

Two Newly Recorded Estuarine Ciliates, *Euplotes vannus* and *E. parawoodruffi* (Ciliophora: Spirotrichea: Euplotida) from Korea

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ABSTRACT

Two euplotid ciliates collected from the estuarine littorals in Korea were identified as *Euplotes vannus* (Müller, 1786) and *E. parawoodruffi* Song and Bradbury, 1997. These species are reported taxonomically for the first time from Korea. These two species are redescribed with illustrations, photos and biometry based on live and silver impregnated specimens. Diagnostics of each species are as follows. *E. vannus*: size *in vivo* 94-111 × 55-75 µm (average 103 × 60 µm), adoral zone of membranelles (AZM) 70% of cell length with 57-74 adoral membranelles (AM) and terminating close to hook-shape, macronucleus (Ma) C-shaped with twisted foot-like, 10 frontoventral (FVC), 5 transverse (TC), 4-7 (average 5) caudal cirri (CC), 9-10 dorsal kineties (DK), mid-dorsal kinety with 15-22 cilia; silver-line system single vannus type. *E. parawoodruffi*: size *in vivo* 125-163 × 72-100 µm, (average 141 × 87 µm), dorsally strongly arched, body shaped reserved triangular. AZM 67-83% of cell length with 60-85 AMs, 9 FVC, 5 TC, 4 CC, 9 DK; mid-dorsal kinety with 20-30 cilia, double-eurystomus type, T-shaped Ma with equal sized right and left arms or right arm shortened slightly.

Key words: *Euplotes*, estuary, morphology, description, taxonomy

INTRODUCTION

The species belong to the genus *Euplotes* are distributed ubiquitously and can be found in nearly any habitat: aquatic (freshwater, estuarine and marine) and edaphic. They are best known taxa among ciliates. Over 100 species have been listed and about 70% of them are admitted as valid species all over the world, while 6 species of *Euplotes* have been taxonomically recorded in Korea up to now (Curds, 1975; Berger, 2001; Kwon and Shin, 2006). The correct identification of *Euplotes* species is not easy because they have high phenotypic variability, low unique morphological features, insufficient descriptions and unavailable type specimens.

We isolated two *Euplotes* species (*E. vannus* and *E. parawoodruffi*) from the estuarine littorals of Taehwa River in Ulsan, Korea. These two species are new to Korea, and their reality of identity is still controversial due to their morphological variability. Thus we redescribed them as local Korean populations in detail with illustrations and microphotographs, and discussed about their variation of morphology, compared with their congeners (Kahl, 1932; Tuffrau,

1960; Borror, 1968; Curds, 1975; Gates, 1990; Song and Packroff, 1997).

MATERIALS AND METHODS

The specimens of *Euplotes vannus* and *E. parawoodruffi* were collected from the water and debris of the estuarine littoral of Taehwa River in Ulsan, Korea, at October 18th, 2006 and February 13th, 2007. The collected samples were moved to the laboratory maintaining the temperature in ice-box. The ciliates were isolated and cultured in petridish using the boiled water sampled from collection site, with dried wheat grains and malt.

The living and silver impregnated specimens were observed under the microscopes (Zeiss Axioskop 2 and Olympus BX50), and the mobile and still images were captured using camcorder (Sony DCR-PC115) and camera (Zeiss Axiocam MRc). The silver impregnated specimens were prepared by protargol and dry methods (Wilbert, 1975; Foissner, 1992; Shin and Kim, 1993). The morphological characters were analyzed morphometrically and illustrated. The classification schemes established by Lynn and Small (2002) was adopted.

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Table 1. Morphometric data of *Euplotes vannus* (Ev) and *E. parawoodruffi* (Ep). The abbreviations in the table are the same as in the text, and the abbreviations not in the text are as follows: SD=standard deviation; SE=standard error; CV=coefficient of variation in %; n=population size; L=live; S=stained; All measurements of length and width= μm

| Characters | Method | Species | Mean | Med | Min | Max | SD | SE | CV (%) | n |
|----------------------------|--------|---------|------|-----|-----|-----|-----|-----|--------|----|
| Body length | L | Ev | 103 | 104 | 94 | 111 | 5 | 1 | 4.9 | 15 |
| | L | Ep | 141 | 140 | 125 | 163 | 14 | 5 | 9.9 | 7 |
| Body width | L | Ev | 60 | 59 | 53 | 75 | 5 | 1 | 9.0 | 15 |
| | L | Ep | 87 | 88 | 72 | 100 | 11 | 4 | 12.4 | 7 |
| Body length/Body width | L | Ev | 1.7 | 1.8 | 1.5 | 1.9 | 0.1 | 0.0 | 6.1 | 15 |
| | L | Ep | 1.6 | 1.7 | 1.4 | 1.9 | 0.2 | 0.1 | 13.0 | 7 |
| AZM length | L | Ev | 73 | 71 | 68 | 79 | 4 | 1 | 5.0 | 15 |
| | L | Ep | 105 | 100 | 88 | 125 | 12 | 5 | 12.2 | 7 |
| AZM length/Body length (%) | L | Ev | 70 | 71 | 63 | 79 | 5 | 1 | 6.5 | 15 |
| | L | Ep | 75 | 71 | 67 | 83 | 7 | 3 | 9.6 | 7 |
| Body length | S | Ev | 99 | 97 | 85 | 111 | 8 | 2 | 8.3 | 15 |
| | S | Ep | 129 | 125 | 100 | 175 | 23 | 5 | 18.7 | 23 |
| Body width | S | Ev | 60 | 57 | 46 | 79 | 9 | 2 | 15.6 | 15 |
| | S | Ep | 84 | 85 | 63 | 105 | 14 | 3 | 16.3 | 23 |
| Body length/Body width | S | Ev | 2 | 2 | 1 | 2 | 0 | 0 | 12.0 | 15 |
| | S | Ep | 2 | 2 | 1 | 2 | 0 | 0 | 9.6 | 23 |
| AZM length | S | Ev | 71 | 71 | 60 | 83 | 5 | 1 | 7.6 | 15 |
| | S | Ep | 100 | 103 | 75 | 140 | 18 | 4 | 18.0 | 23 |
| AZM length/Body length (%) | S | Ev | 72 | 73 | 60 | 82 | 6 | 2 | 8.7 | 15 |
| | S | Ep | 78 | 78 | 70 | 86 | 4 | 1 | 5.7 | 23 |
| FVC number | S | Ev | 10 | 10 | 10 | 10 | 0 | 0 | 0.0 | 15 |
| | S | Ep | 9 | 9 | 9 | 9 | 0 | 0 | 0.0 | 3 |
| CC number | S | Ev | 5 | 5 | 4 | 5 | 0 | 0 | 5.4 | 14 |
| | S | Ep | 4 | 4 | 4 | 4 | 0 | 0 | 0.0 | 8 |
| TC number | S | Ev | 5 | 5 | 5 | 5 | 0 | 0 | 0.0 | 15 |
| | S | Ep | 5 | 5 | 5 | 5 | 0 | 0 | 0.0 | 8 |
| DK number | S | Ev | 10 | 10 | 9 | 10 | 0 | 0 | 3.6 | 15 |
| | S | Ep | 9 | 9 | 9 | 9 | 0 | 0 | 0.0 | 4 |
| DB number in mid-DK (#5) | S | Ev | 18 | 18 | 15 | 22 | 2 | 1 | 10.1 | 13 |
| DB number in mid-DK (#6) | S | Ev | 19 | 18 | 16 | 21 | 2 | 0 | 8.5 | 13 |
| DB number in mid-DK (#5) | S | Ep | 25 | 25 | 20 | 30 | 5 | 3 | 20.0 | 3 |
| AM number | S | Ev | 68 | 69 | 57 | 74 | 5 | 1 | 7.3 | 13 |
| | S | Ep | 73 | 73 | 60 | 85 | 8 | 3 | 11.6 | 7 |

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
 Subphylum Intramacronucleata Lynn, 1996
 Class Spirotrichea Bütschli, 1889
 Subclass Hypotrichia Stein, 1859
 Order Euplotida Small and Lynn, 1985
 Suborder Euplotina Small and Lynn, 1985
 Family Euplotidae Ehrenberg, 1838
 Genus *Euplotes* Ehrenberg, 1830

1. *Euplotes vannus* (Müller, 1786) (Fig. 1, Table 1)

Kerona vannus Müller, 1786 (cited from Berger, 2001).

Euplotes crassus Kahl, 1932, p. 636; Tuffrau, 1960, p. 52;
 Curds, 1975, p. 11.

Euplotes mutabilis Tuffrau, 1960, p. 54 (in part); Curds, 1975, p. 14 (in part).

Euplotes vannus: Kahl, 1932, p. 636; Tuffrau, 1960, p. 43;
 Curds, 1975, p. 14; Song and Packroff, 1997, p. 344.

Moneuplotes vannus: Borror and Hill, 1995, p. 461.

Description. General morphology and behavior: Body inflexible, cell *in vivo* 94-111 \times 53-75 μm (average 103 \times 60 μm); ratio (body length/width) 1.7, body shape oval and elongated oval with anterior portion slightly wider than posterior portion, broadly rounded both end, flattened dorsoventrally (Fig. 1A, B, E); dorsal surface slightly convex without ribs, ventral surface slightly concave with prominent cirri (Fig. 1E, I, J). Cytoplasm colourless and transparent but bright

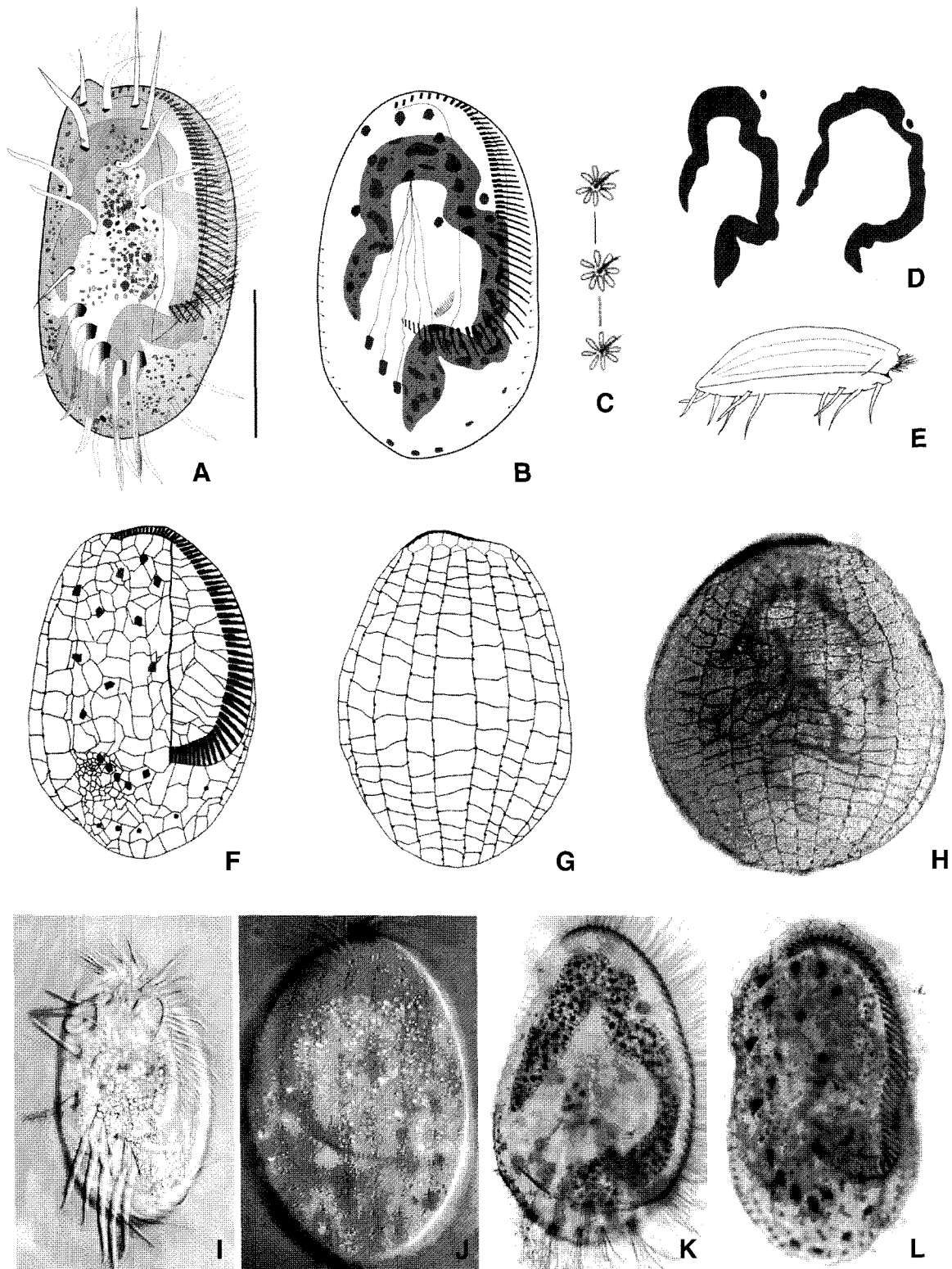


Fig. 1. *Euplotes vannus*. A, entire ventral view; B, infraflagellate after protargol impregnation, ventral view; C, basal body shape of dorsal bristles *in vivo*; D, patterns (shapes) of Ma and Mi; E, lateral view; F, ventral surface of a "Dry" silver-impregnated single cell; G, dorsal surface of a "Dry" silver-impregnated single cell; H, showing single vannus type with eight dorsal kineties; I, entire ventral view *in vivo*; J, dorsal view *in vivo*; K and L, photograph of frontoventral cirrotype and nuclear feature after protargol impregnation, Scale bar=50 μ m.

Table 2. Morphological and morphometric comparison of populations of *Euplotes vannus*. The abbreviations in the table are the same as in the text. All measurements of length and width= μm

| Characters | Data sources | | | |
|-------------------------------|---|---|--|--|
| | <i>E. vannus</i> sensu present study | <i>E. crassus</i> sensu Tuffrau, 1960 and Curds, 1975 | <i>E. vannus</i> sensu Tuffrau, 1960 and Curds, 1975 | <i>E. vannus</i> sensu Song and Packroff, 1997 |
| Size <i>in vivo</i> | 94-111 | 100-130 | 75-100 | 90-140 |
| Body shape | oval and elongated oval | elongated oval | overall oval configuration but slightly curved towards the right | elongated oval |
| Proximal end of AZM | terminating close to hook-shape | terminating close to hook-shape | terminating close to hook-shape | terminating close to hook-shape |
| Number of AM | 57-74 | 50 | 60 | 53-66 |
| CC number | 4-7 | 5-6 | 4 | 4-6 |
| DK number | 9-10 | 10 | 9 | 9-10 |
| Number of cilia in central DK | 15-21 | about 26 | about 22 | 6-21 |
| Ma feature and position | C-shaped with foot-like in the posterior part | C-shaped with foot-like in the posterior part | C-shaped with twisted foot-like extension in the posterior part | C-shaped |

yellow to green by foods, food vacuoles 2-7 μm in diameter scattered entire body irregularly, fluorescent crystal like granules (1-2 μm) mostly scattered in anterior and posterior parts (Fig. 1A, I); large contractile vacuole spherical in shape, 10-13 μm in diameter, positioned posterior right part of body (Fig. 1A, I). Movement slowly to fast crawling on substrate, occasionally gliding and rotating.

Ventral infraciliature: Fronto-ventral cirri (FVC) 10 in number, enlarged, located at anterior ventral surface (Fig. 1A, B, F, I). Adoral zone of membranelles (AZM) with 57-74 (average 68) prominent adoral membranelles (AM), 68-79 μm (average 72.5 μm) in distance between proximal end to distal end of AZM, covering approximately 2/3 of body length. Buccal field large, covering 2/5 of body width, comprising short undulating membrane (UM) of 12-15 μm in length (Fig. 1A, B, F, I). Transverse cirri (TC) v-shaped, positioned at posterior part, 5 in number, enlarged and extending beyond posterior end of body (Fig. 1A, B, F, I).

Dorsal infraciliature: Dorso-lateral kineties (DK) 9-10 in number, of them 7-8 rows located at dorsal surface and 2 rows extending to lateral and ventral surface, mid-dorsal kinety comprising 15-22 (average 18) cilia in number (Fig. 1G, H, J), snow flake shaped clusters of cortical granule (?) around base of dorsal bristles *in vivo* (Fig. 1C, J). Dargyrome pattern single vannus type with most tetragons regularly arranged (Fig. 1G, H). Caudal cirri (CC) 4-7 (average 5) in number, positioned at posterior central and left end of body (Fig. 1A, B, F).

Nuclear apparatus: Macronucleus (Ma) 3- or C-shaped with modification, widest width 8-11 μm , lying along entire of body (Fig. 1A, B, D, K, L). Micronucleus (Mi) one in number, spherically shaped, 2-3 μm in diameter, positioned

close to central left edge of Ma (Fig. 1B, D, K).

Habitat. Estuarine littoral, marine, periphytic.

Distribution. France, USA, North Sea, Somalia, China, Korea.

Remarks. *Euplotes vannus* has been described for a long time since 1786 by Müller (Kahl, 1932; Tuffrau, 1960; Borror, 1968; Curds, 1975; Gates, 1990; Song and Packroff, 1997), but the identification of *E. vannus* continues to be a controversial issue (Song and Packroff, 1997) because of morphological variability of this species. Present specimens have snow flake shaped clusters of cortical granules (?) around the base of dorsal bristles when observed with DIC microscope *in vivo* (Fig. 1C, J).

The characteristics of *Euplotes vannus* populations were compared and summarized with those of previous investigations (Table 2). The cirral distribution, size and shape of body, length of AZM and silverline systems of present specimens resemble those features of previous records of *E. vannus* (Tuffrau, 1960; Curds, 1975) and *E. crassus* (Tuffrau, 1960; Curds, 1975). Despite the difference in number of cilia in mid-DKs and shape of AZM, Song and Packroff (1997) regarded these specimens as variants of *E. vannus*. The present specimens have 9-10 DKs ($n=28$ and $CV=3.2\%$), 57-74 AMs ($n=13$ and $CV=7.3\%$) and 4-7 CCs ($n=29$ and $CV=9.6\%$). Also Chinese population has more or less variable in them (Song and Packroff, 1997; Table 2).

The most closely related species of this species is *E. cristatus* Kahl, 1932. This present species is distinguished from *E. cristatus* by the following characteristics. (1) This species is 85-111 μm in size, while *E. cristatus* is about 60 μm . (2) This species has 4-7 CCs, while *E. cristatus* has 4 CCs. (3) This species has 9-10 DKs, while *E. cristatus* has 8 DKs.

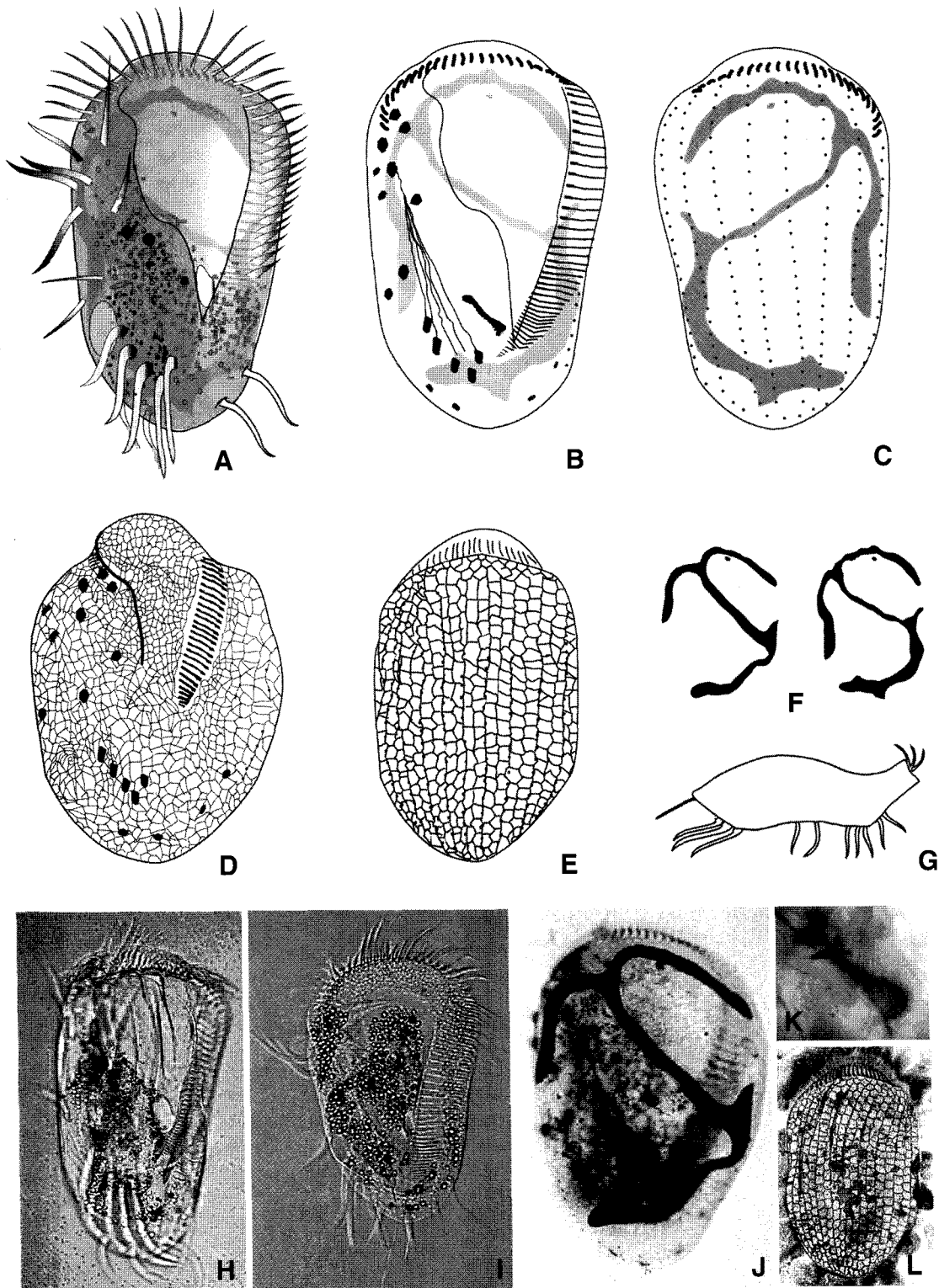


Fig. 2. *Euplates parawoodruffi* from live (A, H, I), protargol stained (B, C, J, K) and silver nitrate impregnated (D, E, L) specimens. A, ventral view of a typical individual; B, ventral view and infraciliature; C, dorsal view; D and E, silver line system on ventral and dorsal surface (AZM shrunken); F, Macronucleus usually more like irregular "T-shape". Upper portion of stem directed to right, two arms similar or unequal length; G, lateral view; H, dorsal view, note the grooves; I and J, Ma; K, comb-like undulating membrane; L, note anterior ends of dorsal kinetids terminated at different distance to the AZM, Scale bar=50 μ m.

(4) This species has 15-21 cilia in mid-dorsal kinety, while *E. cristatus* has 11-15 cilia (Tuffrau, 1960; Curds, 1975).

2. *Euplotes parawoodruffi* Song and Bradbury, 1997 (Fig. 2, Table 1)

Euplotes parawoodruffi Song and Bradbury, 1997, p. 402.

Euplotes woodruffi: Borror, 1963, p. 522 (in part); Maganini and Nobili, 1964, p. 178 (in part); Hill and Reilly, 1976, p. 499 (in part); Dragesco and Dragesco-Kernès, 1986, p. 491 (in part).

Description. General morphology and behavior: Body inflexible, cell *in vivo* 125-163 × 72-100 μm (average 140 × 87 μm); body shape reversed triangular and posterior end narrowed, left side mostly straight and slightly concave, right side almost straight, anterior end broadly rounded and posterior end narrowly rounded, flattened dorsoventrally but strongly arched dorsally, dorsal surface convex without ribs, ventral surface concave with prominent cirri (Fig. 2A-C, G-I); anterior end protruded like collar surrounded by AZM posteriorly (Fig. 2D, E, H). Buccal field spacious and conspicuously long, extending 75-83% of body length, buccal lip indented and along the right border diagonally, buccal lip and AZM forming triangular buccal cavity; buccal cavity extending from anterior most right and left corners to proximal end of AZM (Fig. 2A, B, H); pre-oral pouch teardrop-shaped invagination on dorsal wall of buccal cavity close to proximal end of AZM (Fig. 2H). Cytoplasm colourless and transparent but dark grey by foods, food vacuoles scattered entire body irregularly (Fig. 2H, I); contractile vacuole spherical in shape, positioned posterior right part of body (Fig. 2A). Movement slowly to fast crawling on substrate, occasionally gliding and rotating.

Ventral Infraciliature: Fronto-ventral cirri (FVC) 9 in number, enlarged, located at anterior ventral surface (Fig. 2A, B, D). Adoral zone of membranelles (AZM) with 60-85 (average 73) prominent adoral membranelles (AM), 75-140 μm (average 100 μm, impregnation) in distance between proximal end to distal end of AZM, proximal end of AZM extending to beneath left-most transverse cirrus, anterior distal end of AZM inserting ventrally in between anterior frontal cirri, covering 70-86% of body length (Fig. 2A, B, H, I). Buccal field large, comprising short undulating membrane (UM) of 16-22 μm in length (Fig. 2A, B, K). Transverse cirri (TC) v-shaped, positioned at posterior part, 5 in number, enlarged and prominent, extending beyond posterior end of body (Fig. 2A, B, D, H).

Dorsal infraciliature: Dorsal kineties (DK) 9 in number, mid-DK comprising 20-30 (average 25) cilia in number (Fig. 2C, H, J). Dargyrome pattern double euryostomus type, distance between anterior end of each DK and AZM in collar

short (Fig. 2E, L). Caudal cirri (CC) 4 in number, positioned at posterior left and right end of body (Fig. 2A, B).

Nuclear apparatus: Macronucleus (Ma) irregular T-shaped with modification, two arms similar or unequal length, upper portion of stem directed to right, lying along entire of body (Fig. 2A-C, F, J). Micronucleus (Mi) one in number, spherically shaped, 2-3 μm in diameter, positioned usually under left T-arm of Ma (Fig. 2F).

Habitat. Estuarine littoral, marine.

Distribution. Africa, China, France, Japan, Korea.

Remarks. The present population is identical to *Euplotes parawoodruffi* Song and Bradbury, 1997 concern as usually reversed triangular to rectangular in ventral view, large body size (125-160 × 70-100 μm *in vivo*), very large buccal field (buccal field/body length=70-86%), short distance between anterior ends of dorsal kineties and AZM of collar in dargyrome, and habitats of brackish-water, except the presence of teardrop-shaped invagination on the dorsal wall (Song and Bradbury, 1997). The proximal end of AZM in *E. parawoodruffi* is usually immediately beneath the position of the left-most transverse cirrus on ventral surface, however the present population sometimes anterior to it with an intervening space like a most related *E. woodruffi* (Gaw, 1939; Song and Bradbury, 1997). In *E. parawoodruffi* Song and Bradbury, 1997, the right arm of T-shaped macronucleus is usually half or less the length of the left arm, however in the present population, both arms are about the same length, or the right arm may be slightly longer than left arm. Moreover, micronucleus of the present population is constantly located on inner left arm. This characteristic is different from both typical *E. parawoodruffi* Song and Bradbury, 1997 and *E. woodruffi* Gaw, 1939 (Song and Bradbury, 1997).

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant (KRF-2005-070-C00124) funded by the Korean Government and by a grant (no. 052-052-040) from the Core Environmental Technology Development Project for Next Generation funded by the Ministry of Environment of the Korean Government.

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Received October 8, 2007
Accepted November 7, 2007