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Taxonomy of four scuticociliates (Protozoa: Ciliophora) from coastal waters of South Korea

Jae-Ho Jung*

Department of Biology, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea

**Correspondent: jhjung@gwnu.ac.kr*

The diversity of scuticociliates (subclass Scuticociliatia Small, 1967) had previously been superficially investigated in Korea and only 10 species were reported according to the National Species List of Korea published in 2019. Here, we identify four scuticociliates, collected from three coastal water samples, by observing protargol-impregnated specimens. As a result, the present species belong to the order Pleuronematida Fauré-Fremiet in Corliss, 1956 and their lower taxonomic classifications are as follows: family Eurystomatellidae Miao *et al*., 2010 - *Eurystomatella sinica* Miao *et al*., 2010; family Pleuronematidae Kent, 1881 - *Pleuronema grolierei* Wang *et al*., 2008, *P. setigerum* Calkins, 1902, and *Schizocalyptra aeschtae* Long *et al*., 2007. The family Eurystomatellidae and the genus *Schizocalyptra* Dragesco, 1968 are reported for the first time in Korea. Considering that the scuticociliates are a species-rich group and very common in most habitats (including freshwater and terrestrial habitats), our findings indicate that we are far from understanding the complete diversity of Korean scuticociliates.

Keywords: Ciliate, diversity, marine, protargol impregnation

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Introduction

Scuticociliates are a speciose group and very common in coastal habitats (Carey, 1992; Lynn, 2008). Most of them are free-living, but some parasitize marine metazoans such as fish and crustaceans (Grolière and Leglise, 1977; Lom and Dykova, 1992; Song *et al*., 2003; 2009). According to the list of Korean ciliates made by Kwon *et al*. (2019), only 10 scuticociliate species were reported in Korea, since most Korean taxonomists have been focusing on the class Spirotrichea, which counts for about 60% of the list.

In this study, I focused on the Korean scuticociliates to understand their diversity, resulting in the identification of four species from coastal water samples. Two species belong to the genus *Pleuronema*, which is very common in coastal habitats and comprises more than 20 species (Dujardin, 1841; Carey, 1992; Song *et al*., 2009; Wang *et al*., 2009; Pan *et al*., 2016). In contrast, the other two genera, *Eurystomatella* and *Schizocalyptra*, are relatively uncommon and recently investigated (Long *et al*., 2007; Wang *et al*., 2008b; Miao *et al*., 2010). For instance, the family Eurystomatellidae consists of only two monotypic genera (Fan *et al*., 2009; Miao *et al*., 2010; Zhang *et al*., 2019), while the genus *Schizocalyptra* comprises five species (Dragesco, 1968; Fernandez-Leborans and Novillo, 1994; Long *et al*., 2007; Wang *et al*., 2008b).

Materials and Methods

Three coastal water samples were collected and examined. The locality information, such as coordinates (longitude, latitude), salinity, and temperature, is described in the 'Material examined' section for each species. These samples were transferred to the laboratory and cultured in Petri dishes at ~20°C. In each dish, two or three wheat grains were added to increase bacteria and fungi as food sources.

The protargol-impregnated cells were examined under an optical microscope (Olympus BX53, Japan) and photomicrographs were taken using a digital camera. To prepare protargol slides, <10 mL of the culture water from the Petri dish was fixed using Bouin's fluid and washed 3−5 times using centrifugation. The protargol was synthesized using the method of Pan *et al*. (2013) with slight modification (Kim and Jung, 2017). These concentrated cells were impregnated using the 'procedure A' method

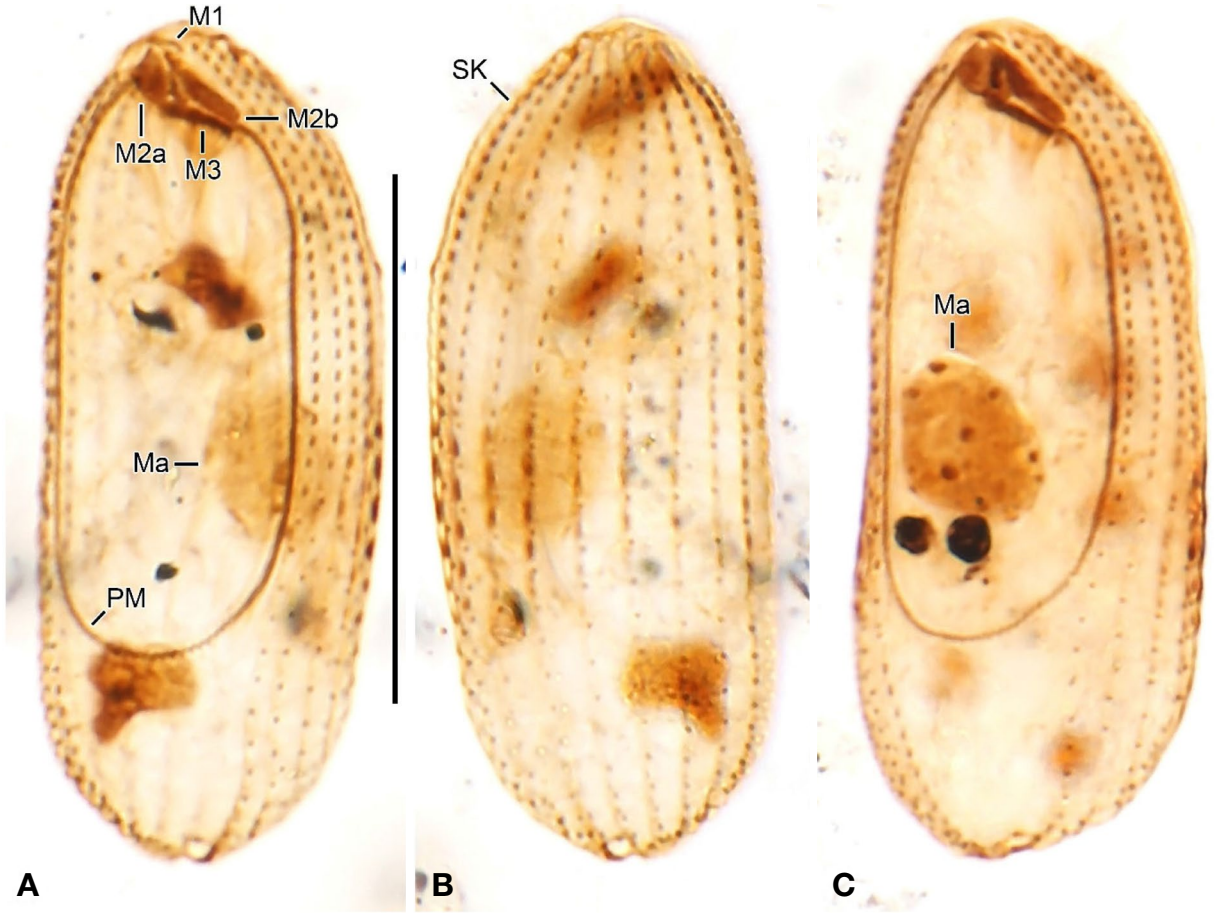


Fig. 1. *Eurystomatella sinica* after protargol impregnation. A, C. Ventral views showing infraciliature and macronucleus. B. Dorsal view showing somatic kineties. Ma, macronucleus; M1, M2a, M2b, M3, oral membranelles; PM, paroral membrane; SK, somatic kineties. Scale bar = 30 μm.

(Foissner, 2014). The differential through-focal images of the protargol-impregnated cells were merged using the software of Helicon Focus 7.6.4 (HeliconSoft Ltd, Ukraine). The general classification follows Lynn (2008).

Results and Discussion

Class Oligohymenophorea de Puytorac *et al*., 1974
Subclass Scuticociliatia Small, 1967
Order Pleuronematida Fauré-Fremiet in Corliss, 1956
Family Eurystomatellidae Miao *et al*., 2010
Genus *Eurystomatella* Miao *et al*., 2010

1. *Eurystomatella sinica* Miao *et al*., 2010 (Fig. 1)

Material examined. Coastal water sample (25.4‰, 21.0°C) collected from Yeosu-si, Jeollanam-do, Republic of Korea (34°2′50″N, 127°17′33″E) on 10 July 2019.
Diagnosis (n = 12). Cell size 42.5−51.7 × 16.5−20.1 μm (47.8 × 18.5 μm on average) in protargol preparations, outline elongated oval with slightly concave right margin, dorsoventrally flattened; 1 elliptical macronucleus in mid-body, 7.9−10.8 × 6.5−9.0 μm (9.9 × 7.9 μm on average) in size; micronucleus not observed; invariably 15 somatic kineties, prolonged caudal cilia; buccal area occupying 69.4−78.8% (72.6% on average) of body length; paroral membrane conspicuous, forms an elliptical ring with anterior opening closed by membranelles 1−3; membranelle 1 (M1) 2-rowed; right membranelle 2 (M2a) about 7−9-rowed; left membranelle 2 (M2b) 3−5-rowed; membranelle 3 (M3) 2- or 3-rowed (it should be noted that these ciliary rows on the membranelles 2 and 3 are arranged too closely, that is the numbers are approximate).
Distribution. China (Miao *et al*., 2010) and Korea (this study).
Remarks. The genus *Eurystomatella* is monotypic (type species: *E. sinica* by original designation) (Miao *et al*., 2010). The Korean population corresponds very well with the type population, but the former shows slightly smaller

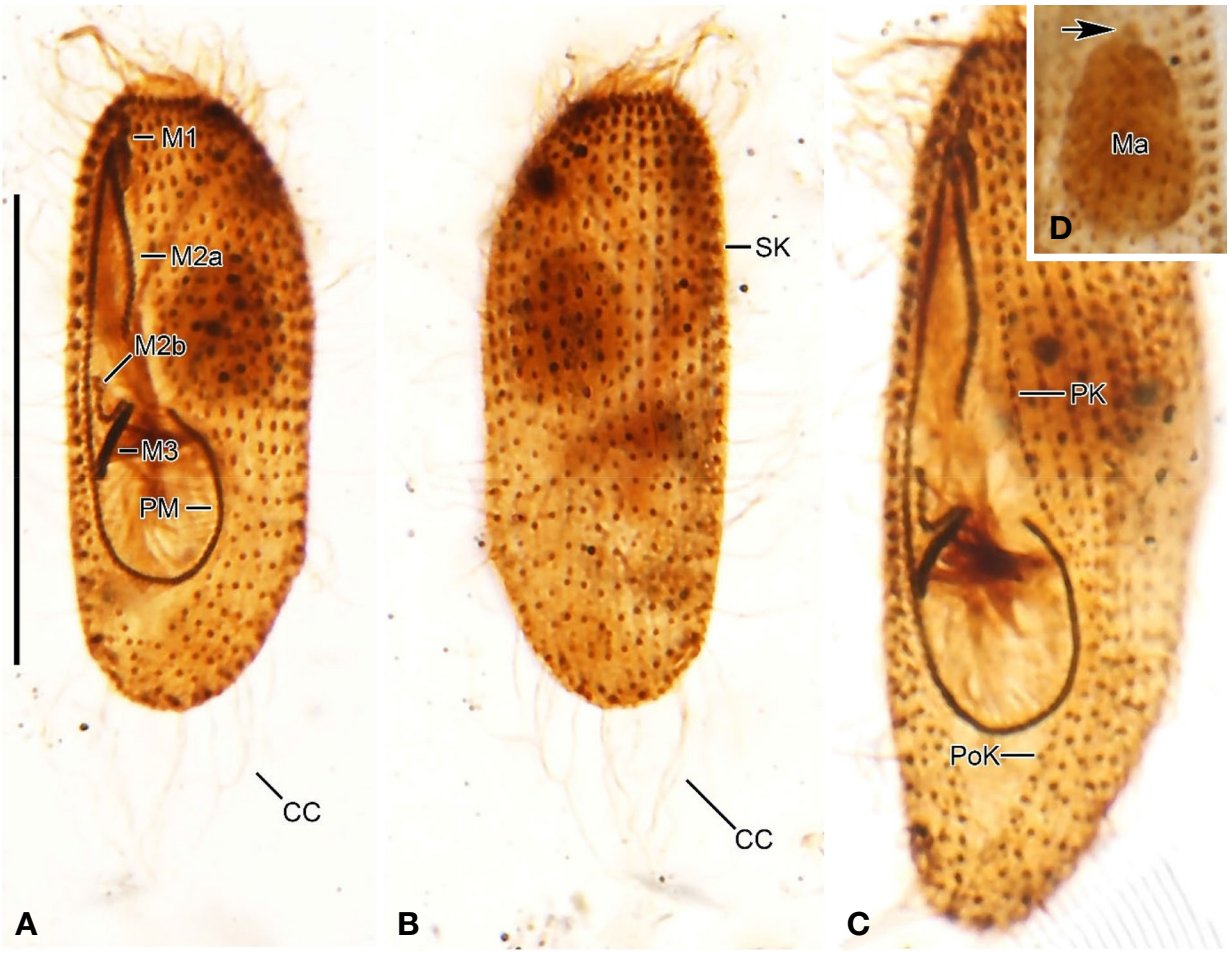


Fig. 2. *Pleuronema grolierei* after protargol impregnation. A, C. Ventral views showing a typical body shape and infraciliature. B. Dorsal view showing somatic kineties. D. Nuclear apparatus, arrow denotes a micronucleus. CC, caudal cilia; Ma, macronucleus; M1, 3, membranelles 1, 3; M2a, anterior part of membranelle 2; M2b, posterior part of membranelle 2; PM, paroral membrane; PK; preoral kineties; PoK, postoral kinety; SK, somatic kineties. Scale bar = 30 μm.

body size (on average, 47.8 × 18.5 μm vs. 57.5 × 34.1 μm in protargol preparations) (Miao *et al*., 2010). The difference may be due to the impregnation method or habitat condition (Foissner, 2014).

Voucher slides. Three slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (GUC002230–2232).

Family Pleuronematidae Kent, 1881
Genus *Pleuronema* Dujardin, 1841

2. *Pleuronema grolierei* Wang *et al*., 2008 (Fig. 2)

Material examined. Coastal water sample (25.4‰, 21.0°C) collected from Yeosu-si, Jeollanam-do, Republic of Korea (34°2′50″N, 127°17′33″E) on 10 July 2019; coastal water sample (33.5‰, 14.5°C) collected from Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°3′2.9″N, 129°22′22.3″E) on 23 November 2020.

Diagnosis (n = 5). Cell size 40.2–53.2 × 15.8–19.6 μm (47.2 × 17.7 μm on average) in protargol preparations, outline slightly oval to spindle-like, dorsoventrally flattened; 1 globular to elliptical macronucleus, 7.8–12.1 × 7.7–9.7 μm (10.5 × 8.3 μm on average) in size; 1 globular micronucleus attached to macronucleus; 1 or 2 preoral kineties (note that 1 out of 5 specimens showed 2 preoral kineties and 1 postoral kinety might have resulted from a fragmentation of somatic kinety 1), 24–26 somatic kineties, prolonged caudal cilia; buccal area occupying 67.1–74.4% (70.4% on average) of body length; paroral membrane conspicuous, hook or ‘6’-shaped; M1 three-rowed; M1:M2a length ratio about 40%, M2a more or less straight without conspicuous structure at posterior end (i.e., ring-like, hook-like structure), 12.0–16.4 μm in length; M2b V-shaped; M3 two-rowed.

Distribution. China (Wang *et al*., 2008a; Pan *et al*., 2015b)

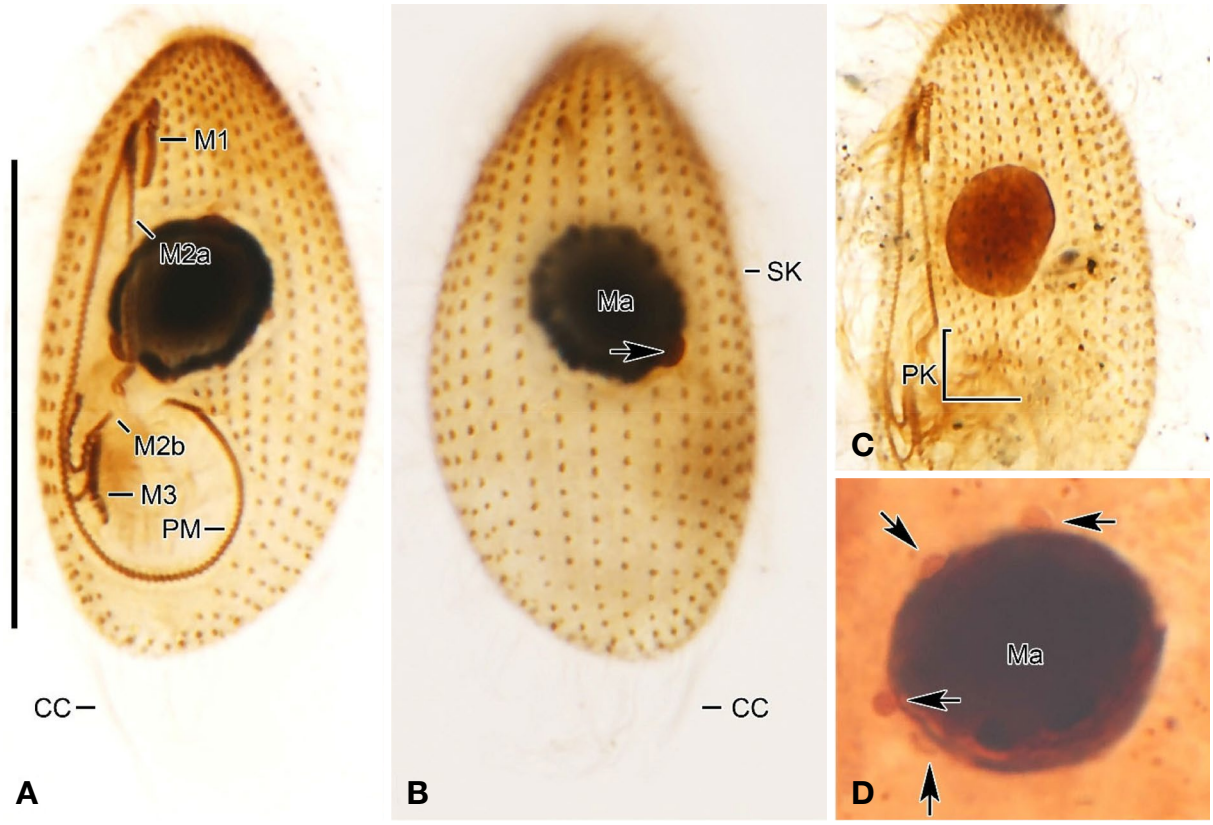


Fig. 3. *Pleuronema setigerum* after protargol impregnation. A, C, D. Ventral views showing infraciliature and nuclear apparatus, arrows denote micronuclei. B. Dorsal view showing somatic kineties and nuclear apparatus, arrow denotes a micronucleus. CC, caudal cilia; Ma, macronucleus; M1, 3, membranelles 1, 3; M2a, anterior part of membranelle 2; M2b, posterior part of membranelle 2; PM, paroral membrane; PK; preoral kineties; SK, somatic kineties. Scale bar = 30 μm.

and Korea (this study).

Remarks. *Pleuronema grolierei* resembles *P. arenicola* Dragesco, 1960, but the former can be distinguished by the inconspicuous paroral membrane (vs. conspicuous), one preoral kinety (vs. 3), and completely two-rowed M2a (vs. two-rowed only in posterior part) (Dragesco, 1960; Wang *et al*., 2008a; Pan *et al*., 2015b). The Korean population differs from the Chinese population (type population) by body length (40.2−53.2 μm vs. 56−71 μm), but this size variation was also observed in *Pleuronema* species (Borror, 1963; Dragesco and Dragesco-Kernéis, 1986; Agatha *et al*., 1993; Song, 2000).

Voucher slides. Three slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (GUC002230−2231, Yeosu population) and the National Institute of Biological Resources, Korea (NIBRPR0000110213, Pohang population).

3. *Pleuronema setigerum* Calkins, 1902 (Fig. 3)

Material examined. Coastal water sample (25.4‰, 21.0°C) collected from Yeosu-si, Jeollanam-do, Republic of Korea (34°2′50″N, 127°17′33″E) on 10 July 2019; coastal water sample (34.4‰, 14.4°C) collected from Gangneung-si, Gangwon-do, Republic of Korea (37°47′55″N, 128°54′59″E) on 16 November 2020.

Diagnosis (n = 8). Cell size 38.1−55.2 × 15.6−33.6 μm (45.5 × 21.0 μm on average) in protargol preparations, outline oval, dorsoventrally flattened; 1 globular macronucleus, 9.1−15.0 × 6.9−13.3 μm (10.7 × 8.7 μm on average) in size; 1−4 micronuclei attached to macronucleus, elliptical to circular; 5 or 6 preoral kineties (note that 1 out of 8 specimens showed 6 preoral kineties and 1 postoral kinety might have resulted from a fragmentation of somatic kinety 1), 20−23 somatic kineties, prolonged caudal cilia; buccal area occupying 58.3−83.8% (73.9% on average) of body length; paroral membrane conspicuous, hook or '6'-shaped; M1 three-rowed, M1:M2a length ratio about 33%; posterior end of M2a ring-like arrangement; M2b V-shaped; M3 three-rowed.

Distribution. USA (Calkins, 1902; Borror, 1963), Europe (Kahl, 1931), China (Pan *et al*., 2010; Pan *et al*., 2015b), and Korea (this study).

Remarks. More than 20 species have been reported in the

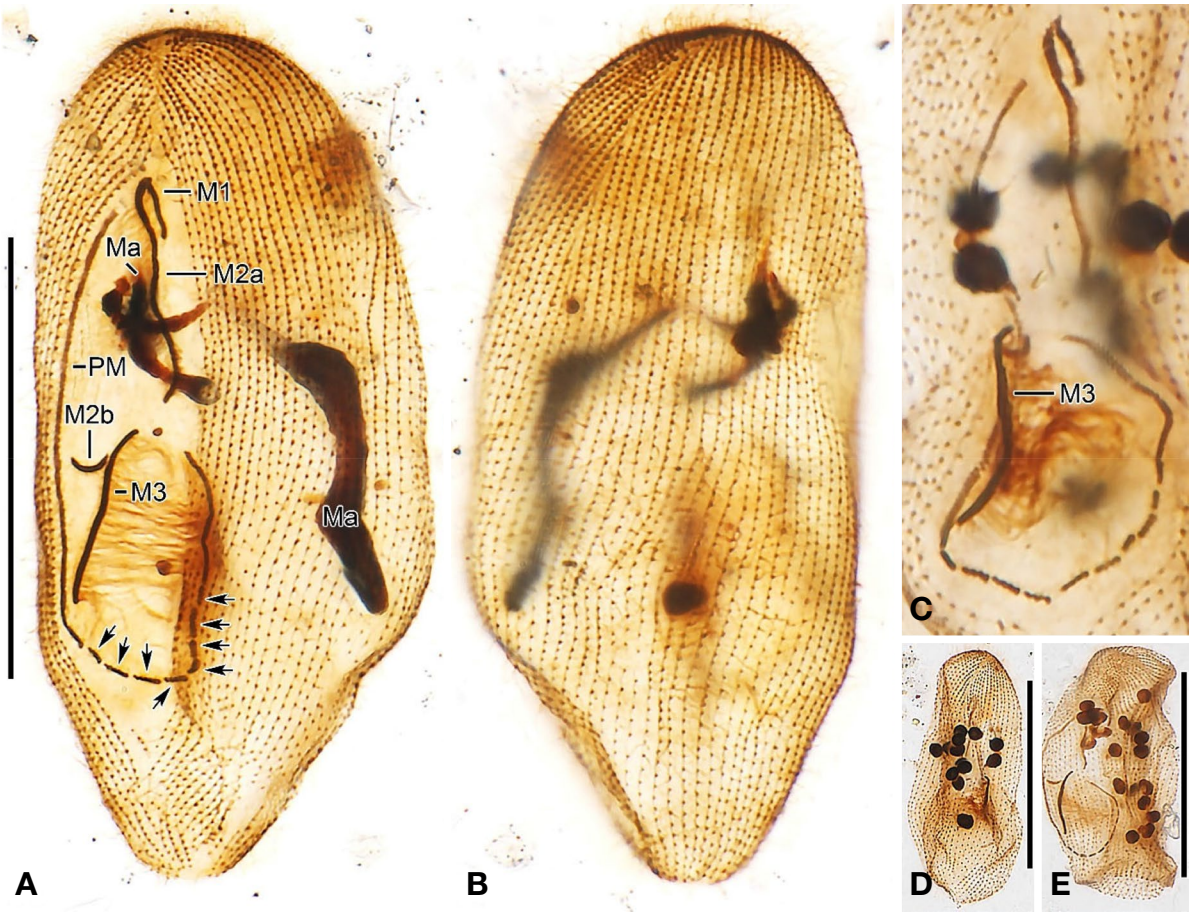


Fig. 4. *Schizocalyptra aeschtae* after protargol impregnation. A, B. Ventral and dorsal view showing a typical morphology arrows denote fragments of paroral membrane. Note the band-like structure of the macronuclear nodules. C. ventral view of the oral apparatus, showing membranelle 3 consisting of three ciliary rows. D, E. Macronuclear nodules. Ma, macronucleus; M1, 3, membranelles 1, 3; M2a, anterior part of membranelle 2; M2b, posterior part of membranelle 2; PM, paroral membrane. Scale bars = 50 μm.

genus *Pleuronema* (Wang *et al*., 2009; Pan *et al*., 2016). Of the congeners, *P. setigerum* can be characterized by the ring-like bosal body arrangement at the posterior end of M2a (Pan *et al*., 2010). *Pleuronema setigerum* highly resembles *P. paucisaetosum* Pan *et al*., 2015 by the number of somatic kineties, oral apparatus, and macronucleus (Pan *et al*., 2015a). According to Pan *et al*. (2015a), *P. setigerum* differs from *P. paucisaetosum* by the structure of M2a (ring-like vs. hook-like posterior end; zigzag vs. one-rowed basal body pattern in middle portion).

Voucher slides. Five slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (GUC002230−2231, 2251−2252, Yeosu population) and the National Institute of Biological Resources, Korea (NIBRPR0000110225, Gangneung population).

Genus *Schizocalyptra* Dragesco, 1968

4. *Schizocalyptra aeschtae* Long *et al*., 2007 (Fig. 4)

Material examined. Coastal water sample (25.4‰, 21.0°C) collected from Yeosu-si, Jeollanam-do, Republic of Korea (34°2′50″N, 127°17′33″E) on 10 July 2019.

Diagnosis (n = 9). Cell size 55.6−118.0 × 30.3−55.3 μm (83.4 × 43.2 μm on average) in protargol preparations, outline slightly oval to elliptical, dorsoventrally flattened; macronuclear nodules highly variable from 1 band-like to 21 spherical nodules, 54−70 somatic kineties; buccal area occupying 54.0−69.2% (59.7% on average) of body length; paroral membrane conspicuous, hook or '6'-shaped, with 7−10 fragments at buccal end; M1 two-rowed; M2a : M3 length ratio about 1 : 1.0−1.6 (1 : 1.4 on average), M2a more and less straight without conspicuous structure at posterior end (i.e., ring-like, hook-like structure), 14.2−32.0 μm in length; M2b slightly curved; M3 two- or three-rowed.

Distribution. China (Long *et al*., 2007) and Korea (this

study).

Remarks. The genus *Schizocalyptra* consists of five species (Wang *et al*., 2008b). They are morphologically very similar to *Pleuronema in vivo*, but clearly distinguishable by the fragmented paroral membrane (vs. non-fragmented) (Dragesco, 1968). *Schizocalyptra aeschtae* highly resembles *S. similis* Wang *et al*., 2008 and *S. sinica* Wang *et al*., 2008 in terms of the body size and the number of somatic kineties (Wang *et al*., 2008b). *Schizocalyptra similis* differs from *S. aeschtae* by the presence (vs. absence) of prolonged somatic cilia (Long *et al*., 2007; Wang *et al*., 2008b). *Schizocalyptra sinica* differs from *S. aeschtae* by the presence (vs. absence) of prolonged somatic cilia and the number of fragments in the PM (12−15 vs. 6−12) (Wang *et al*., 2008b). Wang *et al*. (2008b) re-investigated the type slides of *S. aeschtae* and mentioned that the species might have two or three kinety rows in M3, not just two, as reported by Long *et al*. (2007).

Voucher slides. Three slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (GUC002230−2232).

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