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New record of four ciliates (Protozoa, Ciliophora) collected from rocky intertidal pools of South Korea

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Marine benthic ciliates were collected from four rocky intertidal pools along the eastern and southern coasts of Korea from 2012−2013. Ciliate specimens were examined by observing living and stained cells. Four ciliate species were new to Korea based on morphological characteristics as follows. (1) *Aspidisca polypoda* (Dujardin, 1841): small bean-shaped (ca. 30 μm), eight conspicuous dorsal ridge, polystyla-arrangement of frontoventral cirri; (2) *Epiclintes auricularis auricularis* (Claparède & Lachmann, 1858): tripartite and auriform body (ca. 300 μm), 46 adoral membranelles, one or two frontal and 23 transverse cirri, 12 oblique fronto-midventral rows; 59 left and 71 right marginal cirri; (3) *Pseudochilodonopsis marina* Song, 1991: reniform body (ca. 50 μm), two obliquely positioned contractile vacuoles, seven left and five right kineties, five fragmented preoral kineties; (4) *Dysteria semilunaris* (Gourret and Roeser, 1888): oval-shaped (ca. 30 μm), two longitudinally positioned contractile vacuoles, conspicuous longitudinal grooves on both plates, four or five right kineties, one short row below frontoventral kineties.

Keywords: benthic ciliate, marine, protargol impregnation, redescription, taxonomy

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Introduction

A total of 458 species of ciliates have been discovered in Korea (Kwon *et al*., 2019). A major habitat of the recorded ciliates is coastal waters, representing over 40% of the total number of species. Over 60% of the coastal species numbers were planktonic species, while less benthic ciliates have been recorded in Korean coastal waters.

Here, we report four benthic ciliates collected from intertidal pools as follows: *Aspidisca polypoda* (Dujardin, 1841); *Epiclintes auricularis auricularis* (Claparède & Lachmann, 1858); *Pseudochilodonopsis marina* Song, 1991; *Dysteria semilunaris* (Gourret and Roeser, 1888). Six, two, and six species in genus *Aspidisca*, *Pseudochilodonopsis*, and *Dysteria* were described respectively by taxonomical studies in Korea (Shin, 1988; Shin and Kim, 1988; Li *et al*., 2010; Park and Min, 2015; 2016; Park *et al*., 2017; Kim and Jung, 2017; 2018; Kim *et al*., 2018; Kim and Min, 2018). The genus *Epiclintes* in this study is newly recorded for Korean ciliate fauna.

Materials and Methods

Ciliates were sampled from intertidal pools on basal rock in April of 2012 and in June of 2013. Sampling sites were located on eastern and southern coasts of Korea (Fig. 1). Water temperature, salinity, and pH were measured using a portable YSI (YSI professional plus water quality instrument, USA). Detailed information of sampling locations are described in 'Material examined' of each species. Biofilms covered on surface rock and waters in the pools were collected using brush, pipette, and sponge. The collected samples were transferred to the laboratory, then incubated adding rice grains to proliferate bacteria as food resource. The cultures were maintained in Petri dishes (90 mm in diameter) and incubated at room temperature.

The morphology of cultured specimens was observed under a stereomicroscope (Zeiss Stemi DV4, Germany) and an optical microscope (Zeiss Imager. A2; Germany) at magnifications ranging from 100× to 1000×. The protargol impregnation was performed using 'proce-

dure B' method (Foissner, 2014). All measurements and photomicrographs were obtained using a digital camera (Zeiss AxioCam ICc1 rev. 4, Germany). To create the focus-stacked images of some thick specimens, Helicon Focus software 6.8.18 (HeliconSoft Ltd, Ukraine) was used. Terminology and systematic classification were mainly according to Berger (2006), Gong *et al*. (2007), and Lynn (2008).

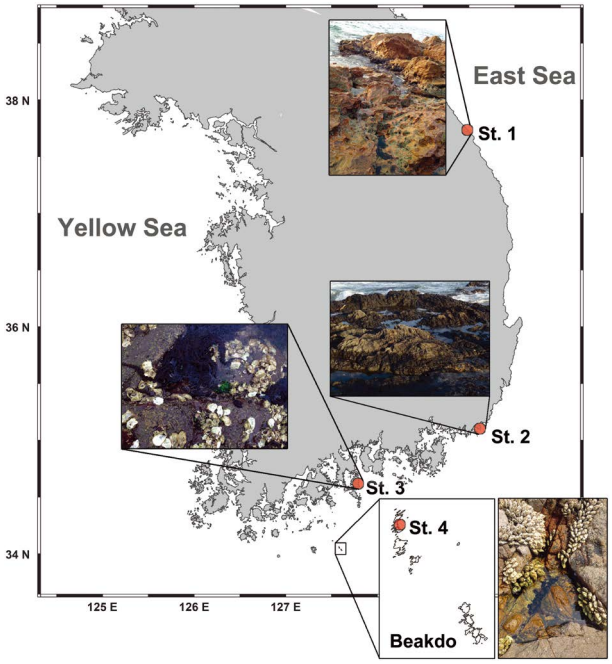

Fig. 1. Map of the sampling stations in South Korea. Each station (marked with red circles) located at three intertidal zones of the Korean Peninsula (St. 1–3) and Baekdo Island (St. 4).

Results and Discussion

Phylum Ciliophora Doflein, 1901
Subphylum Intramacronucleata Lynn, 1996
Class Spirotrichea Bütschli, 1889
Order Euplotida Small & Lynn, 1985
Family Aspidiscidae Ehrenberg, 1830
Genus *Aspidisca* Ehrenberg, 1830

1. *Aspidisca polypoda* (Dujardin, 1841) Kahl, 1932 (Fig. 2)

Material examined. Marine water (temperature 11.5°C; salinity 34.0; pH 8.6) collected from Jeongdongjin Harbor, Jeongdongjin-ri, Gangdong-myeon, Gangneung-si, Gangwon-do, South Korea (37°41′09″N 129°02′32″E) on April 23, 2012 (Fig. 1; St. 1).

Diagnosis. Body size 22–40 × 21–35 μm, length:width ratio about 1.3:1 *in vivo*; 30–40 × 22–32 after protargol impregnation; outline bean-shaped with snout-like

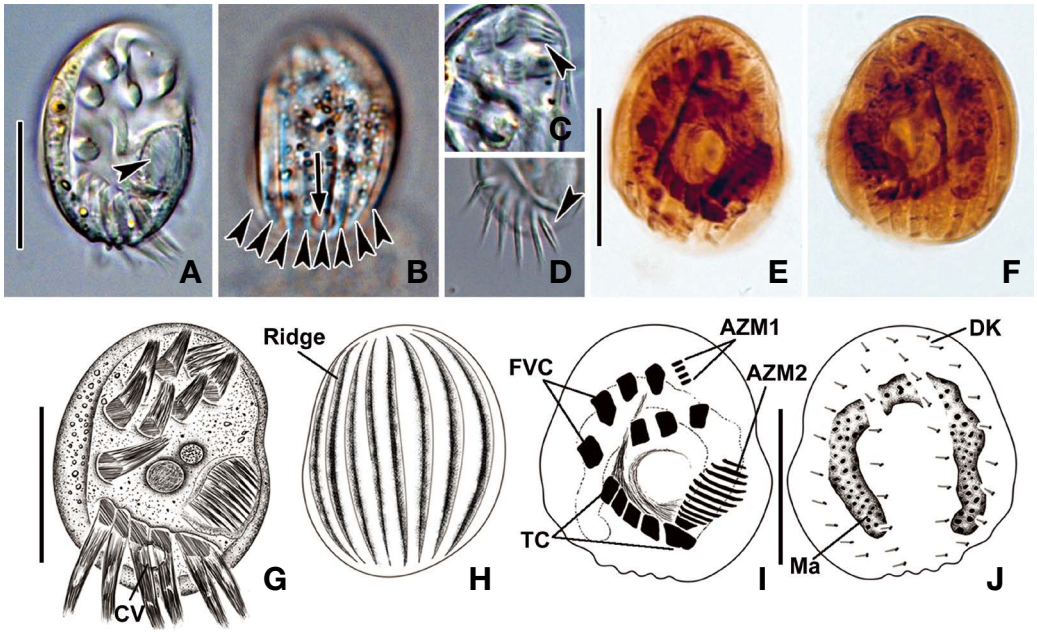

Fig. 2. Photomicrographs of *Aspidisca polypoda* from living specimens (A–D), protargol impregnated specimens (E, F), and illustrations (G–J). A. Ventral view showing posterior part of the adoral membranelles (arrowhead). B. Eight distinctive ridges (arrowheads) and contractile vacuole (arrow). C. Anterior part of the adoral membranelles (arrowhead). D. Separated into two parts of leftmost transverse cirri (arrowhead). E, F. Ventral and dorsal view of stained specimens. G–J. Illustrations showing main characteristics. AZM 1 and 2, adoral zone of membranelles 1 and 2; CV, contractile vacuole; DK, dorsal kineties; FVC, frontoventral cirri; MA, macronucleus; TC, transverse cirri. Scale bars: 20 μm.

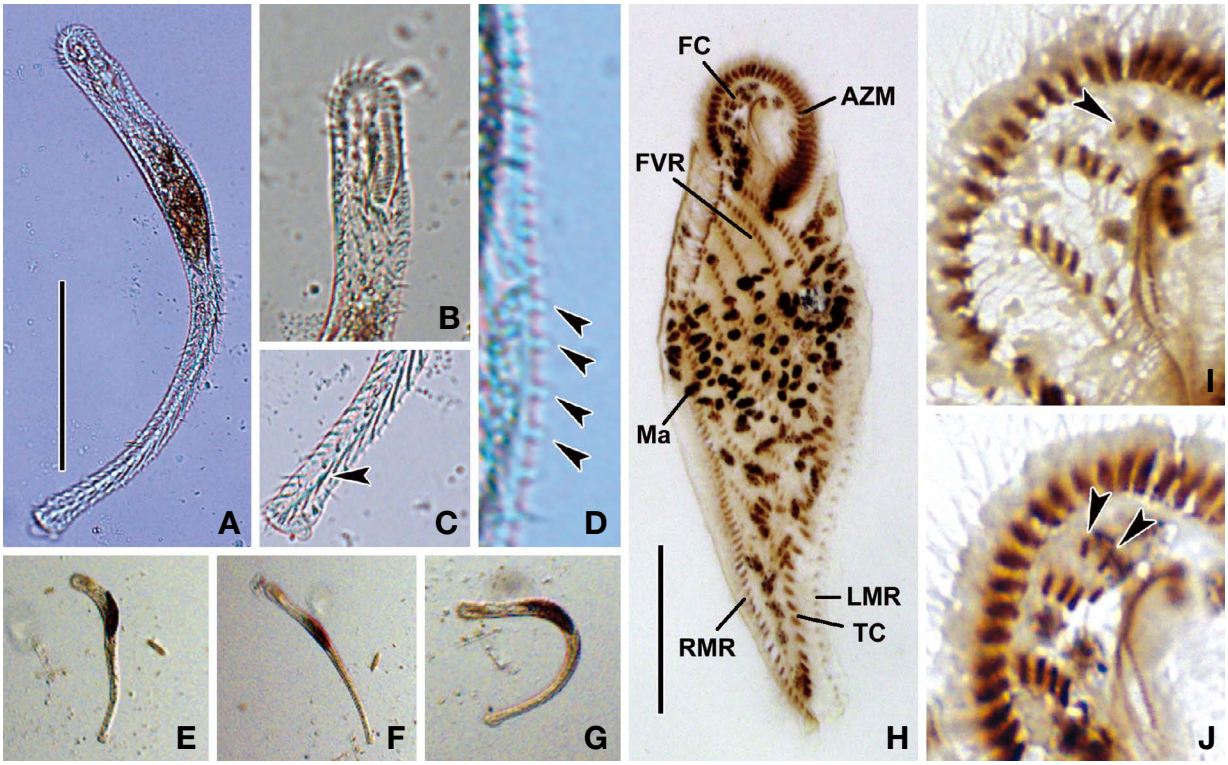

Fig. 3. Photomicrographs of *Epiclintes auricularis auricularis* from living specimens (A–G), protargol impregnated specimens (H–J). A. Ventral view of typical individual. B. Conspicuous adoral zone in anterior portion. C. Transverse cirri (arrowhead) in posterior portion. D. Lateral view showing dorsal bristles located on papillae (arrowheads). E–G. Highly-flexible body. H. Ventral view of stained specimen. I, J. Variation in numbers of leftmost frontal cirri (arrowheads). AZM, adoral zone of membranelles; FC, frontal cirri; FVR, fronto-midventral row; LMR, left marginal row; Ma, macronuclear nodules; RMR, right marginal row; TC, transverse cirri. Scale bars: A = 100 μm, H = 50 μm.

anterior portion; 8 prominent dorsal ridges; 1 inverted U-shaped macronucleus; contractile vacuole below transverse cirri; adoral zone of membranelles split into anterior and posterior part and each with 4–5 and 9–11 membranelles respectively; 7 frontoventral cirri; 6 transverse cirri including almost conjoined leftmost 2 cirri; 4 dorsal kineties each with about 8, 9, 9, 9 basal bodies, from left to right, respectively.

Distribution. Antarctica, France, Germany, Russia, and Korea.

Remarks. *Aspidisca polypoda* is easily distinguished by seven or eight conspicuous dorsal ribs (Dujardin, 1841), which coincides with this Korean population. Our population differs from the Antarctic population by basal body number of dorsal kineties 1–4 (ca. 8, 9, 9, 9 vs. ca. 4, 5, 6, 6, respectively) that might be caused by a distinct sampling localities/habitats (Song and Wilbert, 2002).

Among the other congeners with similar morphology to *A. polypoda*, *A. steini* Buddenbrock, 1920 is distinguishable from *A. polypoda* based on the number of dorsal ridges (absent or four vs. eight). *Aspidisca cicada* (Müller, 1786) Claparède & Lachmann, 1858 differs by pattern of frontoventral cirri (lynceus- arrangement vs. polystyla-arrangement) (Wu and Curds, 1979; Song and Wilbert, 1997).

Voucher slides. Two slides with protargol-impregnated specimens was deposited at National Institute of Biological Resources (NIBRPR0000110829) and Korea Institute of Ocean Science and Technology (KIOST), respectively.

Order Urostylida Jankowski, 1979
Family Epiclintidae Wicklow & Borror, 1990
Genus *Epiclintes* Stein, 1863

2. *Epiclintes auricularis auricularis* (Claparède & Lachmann, 1858) Stein, 1864 (Fig. 3)

Material examined. Marine water (temperature 16.4°C; salinity 34.6; pH 8.2) collected from Baekdo Island, Geomun-ri, Samsan-myeon, Yeosu-si, Jeollanam-do, South Korea (34°03′42″N 127°35′00″E) on June 4, 2013 (Fig. 1; St. 4).

Diagnosis. Body size about 250–350 × 30–40 μm *in vivo*; elongated and tripartite body composed of asymmetric auriform head, slightly wide trunk, and slender tail; dorsal bristle located on papillae; nuclear apparatus composed of

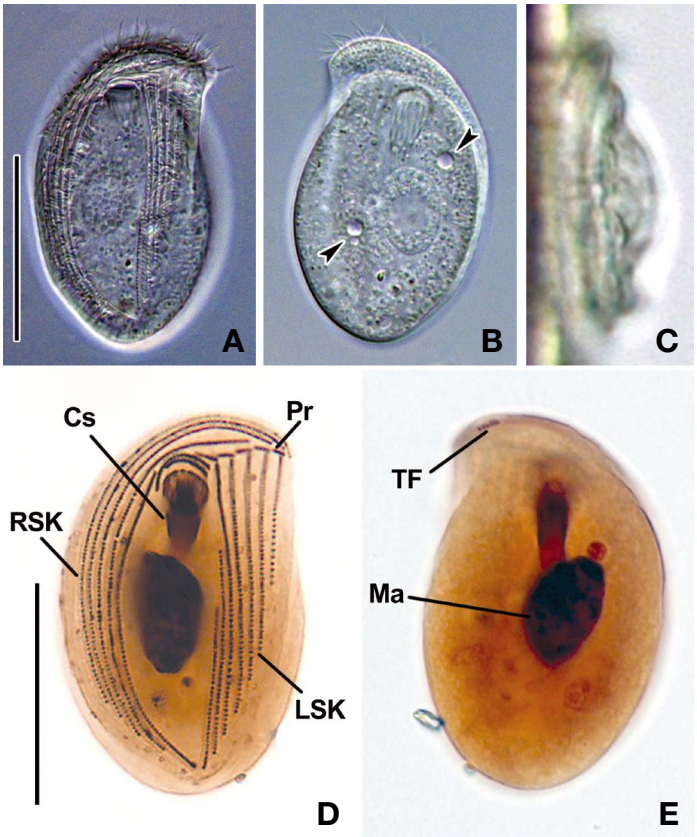

Fig. 4. Photomicrographs of *Pseudochilodonopsis marina* from living specimens (A–C) and protargol impregnated specimens (D, E). A. Ventral view of typical individual. B. Two contractile vacuoles (arrowheads). C. Lateral view. D, E. Ventral and dorsal view of stained specimens. Cs, cytostome; LSK, left somatic kineties; Ma, macronucleus; Pr, preoral kineties; RSK, right somatic kineties. TF, terminal fragment. Scale bars: 30 μm.

90–120 macronuclear nodules (3–8 × 2–5 μm) and several micronuclei (2.5–3.0 μm in diameter); 43–49 adoral membranelles; 1–2 frontal and 22–24 transverse cirri; 11–13 oblique fronto-midventral rows; 1 left and 1 right marginal row each with 57–60 and 68–73 cirri, respectively; three dorsal kineties.

Distribution. Worldwide (China, Egypt, England, France, Germany, Italy, Japan, Mexico, Netherlands, Norway, Poland, Russia, Sweden, USA, and Korea).

Remarks. The Korean population of *E. auricularis auricularis* follows to the original description by Claparède and Lachmann (1858) in following features: auriform body shape, about 300 μm body length; many oblique fronto-midventral rows; short cirri in tail part; short rod-shaped on cortex. Our population slightly differs from the Chinese population in the following features: body width (30–40 μm vs. 45–60 μm); number of frontal cirri (one or two vs. consistently one); number of left marginal cirri (57–60 vs. 49–56) (Song and Warren, 1996; Berger, 2006).

Epiclintes auricularis rarisetus Hu *et al*., 2009 can be distinguished from the Korean population of *E. auricularis auricularis* as follows: (1) number of adoral membranelles (23–33 vs. 43–49); (2) number of left and right marginal cirri (22–31 vs. 57–60 for left and 35–54 vs. 68–73 for right); (3) number of fronto-midventral rows (8–9 vs. 12–15) (Hu *et al*., 2009).

Voucher slides. Two slides with protargol-impregnated specimens was deposited at National Institute of Biological Resources (NIBRPR0000110830) and Korea Institute of Ocean Science and Technology (KIOST), respectively.

Class Phyllopharyngea de Puytorac *et al*., 1974
Order Chlamydodontida Deroux, 1976
Family Chilodonellidae Deroux, 1970
Genus *Pseudochilodonopsis* Foissner, 1979

3. *Pseudochilodonopsis marina* Song, 1991 (Fig. 4)

Material examined. Marine water (temperature 14.5°C; salinity 32.5; pH 8.5) collected from Oryukdo, Yongho-dong, Nam-gu, Busan, South Korea (35°05′58″N 129° 07′25″E) on April 22, 2012 (Fig. 1; St. 2).

Diagnosis. Body size 40–60 × 28–35 μm, length : width

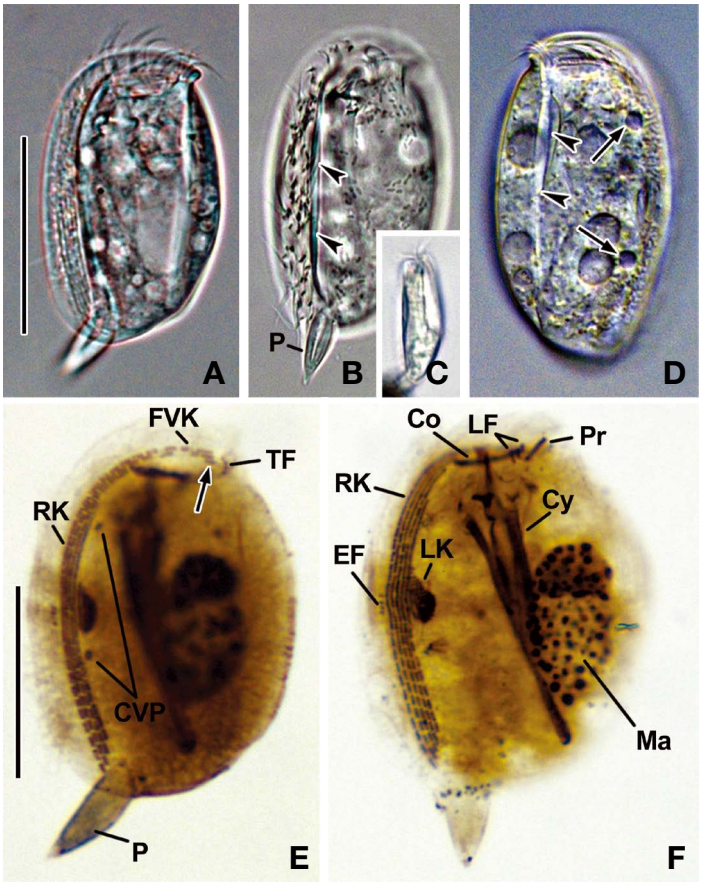

Fig. 5. Photomicrographs of *Dysteria semilunaris* from living specimens (A–D) and protargol impregnated specimens (E, F). A. Left lateral view of typical individual. B. longitudinal groove on left plate (arrowheads). C. Ventral view. D. Right lateral view showing the two contractile vacuoles (arrows) and the longitudinal groove on the right plate (arrowheads). E, F. Left views of stained specimens showing infraciliature, arrow denotes the short row below the end of the frontoventral kineties. Co, circumoral kineties; CVP, contractile vacuole pore; Cy, cytopharynx; EF, equatorial fragment; FVK, frontoventral kineties; LF, left frontal kineties; LK, left kineties; Ma, macronucleus; P, podite; Pr, preoral kinety; RK, right kineties; TF, terminal fragment. Scale bars: 20 μm.

ratio about 1.4 : 1 *in vivo*; 41–65 × 21–38 μm after protargol impregnation; outline reniform; dorsoventrally flattened about 2 : 1; single macronucleus 13–18 × 9–17 μm in size; 2 contractile vacuoles obliquely located; 10–15 nematodesmal rods; 7 left and 5 right kineties on ventral side; 5 fragmented preoral kineties; terminal fragment positioned apically on dorsal side, consisting of 5–7 basal bodies.

Distribution. China and Korea.

Remarks. The Korean population of *P. marina* highly resembles the original description by Song (1991) but, slightly differs in the size of macronucleus (ca. 15.5 × 11.9 vs. ca. 19.5 × 13.3) (Song, 1991; Liu *et al*., 2008).

The closest congener *P. similis* Song & Wilbert, 1989 differs from the Korean population as follows: location of terminal fragment (apical vs. subapical); basal body number of the terminal fragment (5–7 vs. 8–10); number of nematodesmal rods (10–15 vs. 16–18) (Song and Wilbert, 1989).

Voucher slides. Two slides with protargol-impregnated specimens was deposited at National Institute of Biological Resources (NIBRPR0000110831) and Korea Institute of Ocean Science and Technology (KIOST), respectively.

Order Dysteriida Deroux, 1976
Family Dysteriidae Claparède & Lachmann, 1858
Genus *Dysteria* Huxley, 1857

4. *Dysteria semilunaris* (Gourret & Roeser, 1888) Kahl, 1931 (Fig. 5)

Material examined. Marine water (temperature 12.0°C; salinity 33.0; pH 8.5) collected from the beach of Yullim village, Yullim-ri, Dolsan-eup, Yeosu-si, Jeollanam-do, South Korea (34°36′19″N 127°47′22″E) on April 21, 2012 (Fig. 1; St. 3).

Diagnosis. Body size 26–36 × 16–20 μm, length : width ratio about 1.7 : 1, bilaterally flattened about 1 : 2.1 *in*

vivo; outline oval-shaped, broad anterior and narrowed posterior end of the cell, dorsal side convex than ventral side; conspicuous longitudinal grooves presented left and right plate; single macronucleus 14−20 × 6−10 μm in size; 2 longitudinally positioned contractile vacuoles near innermost row of right kineties; about 9 μm podite caudally positioned on left ventral side; 4−5 right kineties, including 2 frontoventral kineties of which extend dorso-apically; 1 short row below the anterior end of the frontoventral kineties; 6−7 left kineties; equatorial fragment composed of 5−9 basal bodies; terminal fragment anterodorsally located, composed of 3−5 basal bodies; 2 parallel circumoral kineties; 3 left frontal kineties; single-rowed preoral kinety.

Distribution. China, France, Germany, and Korea.

Remarks. The descriptions by Gourret and Roeser (1888) and Kahl (1931) did not provide detailed information of body size and infraciliature. Nevertheless, the *D. semilunaris* can be easily identified by the body shape and marine habitat. The Korean population of *D. semilunaris* differs from the two Chinese populations (Qingdao and Shanghai) in number of right kineties (4−5 vs. consistently 4 in both populations), presence of longitudinal groove (left and right plates vs. left only vs. right only), and transverse stripe at the equatorial region (absent in the Korean and Qingdao population vs. present in Shanghai population) (Gong *et al*., 2007; Pan *et al*., 2016).

The closest congeners *D. ovalis* (Gourret and Roeser, 1886) Kahl, 1931 and *D. crassipes* Claparède & Lachmann, 1859 can be distinguished from *D. semilunaris* as follows: (1) absence of an extra short row below the anterior end of the frontoventral kineties; (2) absence of a longitudinal groove on the both plate; (3) subcaudally-located podite (Gong *et al*., 2007; Park and Min, 2015; Pan *et al*., 2016). Additionally, *D. calkinsi* Kahl, 1931 differs from *D. semilunaris* in the following features: arrangement of contractile vacuoles (diagonally positioned in original description but not detected in Antarctic population vs. longitudinally positioned); number of groove on the left plate (two vs. one); extra short row below the end of frontoventral kineties (absent vs. present); number of left frontal kineties (two vs. three) (Kahl, 1931; Song and Wilbert, 2002; Gong *et al*., 2007) .

Voucher slides. Two slides with protargol-impregnated specimens was deposited at National Institute of Biological Resources (NIBRPR0000110832) and Korea Institute of Ocean Science and Technology (KIOST), respectively.

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