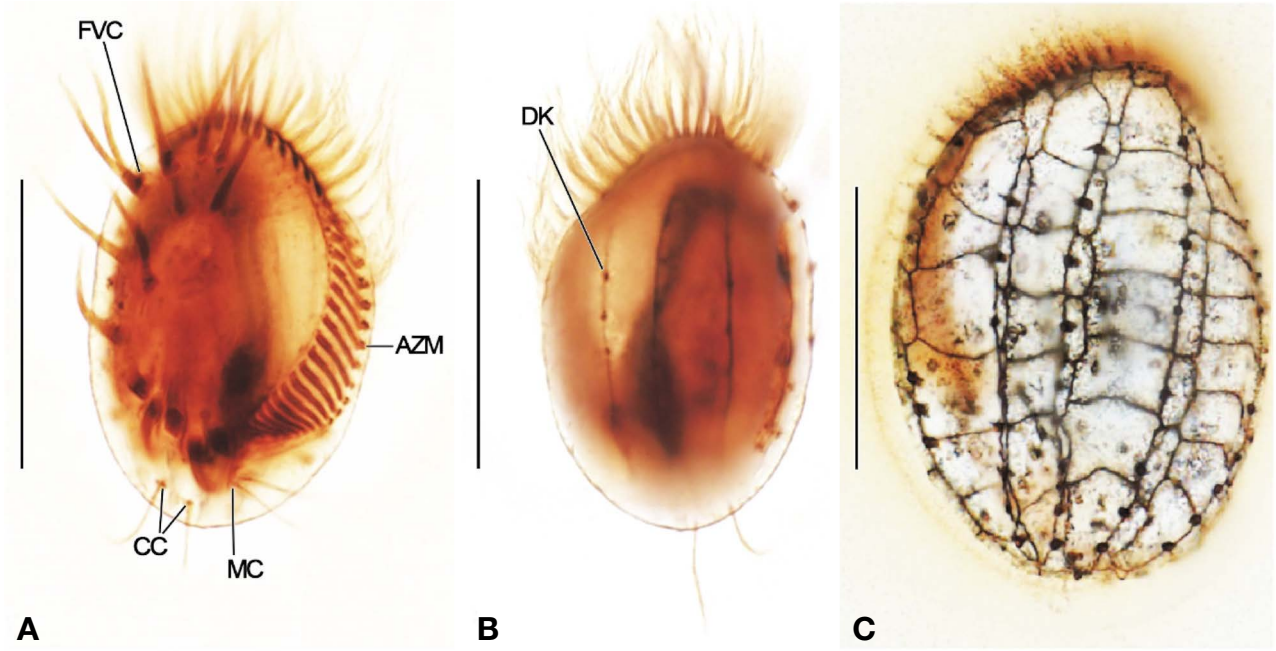

Fig. 4. *Euplotes petzi* after protargol (A, B) and after "wet" silver nitrate impregnation (C). A, B. Ventral (A) and dorsal (B) view of a representative specimen. Note the narrow separation between the two marginal cirri, a distinctive characteristic of this species. C. Dorsal view showing the argyrome in double-*patella* pattern. AZM, adoral zone of membranelles; CC; caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri. Scale bars: 30 μm.

Diagnosis. Body size 38.2−46.2 × 25.3−36.7 μm (on average 42.6 × 29.9 μm) after protargol impregnation, shape ellipsoidal; macronucleus hook-shaped, with one spherical micronucleus attached; 35−45 adoral membranelles; 10 frontoventral, 5 transverse, 2 caudal and 2 marginal cirri; invariably 6 dorsal kineties, of which the middle kinety composed of about 8−10 dikinetids; dorsal argyrome pattern in double-*patella* type.

Distribution. King George Island (Wilbert and Song, 2008) and Korea (present study).

Remarks. The morphology of the Korean and King George Island populations of *Euplotes petzi* (Wilbert and Song, 2008) is mostly similar except cell size (38−46 × 25−37 μm, and 50−80 × 30−50 μm). A distinctive morphological feature of this species is the narrow separation between the two marginal cirri compared to other *Euplotes* species. Di Giuseppe *et al*. (2014) improved the morphological characterization of *E. petzi* and used SSU rDNA gene sequences to relate this species to *E. sinicus* Jiang *et al*., 2010b as the deepest branch at the base of the *Euplotes* phylogenetic tree. Moreover, their investigation revealed notable similarities between these two species in terms of the cell size and key characteristics.

Voucher slides. One slide with protargol-impregnated specimens (NNIBRPR25661) and one slide with wet silver nitrate-impregnated specimens (NNIBRPR25662) were deposited at the Nakdonggang National Institute of Biological Resources.

5. *Euplotes raikovi* Agamaliev, 1966 (Fig. 5)

Material examined. Marine water (salinity 34‰, temperature 26°C) collected from Jongdal harbor, Gujwa-eup, Jeju-si, Jeju-do, Korea (33°29′48″N, 126°54′42″E) on August 19, 2020.

Diagnosis. Body size about 35.2−48.0 × 20.0−28.7 μm (on average 40.5 × 24.6 μm) after protargol impregnation, shape oval, 6 dorsal and 3 ventral ridges; macronucleus C-shaped with a single spherical micronucleus attached to it; 27−31 adoral membranelles; 7 frontoventral [note that the reduced, non-ciliated, frontoventral cirrus (basal plaque) is not counted], 5 transverse, 2 caudal cirri and 1 marginal cirrus; 7 or 8 dorsal kineties, of which the middle one is composed of about 9−11 dikinetids; dorsal argyrome pattern of double-*patella* type.

Distribution. Cosmopolitan.

Remarks. The Korean population of *E. raikovi* highly resembles the type population (Agamaliev, 1966) except for the number of dorsal kineties (7 or 8 vs. 6 or 7). Short time after the original description, Agamaliev (1967) reported a population with 8 frontoventral cirri (vs. 7 in the type), but Jiang *et al*. (2010a) considered it as a different form because the basal plaque is very stable among populations (Washburn and Borror, 1972; Miceli *et al*., 1981; Jiang

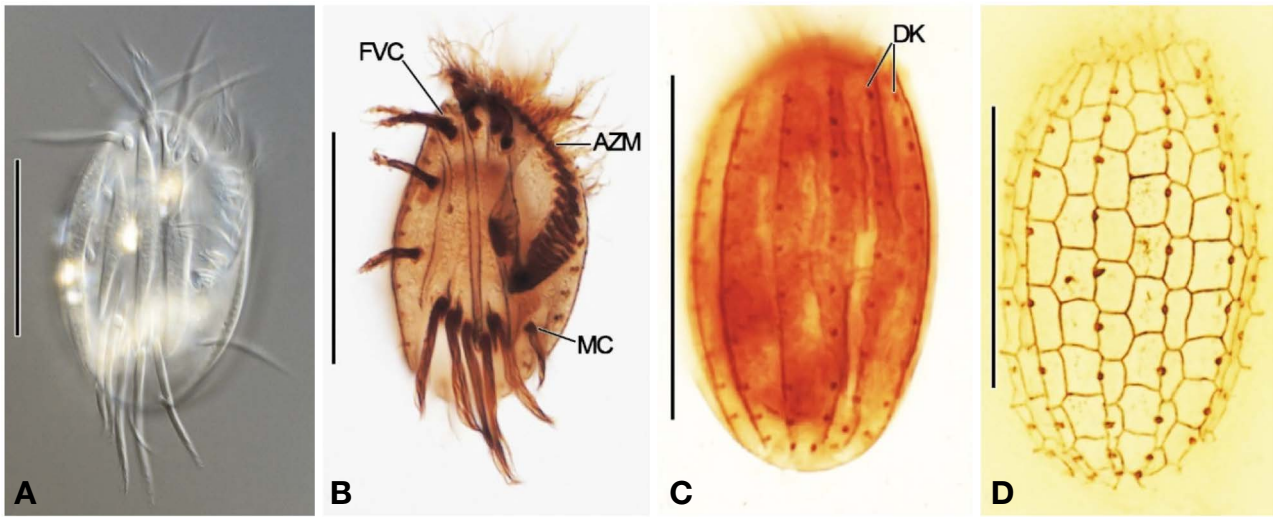

Fig. 5. *Euplotes raikovi* in vivo (A) and after protargol (B, C) and "wet" silver nitrate impregnation (D). A–C. Ventral (A, B) and dorsal (C) views of specimens showing the infraciliature and ventral and dorsal ridges. D. Silverline system on dorsal side of double-*patella* type. AZM, adoral zone of membranelles; DK, dorsal kineties; FVC, frontoventral cirrus; MC, marginal cirri. Scale bars: 30 μm.

et al., 2010a). Additionally, Jiang *et al*. (2010a) reported 8 kineties. Considering the basal plaque and saline habitat, *E. raikovi* is similar to *E. elegans* Kahl, 1932, *E. orientalis* Jiang *et al*., 2010a, *E. pseudoraikovi* Alekperov, 2005, and *E. strelkovi* Agamaliev, 1967. Originally, *E. raikovi* had 8 frontoventral cirri, one of which disappeared and remained as a trace, leaving 7 frontoventral cirri. Only two species within the genus *Euplotes* have 7 frontoventral cirri, i.e., *E. raikovi* and *E. oropensis* (Fernández-Leboráns and Castro de Zaldumbide, 1986), however, they can be distinguished by the dorsal argyrome pattern, double-*patella* in *E. raikovi* vs. double-*eurystomus* in *E. oropensis*.

Voucher slides. One slide with protargol-impregnated specimens (NNIBRPR25663) and one slide with wet silver nitrate-impregnated specimens (NNIBRPR25664) were deposited at the Nakdonggang National Institute of Biological Resources.

A Key to the Species of Korean *Euplotes*

Key to the major groups of species

1. Key to species with a single-*vannus* type dorsal argyrome ····· Section A
2. Key to species with a double-*eurystomus* type dorsal argyrome ····· Section B
3. Key to species with a double-*patella* type dorsal argyrome ····· Section C
4. Key to species with a multiple type dorsal argyrome ·· ····· Section D
5. Key to species with a complex type dorsal argyrome·· ····· Section E

Section A. Key to species with a single-*vannus* type dorsal argyrome

1. 8 dorsolateral kineties ····· *E. cristatus*
– 9 or more dorsolateral kineties ····· 2
2. 9 dorsolateral kineties with 10–12 dikinetids in mid-dorsal kinety ····· *E. minuta*
– 9 or more dorsolateral kineties with 15 or more dikinetids in mid-dorsal kinety ····· 3
3. 9–12 dorsolateral kineties with 15–22 dikinetids in mid-dorsal kinety ····· *E. crassus* or *E. vannus*

Section B. Key to species with a double-*eurystomus* type dorsal argyrome

4. 9 fronto-ventral cirri ····· 5
– 10 fronto-ventral cirri ····· 8
5. 7 dorsolateral kineties ····· *E. eurystomus*
– 8 or more dorsolateral kineties ····· 6
6. 8 dorsolateral kineties ····· *E. aediculatus*
– 9 or 10 dorsolateral kineties ····· 7
7. 9 dorsolateral kineties with about 20–30 dikinetids in mid-dorsal kinety ····· *E. parawoodruffi* or *E. woodruffi*
8. 12 dorsolateral kineties ····· *E. charon*
– 8 or more dorsolateral kineties ····· 9
9. 8–9 dorsolateral kineties with 12 dikinetids in mid-dorsal kinety ····· *E. balteatus*
– 9–10 dorsolateral kineties with 15–20 dikinetids in mid-dorsal kinety ····· *E. focardii* (Fig. 1)

Section C. Key to species with a double-*patella* type dorsal argyrome

10. 7 fronto-ventral cirri ····· *E. raikovi* (Fig. 5)

– 9 or more fronto-ventral cirri ····· 11
11. 9 fronto-ventral cirri ····· 12
– 10 fronto-ventral cirri ····· 13
12. 7 dorsolateral kineties ····· *E. patella*
– 8 dorsolateral kineties ····· *E. octocarinatus* (Fig. 3)
13. 7 or 8 dorsolateral kineties ····· *E. nobilii* (Fig. 2)
– 6 dorsolateral kineties ····· 14
14. 5–7 dikinetids in mid-dorsal kinety ····· *E. rariseta*
– 8–10 dikinetids in mid-dorsal kinety··· *E. petzi* (Fig. 4)

Section D. Key to species with a multiple type dorsal argyrome

15. Soil, 9 fronto-ventral cirri, 9 dorsolateral kineties with 20–30 dikinetids in mid-dorsal kinety····· *E. muscicola*

Section E. Key to species with a complex type dorsal argyrome

16. Coastal, 9 fronto-ventral cirri, 7 dorsolateral kineties, 20–22 dikinetids in mid-dorsal kinety····· *E. encysticus*
– Soil, 9 fronto-ventral cirri, 8 dorsolateral kineties, 22–28 dikinetids in mid-dorsal kinety ····· *E. muscorum*

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