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Redescriptions of *Diophrys appendiculata* and *D. scutum* (Ciliophora: Spirotrichea: Uronychiidae) New to Korea

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ABSTRACT

Specimens of *Diophrys appendiculata* (Ehrenberg, 1838) and *D. scutum* (Dujardin, 1841) have been collected from the coastal and brackish waters around near Ulsan, during 2004-2007. *Diophrys appendiculata* and *D. scutum* are described taxonomically for the first time in Korea. Diagnostic characteristics of these species are as follows. *Diophrys appendiculata*: size *in vivo* 43-68 × 25-50 μm, adoral zone of membranelles (AZM) covering 43-74% of cell length in impregnated and 46-65% *in vivo* specimens with 32-47 adoral membranelles (AM). Paroral membrane is slightly curved. Four to five dorsal kinetal (DK) rows are fragmented and anterior and posterior parts of rows densely ciliated. Two macronuclear nodules (Ma) irregular and elongated oval in shape and widely separated. *D. scutum*: size *in vivo* 125-225 × 75-140 μm, AZM extending to the middle of right border of body and covering 50-60% of cell length with 56-75 AMs. Body shape is typically ovoid with prominent concave margin at right posterio-lateral end, and rather thick and wide longitudinal ridge along lower buccal cavity on ventral side. Two macronuclei shaped like a sausage. five to six dorsal kineties.

Key words: *Diophrys*, redescription, marine, estuarine, morphology

INTRODUCTION

Members of the ciliate genus *Diophrys* are common inhabitants of littoral and sandy habitats, and are found worldwide in marine and estuarine biotopes (Kahl, 1932; Curds, 1975; Petz et al., 1995; Song and Packroff, 1997; Chen and Song, 2002; Song and Wilbert, 2002; Song et al., 2007). *Diophrys* has a typical body shape, well developed adoral zone of membranelles and conspicuous caudal cirri. Seventeen species have been described up to now. Among them, only *D. oligothrix* has been reported from Korea (Kwon and Shin, 2006). In addition, we have discovered two additional species for the first time in Korea: *D. appendiculata* and *D. oligothrix*.

The three Korean species of *Diophrys*, *D. appendiculata*, *D. oligothrix* and *D. scutum* are very common in other countries too. Among them, *D. scutum* is distinguishable from other congeners by the large size and the extension of distal end of AZM (adoral zone of membranelles) to the middle of right border. However, *D. appendiculata* and *D. oligothrix* are still confused to each other, and it's not easy to delimit them (Kahl, 1932; Curds, 1975; Petz et al., 1995; Song and Packroff, 1997; Chen and Song, 2002; Song and Wilbert, 2002; Song et al., 2007).

The aim of the present work is to redescribe *D. appendiculata* and *D. scutum* new to Korea.

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MATERIALS AND METHODS

Specimens of *D. appendiculata* were collected on 27 June 2007 from the coastal seawater in Taejongdae, Pusan (35° 03′23″N, 129° 04′45″E). The specimens of *D. secutum* were collected the estuarine littorals of Taehwa River, Ulsan (35° 32′45″N, 129° 20′20″E) at 19 April 2007. The raw cultures were established at room temperature in laboratory using Petri dishes filled with saline water from the collection site.

Living specimens were isolated and examined under the bright field and differential interference contrast microscopes. The living and silver impregnated specimens were prepared by modified protargol and dry staining methods (Wilbert, 1975; Foissner, 1992; Shin and Kim, 1993). Terminology and taxonomic scheme are mostly according to Lynn (2002).

RESULTS AND DISCUSSION

Family Uronychiidae Jankowski, 1979
Genus *Diophrys* Dujardin, 1841

[1]*1. ***Diophrys appendiculata*** **(Ehrenberg, 1838) (Fig. 1, Table 1)**

Stylonychia appendiculata Ehrenberg, 1838 (cited from Curds and Wu, 1983, p. 204, Fig. 9a).

Diophrys appendiculata: Kahl, 1932, p. 625; Ozaki and Yagiu, 1943, p. 28; Dragesco and Dragesco-Kernéis, 1986, p. 509; Song and Packroff, 1997, p. 22; Chen and Song, 2002, p. 386; Song et al., 2007, p. 294.

Diophrys quadricaudatus Agamaliev, 1967, p. 394.

Diagnosis. Size *in vivo* 43-68 × 25-50 μm, mostly 57 × 35 μm. Adoral zone of membranelles (AZM) covering about 50% of body length *in vivo* and 43-75% of body length in impregnated specimens with 41 adoral membranelles (AM). Paroral membrane (PM) slightly curved. Transverse cirri (TC) five, caudal cirri (CC) three, left marginal cirri (LMC) two and dorsal kineties (DK) 4-5 in number.

Description. General morphology: Size *in vivo* 43-68 × 25-50 μm, mostly 57 × 35 μm. Length/width ratio about 1.6/1 (Table 1). Body shape ovoid (Fig. 1A, D, E), cell conspicuously rigid. The concavity at the posterior right edge conspicuous (Fig. 1A, E). Cytoplasm colourless, but dark grey coloured cytoplasmic inclusion at low magnification. Contractile vacuole (CV) located near the CC.

Frontal and buccal field: Frontoventral cirri (FVC) seven in number (Fig. 1B, F-H and Table 1) and arranged in two distinct group, five at anterior and the rest two at mid-ventral surface and upper transverse cirri (TC) (Fig. 1B, F). Buccal field prominent, extending over about 46-65% of body length *in vivo*, and 43-74% of body length in protargol impregnated specimens. AZM width wide with membrane up to 60 μm and with 32-47 (average 41) AMs, of them, about 14-18 membranelles in distal part of AZM, arching at anterior end to the dorsal surface and back toward ventral surface, proximal part of AZM on ventral side composed of approximately 23-26 membranelles along the left margin of buccal cavity. Two undulating membranes (UM, composed of PM and EM) lying to the right of AZM. PM long, curved at mid part and extending to anterior end of buccal area. Endoral membrane (EM) covering 2/3 of PM, slightly curved. PM and EM originated near proximal end of AZM and divergent from one fourth of PM (Fig. 1B, F) or overlaped nearly (Fig. 1G, H).

Somatic infraciliature: Typical *Diophrys* pattern on ventral side. Five TC and about 37 μm long *in vivo*. LMC two in number and relatively weak and conspicuously separated, posterior MC positioned close to TC (Fig. 1B, F-H). CC on dorsal side of right posterior margin, three in number constantly and prominent, and 18-28 μm long *in vivo*. Dorsal surface bearing 4-5 DKs, rows fragmented and anterior and posterior parts of rows densely ciliated (Fig. 1I).

Nuclear appearance: Macronuclei (Ma) irregular and elongated oval in shape and two in number, 10 μm in diameter (Fig. 1C, G, H). Small nucleoli scattered within Ma. 1-2 small micronuclei (Mi) located at each Ma nodule (Fig. 1C).

Locomotion: Behavior and movement gliding on substrate

Table 1. Biometric characterization of *Diophrys appendiculata* (D.a.) and *D. scutum* (D.s.)

Characters	p	Mean	Med.	Min.	Max.	SD	SE	CV (%)	n
Body length	D.a.	80.3	80.0	70	95.0	6.8	1.7	8.4	15
	D.s.	161.0	157.5	115	225.0	30.3	6.2	18.8	24
Body width	D.a.	52.3	50.0	45	64.0	6.9	2.4	13.2	8
	D.s.	101.6	100.0	65.0	138.0	21.9	4.7	21.6	22
Body length/Body width	D.a.	1.5	1.5	1.2	1.7	0.1	0.0	9.2	8
	D.s.	1.6	1.6	1.3	2.1	0.2	0.0	10.2	22
AZM length	D.a.	50.0	50.0	39.0	60.0	4.4	1.1	8.7	17
	D.s.	96.9	100.0	75.0	112.5	13.3	2.7	13.8	24
AZM length/Body length (%)	D.a.	62.7	65.0	43.0	74.0	8.1	2.2	12.9	13
	D.s.	61.0	60.9	44.4	76.9	6.7	1.4	11.0	24
AM number	D.a.	40.6	42.0	32.0	47.0	3.8	1.1	9.4	13
	D.s.	65.4	65.0	45.0	75.0	6.4	1.4	9.8	21
FVC number	D.a.	7.2	7.0	6.0	9.0	0.8	0.2	10.4	16
	D.s.	7.0	7.0	7.0	7.0	0.0	0.0	0.0	24
CC number	D.a.	3.0	3.0	3.0	3.0	0.0	0.0	0.0	16
	D.s.	3.0	3.0	3.0	3.0	0.0	0.0	0.0	24
DK number	D.a.	4.3	4.0	4.0	5.0	0.5	0.2	11.4	7
	D.s.	5.1	5.0	5.0	6.0	0.4	0.1	6.9	8

All abbreviations of terms are the same as in the text except SD=standard deviation; SE=standard error; CV=coefficient of variation in %; n=population size; Max=maximum; Med=median; Min=minimum; SD=standard deviation; SE=standard error; CV=coefficient of variation in %; n=population size; p=species populations of *Diophrys*. The unit of length and width is μm.

[1]*부유쌍눈썹충 (신칭)

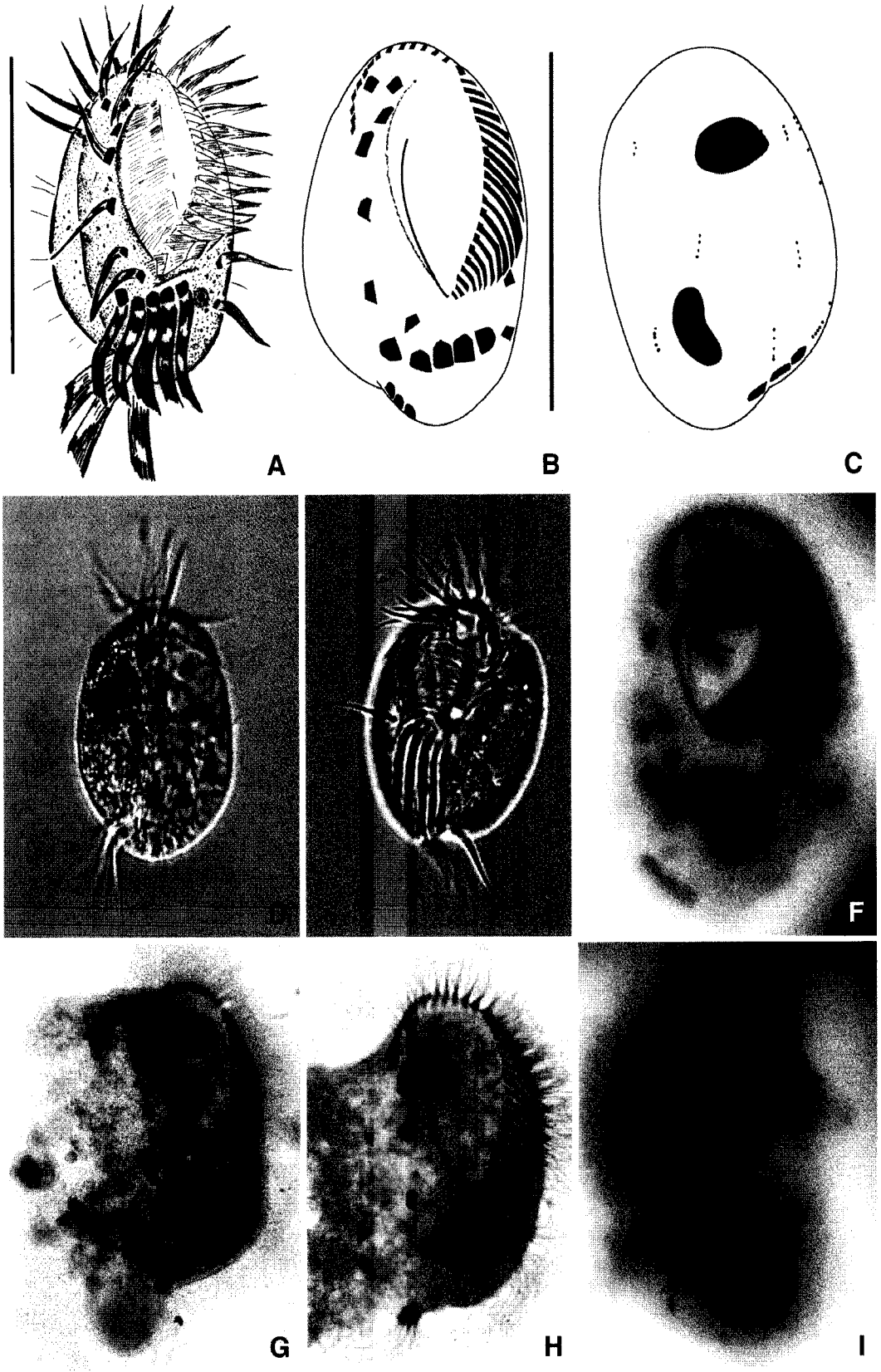

Fig. 1. *Diophrys appendiculata* from living (A, D, E) and protargol impregnated specimens (B, C, F-I). A, habitus; B, F, G and H, ventral view. B and F, divergent paroral and endoral membranes (E, arrowhead mark) and close marginal cirri. D and I, dorsal view, concave posterior right edge with caudal cirri, fragmented dorsal kinetal rows with densely ciliated in posterior part. Scale bars=50 μm.

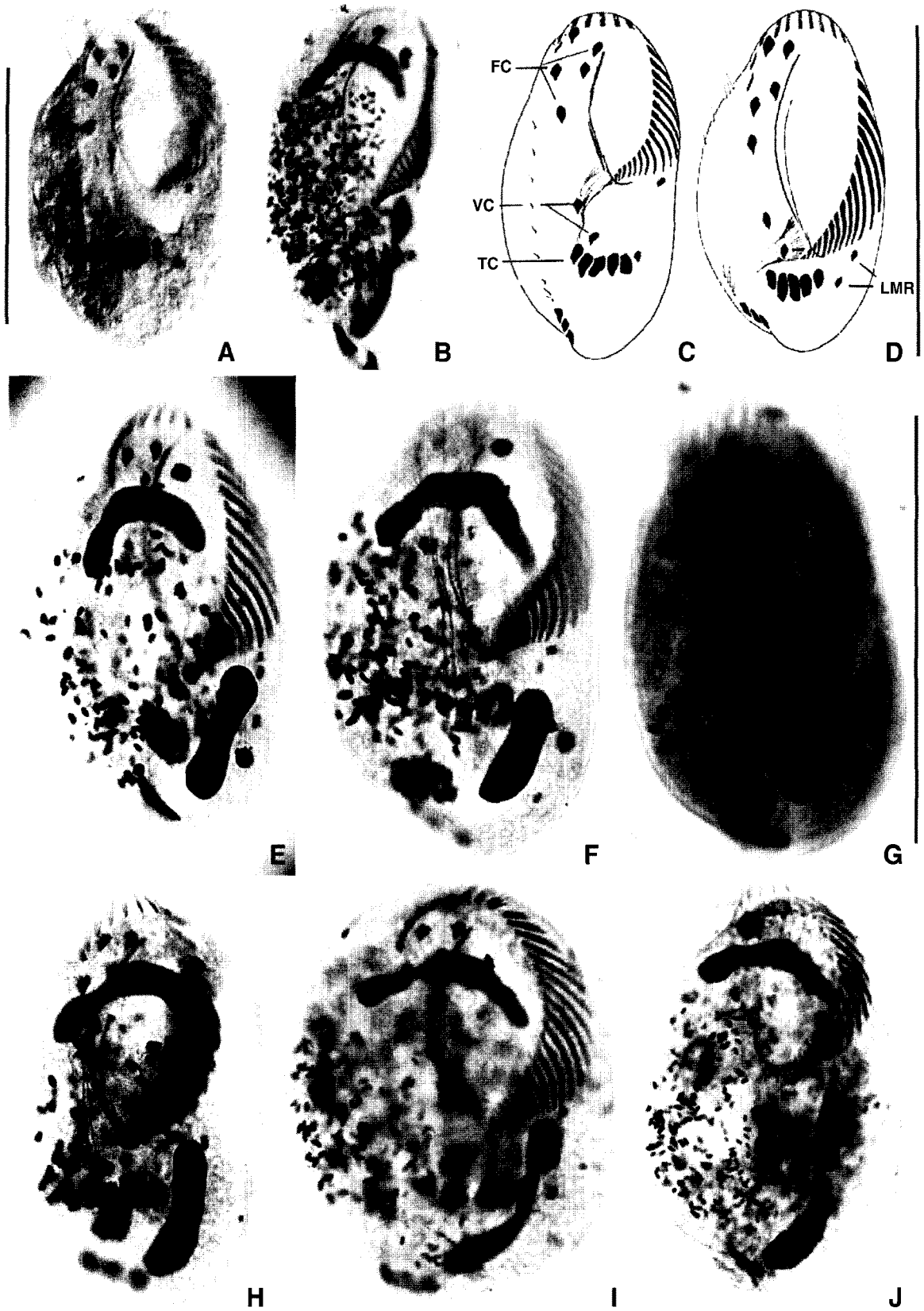

Fig. 2. *Diophrys oligothrix* (A-C, E-J) and *D. appendiculata* (D). A, B and J. typical characters in ventral side; F-J. paroral and endoral membranes are divergent like those of *D. appendiculata*; E-G. short distance between buccal cavity and TC like those of *D. appendiculata*; G. short distance between two left marginal cirri like those of *D. appendiculata*. Scale bars=50 μm.

and benthic crawling on substrate with jumps and speeded like a whirlwind.

Distribution. Africa, America, Europe, China and Korea

Remarks. The Korean population of *Diophrys appendiculata* agrees with the original and subsequent descriptions (Kahl, 1932; Curds and Wu, 1983; Dragesco and Dragesco-Kernéis, 1986; Song and Packroff, 1997; Chen and Song, 2002; Song et al., 2007). Especially wide oval in feature of outline, number of FVC and CC, size of body, fragmented rows of DKs, relatively short distance from buccal field to TC, short distance between both LMC of present specimens resembles that of previous records of *D. appendiculata* (Song and Packroff, 1997; Chen and Song, 2002; Song et al., 2007). But the present population appears that PM and EM are divergent at one fourth of PM or close together.

D. appendiculata is known as most related and similar to *D. oligothrix* in the morphological criteria. The characters delimiting these two species were summerized by Chen and Song (2002) based on Chinese populations as followings: (1) continuity of DKs' ciliary rows on the dorsal side (fragmented into group vs. continuous), (2) shape of macronuclear nodules (short globular vs. sausage), (3) number of adoral membranelles (35-44 vs. 26-35), (4) body shape (wide oval vs. long-ellipse), (5) width of AZM (wide vs. narrow). (6) arrangement of paroral and endoral membranes (divergent vs. parallel, close together) (7) distance between buccal field to TC (shorter vs. longer) and (8) distance between left marginal cirri (shorter vs longer). In Korea, we discovered these two species recently but could not apply the criteria from Chinese population completely. There are several possibilities for these reasons. First of all, some of these characters (5)-(8) are too variable to delimit these species. Secondly, the micro-habitats of each population collected were different, that is, in the case of Chinese population were coastal waters and coastal ponds while the estuarine waters in Korean population, especially in differences of salinity. Thirdly, three species of *Diophry*, *D. appendiculata*, *D. oligothrix* and *D. scutum*, have the same evolutionary affinity and they could be evolved recently, so some characters could be flexible to change according to the environmental factors or resources (Hill, 1981).

In the Korean populations of *D. oligothrix*, we could find some variations in several characters which could not be used as diagnostic characters to delimit between *D. oligothrix* and *D. appendiculata* as followings: 1) Distance between marginal cirri: longer or shorter (Fig. 2A, B, E, G, I). 2) Distance from buccal field to TC: shorter or longer (Fig. 2A, F-I). 3) Arrangement of paroral and endoral membranes: divergent or close together (Fig. 2F, H, I).

[1]*2. *Diophrys scutum* (Dujardin, 1841) (Fig. 3, Table 1)

Ploesconia scutum Dujardin, 1841 (in part, cited from Curds and Wu, 1983, p. 203, Fig. 11a).

Diophrys scutum: Kahl, 1932, p. 624; Ozaki and Yagiu, 1943, p. 26; Curds and Wu, 1983, p. 203; Song and Packroff, 1997, p. 348; Chen and Song, 2002, p. 386; Song and Wilbert, 2002, p. 55; Song et al., 2007, p. 291.

Diophrys scutoides Agamaliev, 1967, p. 391.

Diagnosis. Large body size 125-225 μm × 75-140 μm (average 180 × 120 μm) *in vivo*. Length/width ratio about 1.5/1. AZM extending to the middle of right border of body, AZM width wide with membrane up to 102 μm and with 56-75 (average 66) AMs. AZM length/body length ratio about 58% in fixed specimens. PM and EM not parallel and divergent. Five TC prominent and about 50 μm long *in vivo*. Three CC prominent. Two LMC conspicuously separated. Dorsal surface bearing 5-6 DKs, and about 7 μm long *in vivo* at marginal dorsal bristles (DB).

Description. General morphology: size *in vivo* 125-225 × 75-140 μm, mostly 180 × 120 μm. Length/width ratio about 1.5/1 (Table 1). The largest species of *Diophrys*. Body slenderly ovoid form with a collar (Fig. 3A), cell conspicuously rigid. Concavity at posterior right edge conspicuous (3A, B, D, E). Thick and wide longitudinal ridge along lower buccal cavity on ventral side (Fig. 3A, D, E). Cytoplasm colourless, dark gray coloured cytoplasmic inclusion at low magnification. CV located near CC.

Frontal and buccal field: FVC seven in number and about 25 μm long *in vivo*, arranged in two distinct cirral groups; five cirri at anterior part, and rest two cirri situated at mid-ventral surface and upper TC (Fig. 3B, G, I).

Buccal field large and prominent, extending over about 44-76% of body length. AZM extending to middle of right border of body. AZM wide with membrane up to 102 μm and with 58-75 (average 66) AMs; about 20-26 membranelles in distal part of AZM, arching at anterior end to dorsal surface and back toward ventral surface, proximal part of AZM on ventral side composed of approximately 32-40 membranelles along left margin of buccal cavity. UM composed of EM and PM. PM long, prominently curved around mid-portion, extending to anterior end of buccal area. EM about 2/3 of PM length, originated from proximal end of AZM (Fig. 3B, G, I). PM and EM divergent from 1/3 portion of PM (Fig. 3B, G).

Somatic infraciliature: ventral side ciliary pattern aligned in typical *Diophrys*. Five TC, about 50 μm long and prominent. Usually two relatively weak LMC positioned posterior to the level of TC, aligned transversely, two LMC conspi-

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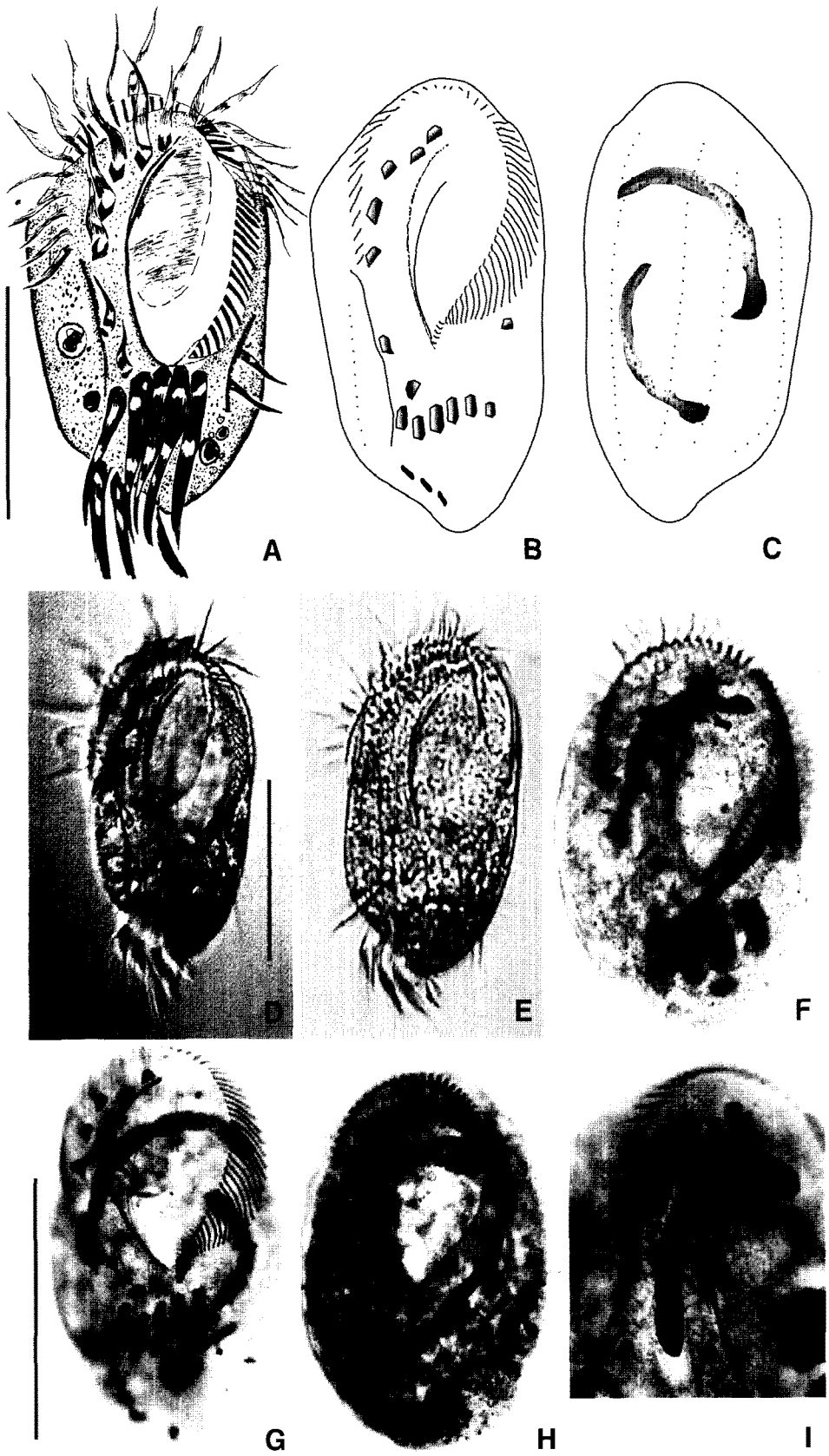

Fig. 3. *Diophrys scutum* from living (A, D, E) and from protargol impregnated specimens (B, C, F-I). A, habitus; B, F, G and H, ventral view. B and F, divergent paroral and endoral membranes; C and F, dorsal view, concave posterior right edge with caudal cirri. Scale bars=100 μm.

cuously well separated. Three CC on dorsal surface of right posterior margin, prominent and about 30-40 μm long. Dorsal surface bearing 5-6 DKs and about 7 μm long in marginal DB.

Nuclear appearance: two irregular, slender and long Ma nodules, about 60-85 × 10 μm (Fig. 3C, G, H). Small nucleoli scattered within Ma. Mi small, 2.5 μm in diameter, located close to each Ma nodule, repectively (Fig. 3I).

Locomotion: Behavior and movement gliding on substrate and benthic. Crawling on substrate with jumps.

Distribution. America, Antarctic, Europe, Middle Asia, China, Japan and Korea.

Remarks. The Korean population of *D. scutum* (Dujardin, 1841) agrees basically well with the subsequent redescriptions, except the number of DKs, i.e. the Chinese population has more rows of DKs (6-9 DKs) than the present population (Kahl, 1932; Curds and Wu, 1983; Song and Packroff, 1997; Chen and Song, 2002; Song and Wilbert, 2002; Song et al., 2007).

D. scutoides Agamaliev, 1967 is considered as a junior synonym of *D. scutum*, because the number of FVC is variable even within the clone (Song and Packroff, 1997; Song and Wilbert, 2002). *D. scutum* is similar to *D. oligothrix* or *D. appendiculata*, concerning the number of FVC, TC and CC, however *D. scutum* differs from *D. oligothrix* or *D. appendiculata* by the followings: 1) the body size of *D. scutum* is three times larger than that of *D. oligothrix* or *D. appendiculata*; 2) the distal end of AZM in *D. scutum* extends to the middle of right border of body, while those of the latters to the anterior border of body; 3) *D. scutum* has about 58-75 AMs, while the latters have 25-45 AMs (Song et al., 2007) and 20-35 AM in the present specimens.

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REFERENCES

Agamaliev, F.G., 1967. Faune des ciliés mésopsammiques de la cote oust de la mer caspienne. Cah. Biol. Mar., 8: 359-402.

Borror, A.C., 1965. Morphological comparisom of *Diophrys scutum* (Duhardin, 1841) and *Diophrys peloetes* n. sp. (Hypotrichida, Ciliophora). J. Protozool., 12: 60-66.

Chen, Z. and W. Song, 2002. Characterization and identification of the *Diophrys* species (Protozoa, Ciliophora, Hypotrichida) based on RAPD fingerprinting and ARDRA riboprinting. Eur. J. Protistol., 38: 383-391.

Curds, R.C. and I.C.H. Wu, 1983. A review of the Euplotidae (Hypotrichida, Ciliophora). Bull. Br. Mus. Nat. Hist. (Zool.), 44: 191-247.

Czapik, A., 1981. La morphogenése chez le cilie *Diophrys oligothrix* Borror. Acta Protozool., 20: 367-372.

Dragesco, J. and A. Dragesco-Kernéis, 1986. Ciliés libres de l'Afrique intertropicale. Faune Trop, 36: 1-559.

Foissner, W., 1992. The dry silver nitrate method. *In* Protocols in protozoology (published by the society of Protozoologists). Allen Press, Kansas, pp. C11.1-C11.4.

Hill, B.F., 1981. The cortical morphogenetic cycle associated with cell division in *Diophry* Dujardin, 1841 (Ciliophora, Hypotrichida). J. Protozool., 28: 215-221.

Kahl, A., 1932. Urtiere oder protozoa I. Wimpertiereoder Ciliata (Infusoria) III. Spirotricha. Die Tierwelt Dtl., 25: 399-650.

Kwon, C.B. and M.K. Shin, 2006. Redescription of previously unknown Euplotine ciliates, *Euplotes charon* and *Diophrys oligothrix* (Ciliophora: Spirotrichea: Euplotida), from Korea. Korea J. Sys. Zool., 22: 29-35.

Lynn, D.H. and E.B. Small, 2002. Phylum Ciliophora. *In* An Illustrated Guide to the Protozoa. Lee, J.J., P.C. Bradbury and G.F. Leedale, eds., Society of Protozoologists, Lawrence, Kansas, pp. 371-656.

Ozaki, Y. and R. Yagiu, 1943. Studies on the marine ciliates of Japan. J. Sci. Hiroshima Univ. Ser. B., 10: 21-52.

Petz, W., W. Song and N. Wilbert, 1995. Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. Stapfia, 40: 1-223.

Shin, M.K. and W. Kim, 1993. New records of three oxytrichid hypotrichs (Ciliophora: Hypotrichida: Oxytrichidae) from the Han river in Seoul, Korea. Korean J. Zool., 36: 223-230.

Song, W. and G. Packroff, 1997. Taxonomische Untersuchungen an marinen Ciliaten aus China mit Beschreibungen von 2 neuen Arten, *Strombidium globosaneum* nov. spec. und *Strombidium platum* nov. spec. (Protozoa, Ciliophora). Arch. Protistenkd., 47: 331-360.

Song, W. and N. Wilbert, 2002. Faunistic studies on marine ciliates from the Antarctic benthic area, including descriptions of one epizoic form, 6 new species and 2 new genera (Protozoa: Ciliophora). Acta Protozool., 41: 23-61.

Song, W., N. Wilbert, K. Al-rasheid, A. Warren, C. Shao, H. Long, Z. Yi and L. Liqiong, 2007. Redescriptions of two marine hypotrichous ciliates, *Diophrys irmgard* and *D. hystrix* (Ciliophora, Euplotida), with a brief revision of the genus *Diophrys*. J. Eukaryot. Microbiol., 54: 283-296.

Wilbert, N., 1975. Eine Vertesserte Technik der Protargoimpragnation für Ciliaten. Mikrokosmos, 64: 171-179.

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