

First Record of Two *Euplotes* Ciliates (Ciliophora: Spirotrichea: Euplotida) from Korea

Mi-Hyun Park¹, Se-Joo Kim^{1,2} and Gi-Sik Min^{1,*}

¹Department of Biological Sciences, Inha University, Incheon 402-751, Korea

²The Research Institute for Natural Sciences, Hanyang University, Seoul 133-791, Korea

ABSTRACT

Two marine euplotid ciliates, i.e. *Euplotes cristatus* Kahl, 1932 and *E. minuta* Yocom, 1930, were collected from the public waterfront of Incheon on the Yellow Sea and from the Songjeong Beach, Busan, in the Strait of Korea, respectively. These two species were verified as unrecorded species in Korea. These species were described based on live observation, protargol impregnation, and silver nitrate impregnation. In addition, the small subunit ribosomal DNA (SSU rDNA) sequences of the two species were compared with previously known sequences of the *Euplotes* species. *Euplotes cristatus* has an elongated oval form, size *in vivo* of 60-84 × 38-68 μm, 35-50 adoral zone of membranelles (AZM), 10 frontoventral cirri (FVC), 5 transverse cirri (TC), 4-5 caudal cirri (CC), 8 dorsal kineties (DK), 10-16 dorsal cilia of middle DK, and silverline system of single-*vannus* type. *Euplotes minuta* has a small ovoid form (44-53 × 26-35 μm *in vivo*), 31-41 AZM, 10 FVC, 5 TC, 4 CC, 9 DK, 10-12 dorsal cilia of middle DK, and silverline system of single-*vannus* type.

Keywords: *Euplotes cristatus*, *Euplotes minuta*, marine euplotid ciliate, SSU rDNA, Korea

INTRODUCTION

Species in the genus *Euplotes* Ehrenberg, 1830 have been known as easily investigated and cultured ciliates in the aquatic ecosystem (Petroni et al., 2002; Schwarz et al., 2007). Over 100 species have been described in this genus from the world (Berger, 2001). After the pattern of the silverline system came to be considered as an important factor for species classification, many elements on the classification of the euplotid species have been newly revised by taxonomists (Tuffrau, 1960; Borror, 1968, 1972; Carter, 1972; Curds, 1974; Gates, 1978).

The morphological characters of *Euplotes*-complex are still disputed. Curds (1975) partitioned the *Euplotes*-complex species into six species groups based on differences in the dargyrome pattern. Gates and Curds (1979) newly divided this species complex into three groups characterized as single-, double-, and multiple- dargyrome types. More recently, Borror and Hill (1995) redefined the *Euplotes*-complex species into four genera (*Euplotes*, *Euplotopsis*, *Euplotoides*, and *Moneuplotes*) following characteristics of cortical structure, endosymbionts, morphometric data, morphogenetic patterns, and ecology. Yi et al. (2009) suggested that the systematics of the *Euplotes*-complex needs be reconsidered based on molecular evidence in some cases of taxonomic

classification.

To date, eight species of *Euplotes* have been reported in Korea: *E. aediculatus*, *E. charon*, *E. eurystomus*, *E. muscicola*, *E. muscorum*, *E. parawoodruffi*, *E. patella*, and *E. vannus* (Shin and Kim, 1988; Shin et al., 1992; Shin and Kim, 1995; Jo and Shin, 2003; Kwon and Shin, 2006; Kwon et al., 2007).

Two species, *Euplotes cristatus* and *E. minuta*, are firstly reported to Korean fauna. Two species are described based on their living morphology, protargol impregnation, silver nitrate impregnation, and analysis of small subunit ribosomal RNA (SSU rRNA) gene sequences.

MATERIALS AND METHODS

Collection and identification

Euplotes cristatus and *E. minuta* were collected from coastal areas of Korea using both of PEFS (the polyurethane foam enveloped) system (Xu et al., 2009) and natural slides (Kim and Min, 2009b). Rice grains were used to enrich bacterial growth in the cultures. The specimens were observed under the light microscope (Leica DM2500). The infraciliature was impregnated with the protocol of Foissner (1991). The Chatton-Lwoff method was used for revealing the silverline systems (Corliss, 1953; Song and Wilbert, 1997). Counts and measurement on stained specimens were performed at 1,000 magnification, and drawings were made with a camera

*To whom correspondence should be addressed

Tel: 82-32-860-7692, Fax: 82-32-874-6737

E-mail: mingisik@inha.ac.kr

lucida. The classification scheme followed Lynn and Small (2002). Terminology is mainly according to Curds (1975) and Song et al. (2009). Abbreviations were AZM, adoral zone of membranelles; CC, caudal cirri; CV, contractile vacuole; DK, dorsolateral kineties; FVC, frontoventral cirri; Ma, macronuclear nodule; Mi, micronucleus; PM, paroral membrane; TC, transverse cirri.

Molecular identification

Single living individual specimens were isolated under a dissecting microscope (Olympus SZX12) and washed five times in sterile seawater of the appropriate salinity and then transferred into 1.5 mL microcentrifuge tubes. Genomic DNA extraction was carried out using RED Extract-N-Amp™ Tissue PCR kit (Sigma-Aldrich). Amplification of SSU rRNA gene and sequencing of the purified PCR products were performed according to Kim and Min (2009a). Sequencing was carried out using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems) using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequence variations among the species were calculated using MEGA4 software (Tamura et al., 2007) with a Kimura 2-parameter.

SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901
 Class Spirotrichea Bütschli, 1889
 Subclass Hypotrichia Stein, 1859
 Order Euplotida Small & Lynn, 1985
 Family Euplotidae Ehrenberg, 1838
 Genus *Euplotes* Ehrenberg, 1830

¹**Euplotes cristatus* Kahl, 1932 (Fig. 1 and Table 1)

Euplotes cristatus Kahl, 1932, p. 633, Figs. S. 638, 20-21; Tuffrau, 1960, p. 37, Figs. 20-21; Carter, 1972, p. 468, Figs. 1-2; Curds, 1975, p. 12, Fig. 5; Carey, 1992, p. 206, Fig. 819.

Moneuplotes cristatus (Kahl, 1932) Borrer and Hill, 1995, p. 461.

Material examined. Specimens were collected from the public waterfront, Incheon (37° 26'N and 126° 35'E) on the Yellow Sea of Korea on May 21-29, 2007 (18°C, ca. 29‰, and pH ca. 7.8).

Description. Cell size *in vivo* 60-84 × 38-68 μm in length; body shape constant, elongate oval as shown in Fig. 1A, E-F; anterior end narrowly rounded while posterior end widely

rounded; dorso-ventrally flattened (Fig. 1H-I); buccal field narrow, extending about 67% of body length (Fig. 1A-B, E, J). Cytoplasm hyaline, central part opaque due to numerous yellow to green-coloured and different-sized lipid droplets and a few food vacuoles and granules (Fig. 1A, E-J). CV adjacent to the rightmost TC, about 7 μm in diameter (Fig. 1J, arrowhead).

Ma curved C-shaped, containing numerous large nucleoli; one spherical Mi located on the upper left border of the Ma (Fig. 1B, M-N).

Infraciliature as shown in Fig. 1A-C, F-G, J, M, O. Consistently 10 FVC arranged in normal pattern and located on anterior ventral surface (Fig. 1A-C, M, O); 5 strong TC positioned at posterior region (Fig. 1A-C, E); 4 to 5 CC present at posterior end (Fig. 1A-C, F, M; 1F, arrowheads). AZM prominent, proximal portion curved at about 90° in direction of right, and composed of 35-50 membranelles (ca. 15 μm in length) (Fig. 1B, F-G, J). PM small, beneath buccal lip (Fig. 1B, J; 1J, arrow).

Dorsal surface detected ridges (Fig. 1K, arrowheads). DK distinct, 8 in number, extending of body length; 6 DK located on dorsal side and each one DK located on both margins of ventral surface (Fig. 1B, D, L, M-N, P; 1L, arrowheads); composing about 10-16 dikinetids in middle DK (Fig. 1D). Silverline system typical *vannus* type, single-type dargyrome, irregular pattern on ventral side (Fig. 1C-D, O-P).

The SSU rDNA sequence of this population is 1,856 bp in length, and deposited in GenBank accession number GU953667. Inter-specific variation is 0.8% and 1.8% between this population and two other species, *E. minuta* (AJ305244-6 and EF094958) and known *E. vannus* (AY361854, AY361856, and AJ305241), respectively.

Distribution. France, Germany, USA, and Korea (this study).

Remarks. *Euplotes cristatus* was originally described by Kahl (1932) based on living observation. However in this original description, the important information for species identification such as the exact cirral pattern (number of AZM, FVC, TC, DK, and dikinetids of mid-DK) and silverline system was not provided. Later, Tuffrau (1960) re-described their population using the Chatton-Lwoff staining method (Chatton and Lwoff, 1930, 1935). The description of the present population is almost identical to that of Tuffrau's (1960) population in size of body (55-75 μm in long, size strongly overlapped); number of FVC (10), TC (5), DK (8); type of dargyrome (single-*vannus*) with exception of shape of body; number of AZM, CC, and cilia of mid-DK. Tuffrau (1960) noted the form of body, clearly ovoid, while this population has an elongated oval form. Tuffrau (1960) did not describe the number of AZM and dikinetids of mid-

¹*벼슬머리자라하모충 (신칭)

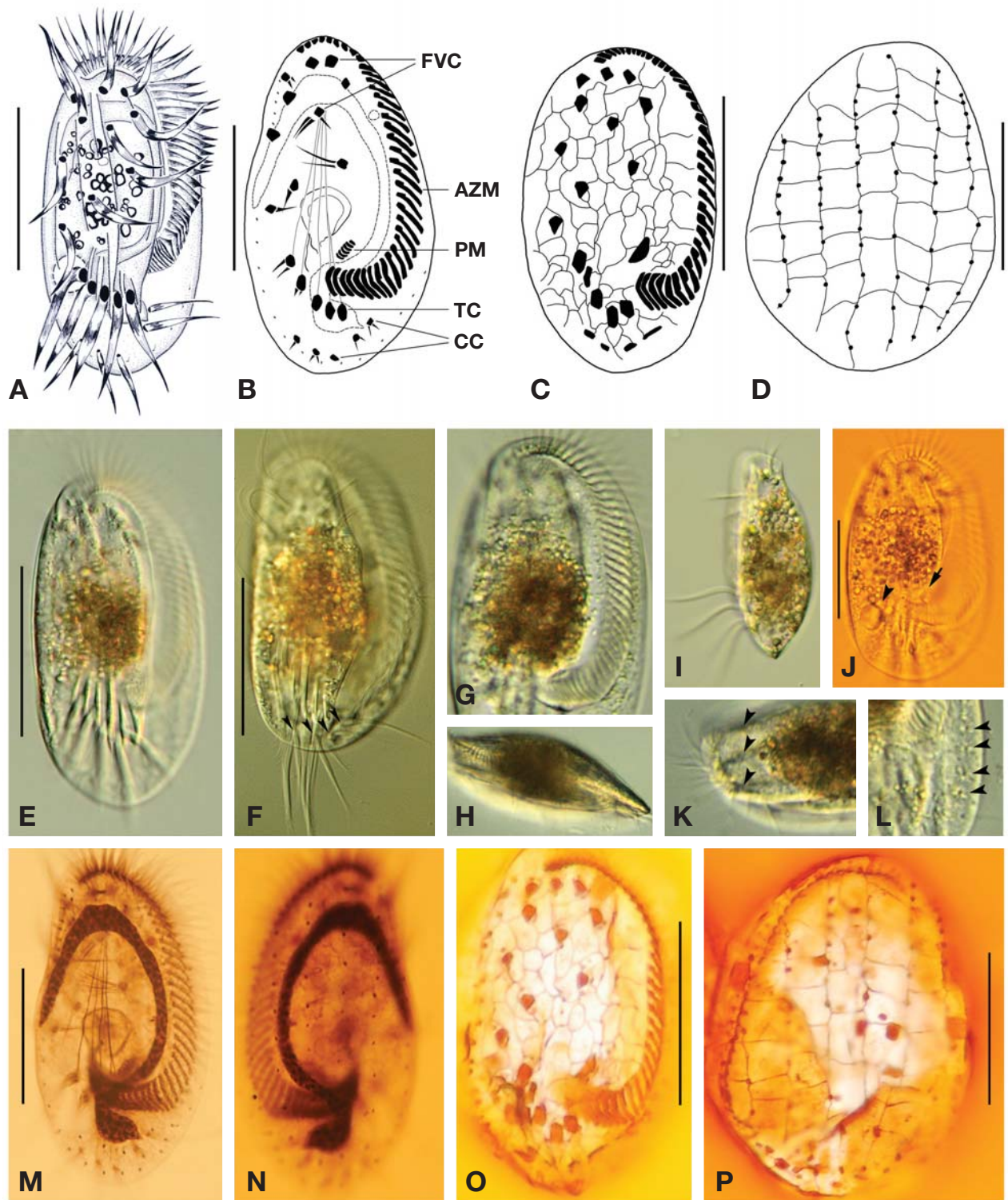


Fig. 1. Morphology and infraciliature of *Euplotes cristatus* Kahl, 1932 *in vivo* (A, E-L), protargol impregnation (B, M-N), and silver nitrate impregnation (C-D, O-P). A, E-F, ventral view of live, arrowheads to show caudal cirri (F); B, infraciliature of ventral side; C-D, O-P, silverline system on ventral (C, O) and dorsal (D, P) sides; G, adoral zone of membranelles of live; H-I, lateral view of live; J, ventral view of live, note contractile vacuole (arrowhead) and paroral membrane (arrow); K, dorsal view, to show the dorsal ridges (arrowheads); L, detailed view to show the dorsolateral cilia (arrowheads); M-N, ventral (M) and dorsal (N) sides of protargol impregnation. Scale bars=30 μm.

Table 1. Morphometric data of *Euplotes cristatus* (upper row) and *E. minuta* (lower row). Data are based on protargol impregnated specimens. Abbreviations are described in the text.

Characteristics	Min.	Max.	Mean	SD	SE	CV	n
Body length	65.6	84.8	75.52	5.65	1.26	7.49	20
	40	52.5	45.5	3.77	0.84	8.29	20
Body width	30.4	51.2	42.08	5.52	1.23	13.12	20
	22.5	37.5	30.44	3.42	0.77	11.25	20
Number of AZM	35	50	43.65	3.86	0.86	8.84	20
	31	41	36.15	3.54	0.79	9.8	20
Number of FVC	10	10	10	0	0	0	20
	10	10	10	0	0	0	20
Number of TC	5	5	5	0	0	0	20
	5	5	5	0	0	0	20
Number of CC	4	5	4.45	0.51	0.11	11.47	20
	4	4	4	0	0	0	20
Number of DK	8	8	8	0	0	0	20
	9	9	9	0	0	0	20
Number of dikinetids in middle DK (no. 4)	10	16	12.75	1.68	0.38	13.19	20
	–	–	–	–	–	–	–
Number of dikinetids in middle DK (no. 5)	9	14	12.15	1.35	0.30	11.1	20
	10	12	10.55	0.69	0.15	6.5	20

All measurements in μm . Abbreviations not described in the text are as follows: CV, coefficient of variation in %; Max., maximum; Min., minimum; n, sample size; SD, standard deviation; SE, standard error of mean.

DK, while specimens of this description have 35-50 AZM and 10-16 dikinetids of mid-DK in number, similar to the results of other previous studies (Carter, 1972; Curds, 1975; Carey, 1992). This population has a more variable number of CC (4-5), whereas the other previous studies described only 3 CC (Kahl, 1932) or 4 CC (Tuffrau, 1960; Carter, 1972; Curds, 1975; Carey, 1992).

Euplotes cristatus is morphologically similar to *E. vannus* and *E. minuta* and all these three species have dorsal silver-line system of single-*vannus* type. *Euplotes vannus* (Kwon, et al., 2007) has a larger size of body ($94\text{-}111 \times 53\text{-}75$ vs. $60\text{-}84 \times 38\text{-}68 \mu\text{m}$); a higher number of AZM (57-74 vs. 35-50), CC (4-7 vs. 4-5), DK (9-10 vs. 8), and dikinetids of mid-DK (15-22 vs. 10-16) than *E. cristatus* of this population. *Euplotes minuta* has a smaller size of body ($44\text{-}53 \times 26\text{-}35 \mu\text{m}$); a higher number of DK (9); and a lower number of AZM (31-41) than *E. cristatus* of the present population.

¹*Euplotes minuta* Yocom, 1930 (Fig. 2 and Table 1, 2)

Euplotes minuta Yocom, 1930, p. 242, Figs. 1-2; Borror, 1962, p. 271, Figs. 1-4; Katter, 1970, p. 185, Fig. 31; Curds, 1975, p. 13, Fig. 6; Carey, 1992, p. 207, Fig. 828; Song and Wilbert, 1997, p. 431, Fig. 11.

Moneuplotes minuta (Kahl, 1932) Borror and Hill, 1995, p. 461.

Material examined. Specimens were collected from Songjeong Beach, Busan ($35^{\circ} 10' \text{N}$ and $129^{\circ} 12' \text{E}$) on the Strait of Korea on December 17, 2007 (18.5°C , ca. 28.8‰, and pH ca. 8.7).

Description. Cell size *in vivo* $44\text{-}53 \times 26\text{-}35 \mu\text{m}$ in length; small and generally oval-outlined, as shown in Fig. 2A-F, K-N; the right margin is slightly convex to nearly straight, the left margin is more convex (Fig. 2A, E-F); both ends are rounded; dorso-ventrally highly flattened with dorsal side slightly arched while the ventral side is concave. Buccal field approximately 3/4 of body length (Fig. 2A, E-F). Cytoplasm hyaline, containing some shining globules and food (Fig. 2E, F). CV posterior to the rightmost TC, ca. $6 \mu\text{m}$ in diameter (Fig. 2H, arrow).

Ma C-shaped, containing numerous large nucleoli (Fig. 2A-B, I, K-L; I, arrow); Mi spherical.

Ventral surface exhibits most of diagnostic features. AZM prominent, composed of 31-41 membranelles, about $10 \mu\text{m}$ in length (Fig. 2B-C, K, M). PM small, beneath cytoplasmic lip (Fig. 2B). Cirral pattern rather stable, FVC 10 in number, enlarged, positioned at anterior ventral surface; 5 TC and 4 CC located at posterior part (Fig. 2A-C, K, M).

On dorsal surface, ellipsoid formed granules are packed together around the dorsal cilia beneath pellicle in a flower-pattern (Fig. 2G-H; 2H, arrowheads). Nine DK extend entire length of cell (Fig. 2J, arrowheads), 7 DK located on dorsal

¹*작은자라하모충 (신칭)

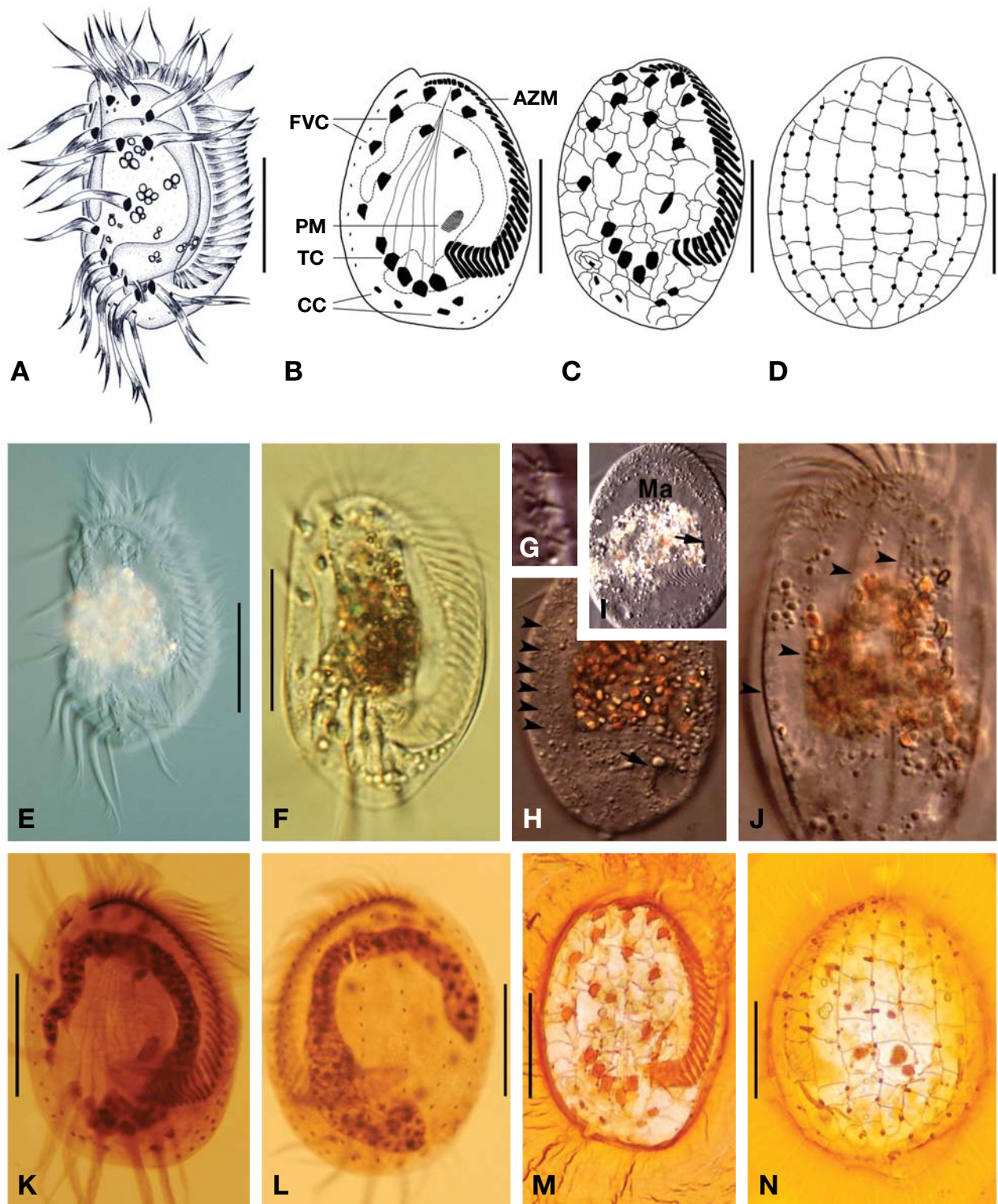


Fig. 2. Morphology and infraciliature of *Euplotes minuta* Yocom, 1930 *in vivo* (A, E-J), protargol impregnation (B, K-L), and silver nitrate impregnation (C-D, M-N). A, E-F, ventral view of live; B, infraciliature of ventral side; C-D, M-N, silverline system on ventral (C, M) and dorsal (D, N) sides; G-H, detailed view to show the dorsolateral cilia (H, arrowheads); I, macronucleus of live (Ma, arrow); J, dorsal view, to show the dorsal ridges (arrowheads); K-L, ventral (K) and dorsal (L) sides of protargol impregnation. Scale bars=20 μ m.

Table 2. Morphological and morphometric comparison of *Euplotes minuta* in present and previous descriptions. The abbreviations in the table are the same as those in the text.

Characters	<i>E. minuta</i> sensu this study	<i>E. minuta</i> Yocom, 1930	<i>E. minuta</i> sensu Borrer, 1962	<i>E. minuta</i> sensu Curds, 1975	<i>E. minuta</i> sensu Song and Wilbert, 1997
Body length <i>in vivo</i>	44-53	44-55	44-64	54	50-70
Body width <i>in vivo</i>	26-35	27-32	25-31	28	40-55
Body shape	small and oval, left margin more convex	oval, left edge more convex	left margin more convex	oval, right margin almost straight	elongated oval, left margin more convex
Number of AZM	31-41	–	31-40	30-40	33-41
Number of CC	4	4-5	4	4	4
Number of DK	9	–	9	9	7-9
Number of dikinetids in middle DK	10-12	–	–	12-13	9-11
Number of dikinetids in leftmost DK	5	–	7-10	–	5-6
Ma feature	C-shaped	G-shaped	G-shaped	C-shaped	C-shaped

All measurements of length and width= μm .

side and each one DK located on both margins of ventral surface (Fig. 2B, D, K, L); middle DK with about 10-12 dikinetids; leftmost kinety containing only about 5 dikinetids (Fig. 2B). Silverline system typical *vannus* type, single-type dargyrome, irregular pattern on ventral side (Fig. 2C-D, M-N).

The SSU rDNA sequence of this population is identical for known *E. minuta* (AJ305244-6 and EF094958), 1,856 bp in length, and deposited in GenBank accession number GU953668. Inter-specific variation is 1.8% and 2.0% between this population and two other species, *E. cristatus* (GU953667, this study) and known *E. vannus* (AY361854, AY361856, and AJ305241), respectively.

Distribution. Brasil, China, Italy, USA, and Korea (this study).

Remarks. The population of this study corresponds well with the description in the Yocom (1930) and in the later studies (Borrer, 1962; Curds, 1975; Song and Wilbert, 1997). The characteristics of *Euplotes minuta* populations were compared and summarized with those of previous investigations (Table 2). The present population is very similar to that of Curds (1975) in most aspects, such as size and shape of body; number of AZM, CC, DK, and cilia in mid-DK; and Ma feature. These specimens differ only slightly from those of other previous studies, as follows: 1) Yocom (1930) described 4-5 CC in number and Ma G-shaped while this population is 4 CC in number and Ma C-shaped in this investigation; 2) Borrer's (1962) specimens have a higher number of cilia in leftmost DK (7-10 vs. 5) and different shapes of Ma (G-shape vs. C-shape) than the present specimens. 3) Song and Wilbert's (1997) population has a larger size of body (50-70 \times 40-55 vs. 44-53 \times 26-35 μm) and a more variable number of DK (7-9 vs. 9) than this population.

The most closely related species to this species is *E. van-*

nus (Muller, 1786). However *E. minuta* has some remarkable differences from *E. vannus* (Kwon et al., 2007), such as the body size (44-53 \times 26-35 vs. 94-111 \times 53-75 μm); number of AZM (31-41 vs. 57-74), CC (4 vs. 4-7), DK (9 vs. 9-10), and dorsal cilia of mid-DK (10-16 vs. 15-22).

ACKNOWLEDGEMENTS

This work was supported partially by grants from the Korea Research Foundation Grant (KRF-2007-C00265), the Ministry of Environment of the Korean Government (NIBR 074-1800-1844-326-260-00), and the Eco-technopia 21 project.

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Received February 24, 2010
Accepted March 11, 2010