

Redescription of Previously Unknown Euplotine Ciliates, *Euplotes charon* and *Diophrys oligothrix* (Ciliophora: Spirotrichea: Euplotida), from Korea

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ABSTRACT

Two ciliates of suborder Euplotina collected from the two habitats (estuarine littoral and sewage treatment plant) in Ulsan, Korea were *Euplotes charon* (Müller, 1773) and *Diophrys oligothrix* Borror, 1965. These two species are reported for the first time from Korea. The description was based on the observation of living and silver impregnated specimens. Diagnostic characteristics of these species are as follows. *E. charon*: size *in vivo* about $90\text{--}130 \times 65\text{--}80\text{ }\mu\text{m}$, adoral zone of membranelles over 79.5% of cell length with 54–80 adoral membranelles; right margin of the peristome shaped sinusoidal form and passed through adoral zone of membranelles; buccal cavity wide anteriorly; 10 frontoventral, 5 transverse, 4 caudal cirri, 12 dorsal kineties, mid-dorsal kinety with 21–25 dorsal bristles; silver-line system double-eurystomus type. *D. oligothrix*: size *in vivo* about $80\text{--}90 \times 30\text{--}70\text{ }\mu\text{m}$; body shape ovoid with prominent right concave postero-lateral end, two irregular elongated macronuclei with one micronucleus, respectively; 7 fronto-ventral, 5 transverse, 2 left marginal and 2 caudal cirri, 4 dorsal kineties with prominent bristles about $9\text{--}14\text{ }\mu\text{m}$ long *in vivo*.

Key words: *Euplotes*, *Diophrys*, sewage treatment plant, estuary, morphology

INTRODUCTION

The diagnostic characteristics of suborder Euplotina are ovoid shape and absence of right marginal cirri. The euplotines represent a monophyletic group including five families of hypotrich ciliates and united as a suborder by their similar morphology, stomatogenesis, ultrastructure, cyst structure, and behavior (Borror and Hill, 1995). The genera *Diophrys* and *Euplotes* are closely related due to the exhibition of morphogenetic characteristics during cell division, but *Euplotes* differs from *Diophrys* in some respects, like patterns of caudal cirri, undulating membrane and habitat (Curds, 1975; Curds and Wu, 1983).

Members of *Euplotes* are among the best known in Ciliophora. In the literature over 80 species have been described all over the world and six species of *Euplotes* ciliates have been reported from Korea (Jo and Shin, 2003). *Euplotes charon* has been described for a long time since 1773 by Müller (Borror, 1968; Curds, 1975; Dragesco and Dragesco-Kernéis, 1986; Kahl, 1932; Song and Packroff, 1997; Tuffrau, 1960), but identification of *E. charon* continues to be a controversial issue (Song and Packroff, 1997) because of morphological variability of this species. The species of *Diophrys* is well-known as a marine species. In the litera-

ture, over 17 species have been described all over the world, and the species of *Diophrys* is reported here for the first time from Korea. *Diophrys* has typical body shape, well developed adoral zone of membranelles and conspicuous caudal cirri.

MATERIALS AND METHODS

The specimens of *Euplotes charon* and *Diophrys oligothrix* were collected from the estuarine littorals of Taehwa River, in Ulsan, May 5, 2005 and from the water of aeration tank in the sewage treatment plant around Ulsan, November, 2004. The collected samples were moved to the laboratory maintaining the temperature in icebox. The ciliates were isolated and cultured in petridish using commercial mineral water with dried wheat grains. The living and silver impregnated specimens were prepared by modified protargol and dry staining methods (Wilbert, 1975; Foissner, 1992; Shin and Kim, 1993), and observed under the microscopes (Zeiss Axioskop 2 and Olympus BX50) using camcorder (Sony DCR-PC115) and camera (Zeiss Axiocam MRc). The protargol impregnated specimens were deposited in the Dept. of Biology, University of Ulsan, Korea. The morphological characters were analyzed biometrically and illustrated. We adopted the classification schemes established by Lynn and Small (2002).

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Table 1. Morphometric data of *Euplotes charon* (Eu) and *Diophrys oligothrix* (Di)

| Characters | Method | Species | Mean | Med. | Min. | Max. | SD | SE | CV (%) | n |
|----------------------------|--------|---------------------------|-------|------|------|------|------|------|--------|----|
| Body, length | L | Eu | 110.7 | 110 | 90 | 130 | 11.0 | 2.8 | 9.9 | 15 |
| | L | Di | 70.4 | 75 | 50 | 88 | 7.9 | 1.4 | 11.2 | 30 |
| Body, width | L | Eu | 78.7 | 80 | 60 | 90 | 7.4 | 1.9 | 9.5 | 15 |
| | L | Di | 43.8 | 40 | 33 | 70 | 9.3 | 1.7 | 21.2 | 30 |
| Body length/Body width | L | Eu | 1.4 | 1 | 1 | 2 | 0.1 | 0.0 | 7.4 | 15 |
| | L | Di | 1.7 | 2 | 1 | 2 | 0.2 | 0.0 | 16.1 | 30 |
| AZM, length | L | Eu | 87.3 | 90 | 70 | 95 | 7.3 | 1.9 | 8.3 | 15 |
| | L | Di | 31.0 | 30 | 20 | 45 | 5.2 | 1.0 | 16.9 | 30 |
| AZM length/body length (%) | L | Eu | 79.5 | 79 | 58 | 100 | 9.00 | 2.3 | 11.3 | 15 |
| | L | Di | 44.3 | 44 | 33 | 60 | 7.1 | 1.3 | 16.1 | 30 |
| AZM length/body length (%) | S | Eu | 73.5 | 73 | 50 | 93 | 8.9 | 1.1 | 12.1 | 62 |
| | S | Di | 55.5 | 55 | 43 | 67 | 7.0 | 1.7 | 12.6 | 16 |
| AM, number | S | Eu | 65.0 | 64 | 54 | 80 | 6.7 | 1.0 | 10.3 | 42 |
| | S | Di | 25.6 | 26 | 19 | 35 | 3.8 | 1.00 | 14.8 | 16 |
| Um, length | S | Eu | 15.7 | 15 | 10 | 30 | 3.5 | 0.5 | 22.9 | 59 |
| Mi, number | S | Di _(anterior) | 1.3 | 1 | 1 | 2 | 0.5 | 0.1 | 35.6 | 23 |
| | S | Di _(posterior) | 1.1 | 1 | 1 | 2 | 0.2 | 0.1 | 21.3 | 20 |
| FVC, number | S | Eu | 10.0 | 10 | 10 | 10 | 0.0 | 0.0 | 0.0 | 32 |
| | S | Di | 7.0 | 7 | 7 | 8 | 0.2 | 0.0 | 2.8 | 26 |
| CC, number | S | Eu | 4.0 | 4 | 4 | 4 | 0.0 | 0.0 | 0.0 | 63 |
| | S | Di | 3.0 | 3 | 3 | 3 | 0.0 | 0.0 | 0.0 | 30 |
| DK, number | S | Eu | 12.0 | 12 | 12 | 12 | 0.0 | 0.0 | 0.0 | 45 |
| | S | Di | 3.7 | 4 | 3 | 4 | 0.5 | 0.1 | 13.5 | 17 |
| DB, number in mid-DK | S | Eu | 22.2 | 22 | 19 | 26 | 1.9 | 0.3 | 8.5 | 40 |
| | S | Di | 8.0 | 8 | 6 | 11 | 1.7 | 0.7 | 20.9 | 6 |
| DB, length | L | Di | 11.4 | 12 | 9 | 14 | 1.9 | 0.9 | 17.1 | 5 |

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 measurement of length and width= μm .

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 charon* (Müller, 1773) (Fig. 1, Table 1)

Trichoda charon Müller, 1773 (cited from Borror, 1968).

Euplotes charon: Kahl, 1932, p. 633; Borror, 1968, p. 803;

Song and Packroff, 1997, p. 343; Tuffrau, 1960, p. 57.

Description. General morphology and behavior: Body stiff and inflexible, cell *in vivo* 90-130 \times 60-90 μm (average

110.7 \times 78.7 μm); body shape asymmetrically oval with anterior portion slightly wider than posterior and less triangular (Fig. 1B, D, E) or somewhat rectangular (Fig. 1A, C, L); left margin usually less convex than right one (Fig. 1D); convex dorsally and concave ventrally (Fig. 1G), dorsally on anterior margin hyaline collar-like plasmatic protrusion at base of adoral zone of membranelles (AZM), the dorsal ridges single-edged (Fig. 1L). Numerous ellipsoidal granules, extremely densely packed and forming mosaic pattern immediately beneath dorsal surface (Fig. 1M). Cytoplasm colourless, food vacuoles containing green algae and bacteria (Fig. 1B); large contractile vacuole located at right of transverse cirri (Fig. 1A-C). Locomotion slowly crawling on substrate.

Frontal and buccal fields: Fronto ventral cirri (FVC) 10 in number, enlarged, situated at ventral surface of anterior part (Fig. 1D). AZM covering 79.5% of cell length with 54-80 (average 65.0) adoral membranelles (AM), buccal field

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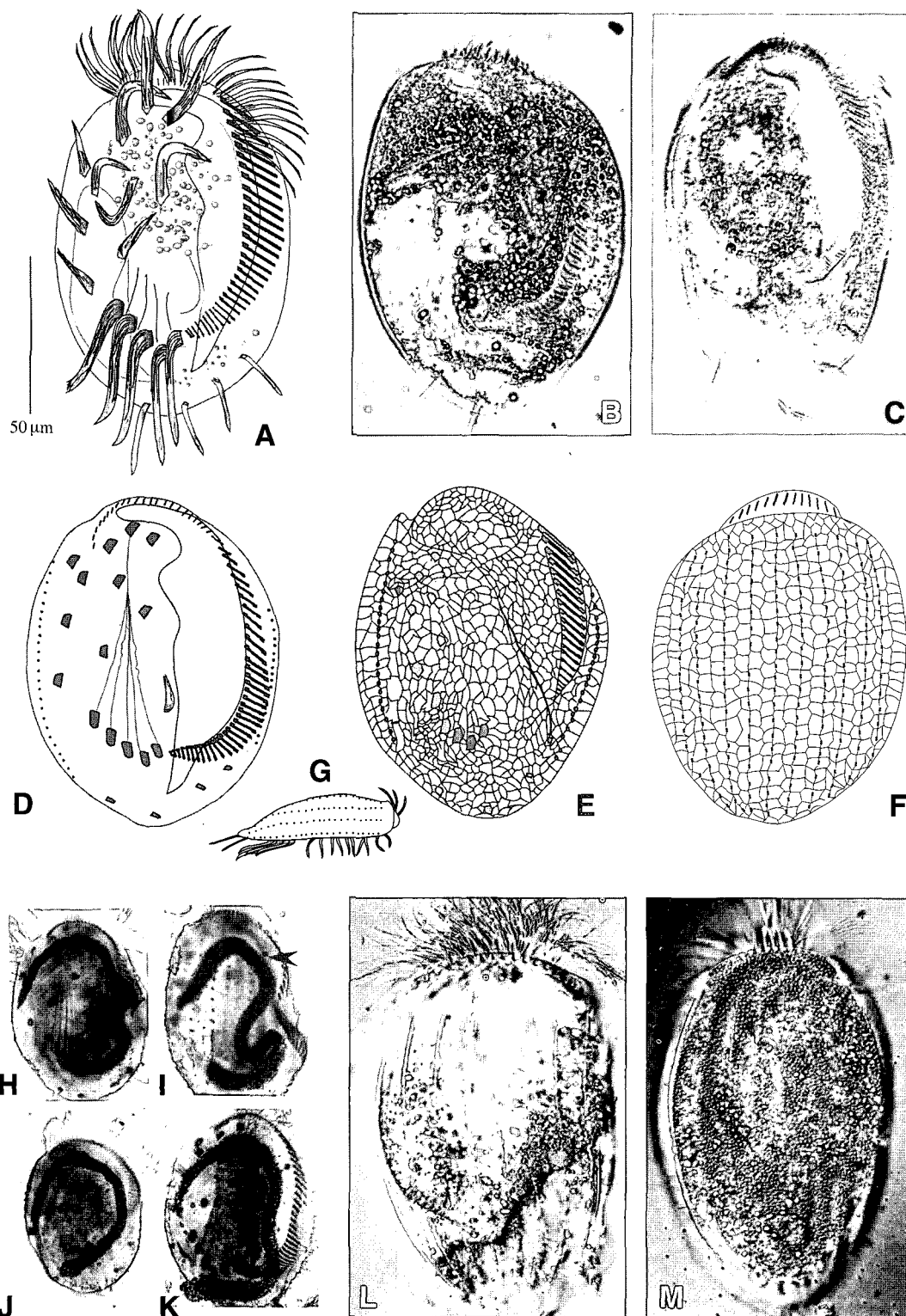


Fig. 1. *Euplotes charon* from live (A-C, G, L and M) and protargol stained (D, G-K) and silver nitrate impregnated (E, F) specimens. A. Ventral view of a typical individual; B. Cytoplasm colourless, food vacuoles containing green algae, bacteria and large contractile vacuole; C. Arrow marks the right margin of the peristome; D. Ventral view and infraciliature; E-F. Silver line system on ventral and dorsal surface; G. Lateral view; H-K. C and 3-shaped macronucleus containing small nucleoli. Micronucleus is located usually top of right portion of macronucleus (arrow). L; The dorsal ridges are single-edged. M; Granules at the dorsal surface.

Table 2. Comparisons of diagnostic characteristics of *E. charon* in present and previous specimens. The abbreviations in the table are the same as in the text

| Characters | Present record | Borror, 1968 | Tuffrau, 1960 & Curds, 1975 | Dragesco & Dragesco-Kernéis, 1986 | Song & Packroff, 1997 |
|--------------------------------|----------------|--------------|-----------------------------|-----------------------------------|-----------------------|
| Habitat | Estuary | Marine | Marine | Marine | Marine |
| Size, length (μm) | 90-130 | 72-87 | 70-96 | 70-96 | 70-110 |
| DK, number | 12 | 12 | 12 | 12 | 9.5 (9-10) |
| DB, number in mid-DK | 21-25 | 18-21 | 35-40 | 18-21 | 10-25 |
| CC, number | 4 | 5 | 5-8 | 6-8 | 4-7 |
| AZM/body length ratio (%) | 73.5 | 65-70 | 66.7 | 66.7 | 77.9 |
| AM, number | 65 | unclear | 70 | 70 | 54.5 (51-60) |
| Ma, shape | C or horseshoe | C | C | C | Horseshoe |

large, right margin of peristome shaped sinusoidal form and passing through AZM (Fig. 1A, D). Short undulating membrane (UM), 15-30 μm (average 15.7 μm) (Fig. 1A, D).

Somatic infraciliature: Caudal cirri (CC) four in number, situated on dorsal surface of posterior central and left border (Fig. 1A, C, D). Transverse cirri (TC) five in number, enlarged and prominent, situated at ventral surface of mid-posterior part (Fig. 1A). Dorsal surface bearing 12 dorsal kineties (DK); mid-DK with 21-25 (average 23.2) dorsal bristles (DB), silverline system on dorsal surface regular double eurytismus type with small polygons arranged two rows between kineties (Fig. 1F).

Nuclear appearance: Macronucleus (Ma) usually more like irregular C- and less 3-shaped containing small nucleoli. One micronuclei (Mi) 2-3 μm in diameter, located usually at top of right portion of Ma (Fig. 1H-K).

Distribution. America, Europe, China and Korea.

Remarks. *Euplotes charon* has been described for a long time since 1773 by Müller (Borror, 1968), but identification of *E. charon* continues to be a controversial issue (Song and Packroff, 1997) because of the morphological variation. The specimens of present *Euplotes charon* were compared and summarized with those of previous investigations (Table 2). The cirral distribution, size and shape of body, shape of buccal field, length of AZM and silverline systems of present specimens resemble those features of previous records of *E. charon* (Tuffrau, 1960; Borror, 1968; Curds, 1975). Despite the difference in number of DK, mid-dorsal bristles and shape of AZM and buccal field, Song and Packroff (1997) regarded their specimens as a variants of *E. charon*. The present specimens have 12 DKs ($n=45$ and $CV=0.0\%$) and 4 CC ($n=63$ and $CV=0.0\%$). These two characters were very constant in this population, but the China population had more or less variable in them (Song and Packroff, 1997, Table 2).

The most closely related species of this species is *E. harpa* Stein, 1859. This species is distinguished from *E.*

harpa by the following characteristics. (1) This species is 70-130 μm in size, while *E. harpa* is 150-160 μm in size. (2) This species has 4-8 CC, while *E. harpa* has 4 CC. (3) This species has 9-12 DK, while *E. harpa* has 13. (4) This species has 10-40 DB in mid-dorsal kinety, while *E. harpa* has 40-45 DB (Kahl, 1932; Tuffrau, 1960; Borror, 1968; Curds, 1975; Dragesco and Dragesco-Kernéis, 1986; Song and Packroff, 1997).

Family ¹*Uronychiidae Jankowski, 1979

Genus ²**Diophrys* Dujardin, 1841

³**Diophrys oligothrix* Borror, 1965 (Fig. 2, Table 1)

Diophrys oligothrix Borror, 1965, p. 559; Czapik, 1981, p. 367; Song and Packroff, 1997, p. 351; Song and Wilbert, 2002, p. 54.

Description. General morphology and behavior: Cell size *in vivo* 50-90 \times 30-70 μm (average 70.3 \times 43.8 μm). Length/width ratio about 1.6/1 (Table 1). Body slenderly ovoid form with a collar (Fig. 2A, D, E). The concavity at the posterior right edge conspicuous and CC positioned here (Fig. 2D). The dorsal surface smooth and round (Fig. 2H and I). Two longitudinal ridges on ventral side, one on right side, another at left side (Fig. 2A). Cytoplasm colourless, contractile vacuole located near the CC. Behavior and movement like *Euplotes*, gliding on substrate and benthic, swimming freely in tight counterclockwise helix.

Frontal and buccal fields: FVC seven in number enlarged and prominent, arranged in two distinct groups; five cirri at anterior part and rest two cirri situated at mid-ventral surface and upper TC (Fig. 2A, B). AZM wide, with prominent membranelles 31 μm long and having 19-35 (average 25.6) AM in number, AZM/body length ratio about 44.3% (Table 1). Paroral and endoral membranes lying at right of AZM. UM parallel and extending anteriorly from cytostome along edge of right buccal overture.

Somatic infraciliature: CC three in number situated on

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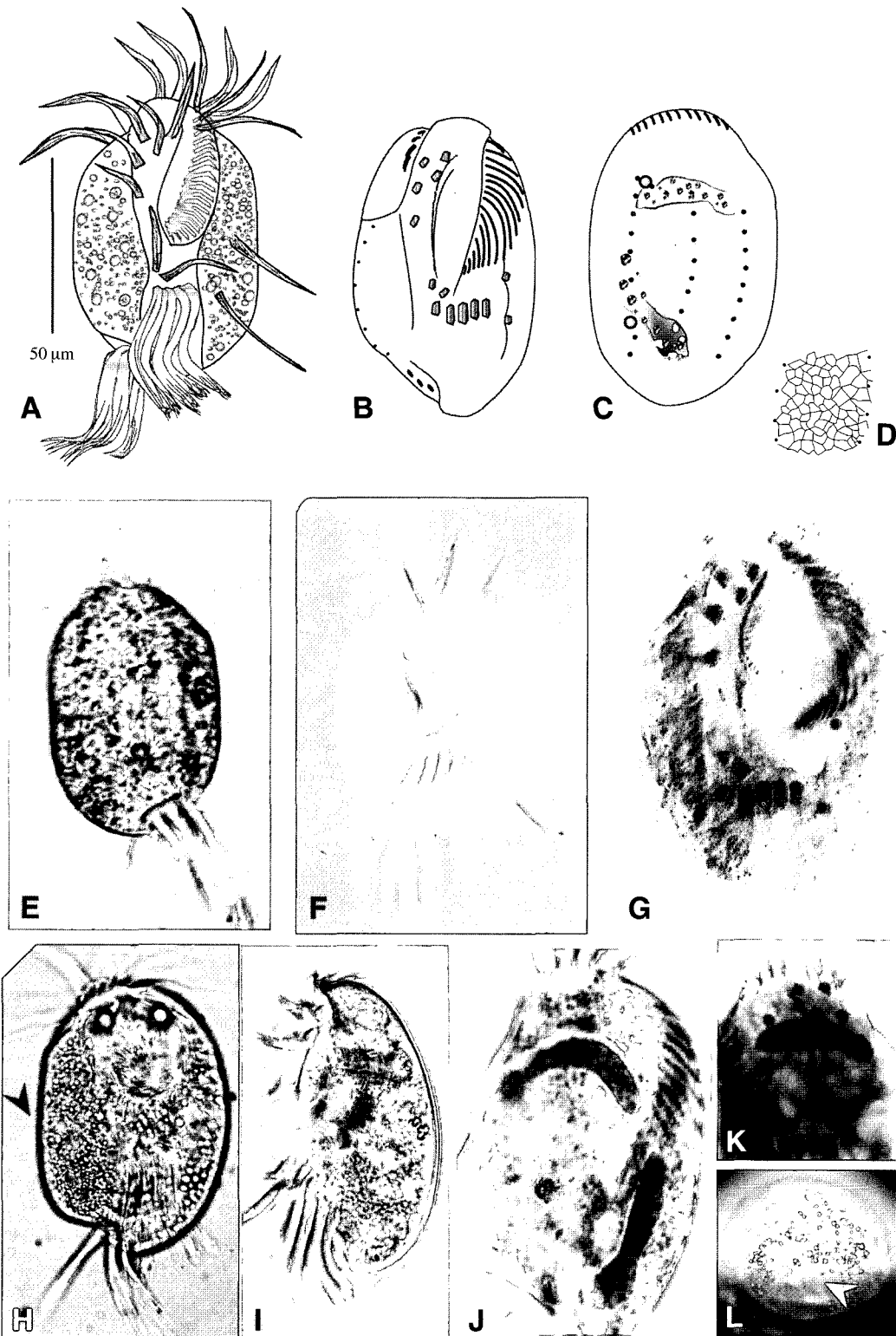


Fig. 2. *Diophrys oligothrix* from live (A, E, F, H, I and L) and protargol stained (B, C, G, J and K). A, E and F. Ventral view of a typical individual. Body slender ovoid form with a collar. The concavity at the posterior right edge is conspicuous and caudal cirri positioned; B and G. ventral view, to show the infraciliature; C and H. Dorsal kineties with prominent long bristles (arrow); D and L. Arrows mark fine mesh like argyrome on dorsal surface; K. Paroral and endoral membranes parallel and closely located; I. Lateral view, smooth dorsal surface; J. Two irregular elongated macronuclei with one micronucleus.

Table 3. Comparisons of diagnostic characteristics of *D. oligothrix* in present and previous specimens. The abbreviations in the table are the same as in the text

| Characters | Present record | Borror, 1965 | Song & Packroff, 1997 | Song & Wilbert, 2002 |
|--------------------------------|------------------------|------------------|-----------------------|-------------------------|
| Habitat | Sewage treatment plant | Tidal marsh pond | Coastal sea | Antarctic littoral pool |
| Size, length (μm) | 50-90 | 79-106 | 50-100 | 60-80 |
| Silverline system | fine mesh like | fine mesh like | no mention | no mention |
| DK, number | 3-4 | 4 | 4-5 | 4-5 |
| DB, number in mid-DK | 6-11 | 35-40 | 18-21 | 9-14 |
| DB, length (μm) | 9-14 | 6-7 | 2-6 | 5-7 |
| CC, number | 3 | 3 | 3 | 5-8 |
| AZM/body length ratio (%) | 44.3 | 50 | 65-70 | 50 |
| AM, number | 19-35 | 40-45 | 35-44 | 31-37 |
| Mi, number in total | 2 | 4 | 3-6 | 4 |

right, very prominent and $20\ \mu\text{m}$ long. Number of CC constant. TCs five and larger as prominent than those of *Euplotes*. Dorsal surface bearing 3-4 DK; mid-DK of cilia with 6-11 (average 8). Dorsal cilia are $9-14\ \mu\text{m}$ long, on the average $11.4\ \mu\text{m}$. First DK located on right side of ventral surface while other rows on dorsal surface (Fig. 2B and C). Fine mesh argyrome on dorsal side (Fig. 2D and L).

Nuclear appearance: Two parts of macronuclei arranged in C-shape and irregular and elongated with small nucleoli. Mi one or two in number (Table 1).

Distribution. America, Antarctic, Europe, China and Korea.

Remarks. This *Diophrys* specimens agree basically well with the original, subsequent descriptions and the variability of their characters (Borror, 1965; Czapik, 1981; Curds and Wu, 1983; Song and Packroff, 1997, Song and Wilbert, 2002). But the length of dorsal bristles and the number of micronuclei in the present specimens were compared with those of former studies especially (Table 3). The present specimens have longer dorsal bristles ($9-14\ \mu\text{m}$) compared to those of previous records ($2-7\ \mu\text{m}$), 2 micronuclei in number in the present specimens compare to 4 or 3-6 in number of micronuclei.

D. oligothrix Borror, 1965 is similar to *D. appendiculata* (Ehrenberg, 1838) as concern the number of FVC and CC, but the former (*D. oligothrix*) differs from the latter (*D. appendiculata*) in followings: (1) The former is bigger than the latter in body size. (2) The former has long ellipsoidal while the latter wide oval in feature of outline. (3) The former has narrow base of buccal field while the latter wide. (4) The former has parallel paroral and endoral membranes close together while the latter divergent (5) The former has narrow AZM while the latter wide. (6) The dorsal kinety rows are continuous in the former, but broken in the latter (Chen and Song, 2002; Petz et al, 1995; Song and Packroff, 1997).

This species was well-known as marine species, but our population occurred in the water of the sewage treatment plant which is very close to coastal sea and has very low

salinity about 1.6‰.

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