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New records of two pleurostomatids (Ciliophora: Litostomatea) from Korea

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Two pleurostomatid ciliates, *Amphileptus litonotiformis* Song, 1991 and *Kentrophyllum setigerum* (Quennerstedt, 1867), were collected from the coastal waters of Korea. Their morphologies are described based on live observation and protargol impregnation, and their diagnoses and morphometrics are provided. Additionally, sequence information of small subunit ribosomal RNA obtained from two species were reported.

Keywords: *Amphileptus litonotiformis*, East Sea, *Kentrophyllum setigerum*, Korea, Pleurostomatida, Yellow Sea

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Introduction

Free living pleurostomatids are characterized by leaf-like body shape and cytostome on the lateral side, and about 200 morphospecies have been described (Wu *et al*., 2015). They were confirmed the monophyletic taxon with molecular phylogenetic studies, and classified into 12 genera belonging to three families by the differences of perioral kineties, somatic ciliature, distribution of extrusomes, and status of suture, etc. (Lynn and Small, 2002; Lynn, 2008; Song *et al*., 2009; Wu *et al*., 2015).

In the present study, two species belonging to unreported genera of pleurostomatids, *Amphileptus* Ehrenberg, 1830 and *Kentrophyllum* Petz, Song et Wilbert, 1995, in Korea were described and illustrated morphological characteristics using live observation and protargol impregnation. Additionally, the accession numbers of their SSU rDNA sequences were reported.

Materials and Methods

Samples were collected from coastal areas of Korea using PEF-S (Xu *et al*., 2009). They were transferred to a laboratory and then continually maintained in petri dishes at 17°C (light-dark 12 : 12 cycles) with rice grains as a food source to enhance bacterial growth. Isolations, microscopical observations, and staining were according to Kim and Min (2015). Terminologies follow Lynn (2008). Abbreviations were as follows: CV, contractile vacuole; DB, dorsal brush kinety; Ex, extrusome; Fu, furrow; LSK, left somatic kinety; Ma, macronuclear nodule; Mi, micronucleus; Nd, nematodesmata; PK, perioral kinety; RSK, right somatic kinety.

Isolation of single cell, DNA extraction, amplification of the SSU rDNA, and sequencing were performed according to Kim and Min (2009). Sequences were aligned using CulstalX (Thompson *et al*., 1997) and further modified manually using Bioedit 7.2 (Hall, 1999). The new sequences were submitted on the database of GenBank, and then their accession numbers were received.

Results

Phylum Ciliophora Doflein, 1901
Class Litostomatea Small and Lynn, 1981
Subclass Haptoria Corliss, 1974
Order Pleurostomatida Schewiakoff, 1896
Family Amphileptidae Bütschli, 1889
Genus *Amphileptus* Ehrenberg, 1830
연리지섬모충속

***Amphileptus litonotiformis* Song, 1991**
해변연리지섬모충 (Table 1, Fig. 1)
Amphileptus litonotiformis Song, 1991, p. 300, Figs. 1, 2.

Material examined. Specimens were collected from the Incheon public waterfront (37°26′N and 126°35′E), Incheon in the Yellow Sea on 21-29 Jul 2007.
Description. Live cell size 150-225 × 50-75 μm, rugby ball-shaped in outline, beak-like anterior end bent toward dorsal side; body flexible and contractile; laterally compressed at a ratio of about 2 : 1; conspicuous longitudinal Fu on right side (Fig. 1A, B, E-H).

Two Ma connected, leaned on dorsal side of body center, usually detectable in live specimens; each nodule egg-shaped, about 22 × 18 μm after fixation; Mi not observed (Fig. 1H). One CV recognized at posterior end (Fig. 1G).

Ex bar-shaped, slightly curved, about 10 μm long; recognizable in live cells under optimal conditions; distributed in front of oral region; some scattered along Nd (Fig. 1B, I).

Cytoplasm slightly grayish colored, with many brown globules and particles; cortical granules detected on pellicle, densely placed between rows of RSK (Fig. 1E, G). Suture formed on anterior region of right side (Fig. 1D, F). Cirri and membranelles of feeding hypotrichs detected after fixation (Fig. 1J). Movement by slow gliding, flexible crawling along substrates, or swimming with slow rotation.

Ciliary pattern typical of *Amphileptus* (Fig. 1C, D, F, G). Two PK placed around oral slit, PK1 on left, and PK2 on right; kinetid rows more tightly packed than somatic kineties; PK1 contains dense dikinetids in anterior 1/2, and a continuous row of monokinetids reaching to posterior end; PK2 composed of dikinetids, reaches down posterior. DB situated on dorsolateral area, with dense dikinetids in anterior 2/3, and a continuous row of monokinetids, reaches to posterior end. All somatic kineties composed of monokinetids; 10-11 LSK, with PK1 and DB; 20-21 RSK, including PK2. Nd well developed.
Distribution. China and Korea (this study).
Deposition. A voucher slide with protargol-impregnated specimens was deposited in the National Institute of Biological Resources in Korea (NIBRPR0000107165).
Accession number of SSU rDNA. KX581241-KX581242.
Remarks. *Amphileptus litonotiformis* was originally described by Song (1991). Characteristics of the Korean population correspond very well with the Chinese population (Song, 1991) in most respects, such as habitat, body shape and size, the number of nuclei, contractile vacuoles, sutures, somatic kineties. However, the Korean population slightly differs from the Chinese population in the distribution of extrusomes (1/2 of oral region vs. whole oral region).

Family Kentrophyllidae Wu *et al*., 2015
Genus *Kentrophyllum* Petz, Song et Wilbert, 1995
가시나뭇잎섬모충속

Table 1. Morphological characterization of *Amphileptus litonotiformis* (1st line) and *Kentrophyllum setigerum* (2nd line) from protargol-impregnated specimens.

Characters	Min.	Max.	Mean	SD	SE	CV	n
Body length	100	275	172.50	45.39	10.15	26.31	20
	79	160	107.76	25.39	6.16	23.56	17
Body width	50	87.5	64.75	10.09	2.26	15.59	20
	30	63	43.71	8.82	2.14	20.19	17
Length of Nd	80	160	107.44	22.48	5.03	20.93	20
	55	115	76.29	17.20	4.60	22.55	14
Number of LSK[a]	10	11	10.42	0.51	0.15	4.94	12
	29	32	31.07	1.00	0.27	3.21	14
Number of RSK[b]	20	21	20.75	0.44	0.10	2.14	20
	34	40	35.88	2.10	0.74	5.85	8
Number of Ma	2	2	2	0	0	0	20
	4	11	6.24	1.75	0.42	28.08	17
Length of Ma	14.4	32	21.96	5.29	1.18	24.09	20
	5	15	9.82	2.88	0.70	29.29	17
Width of Ma	14.4	25.6	18.48	3.18	0.71	17.20	20
	5	11	7.06	1.95	0.47	27.65	17
Length of the Ex	4.8	12.8	9.90	1.82	0.41	18.36	20
	10	14	11.71	1.26	0.31	10.79	17

All measurement in μm. [a] PK1 and DB included. [b] PK2 included.
CV, coefficient of variation in %; Max. maximum; Min, minimum; n, sample size; SD, standard deviation.

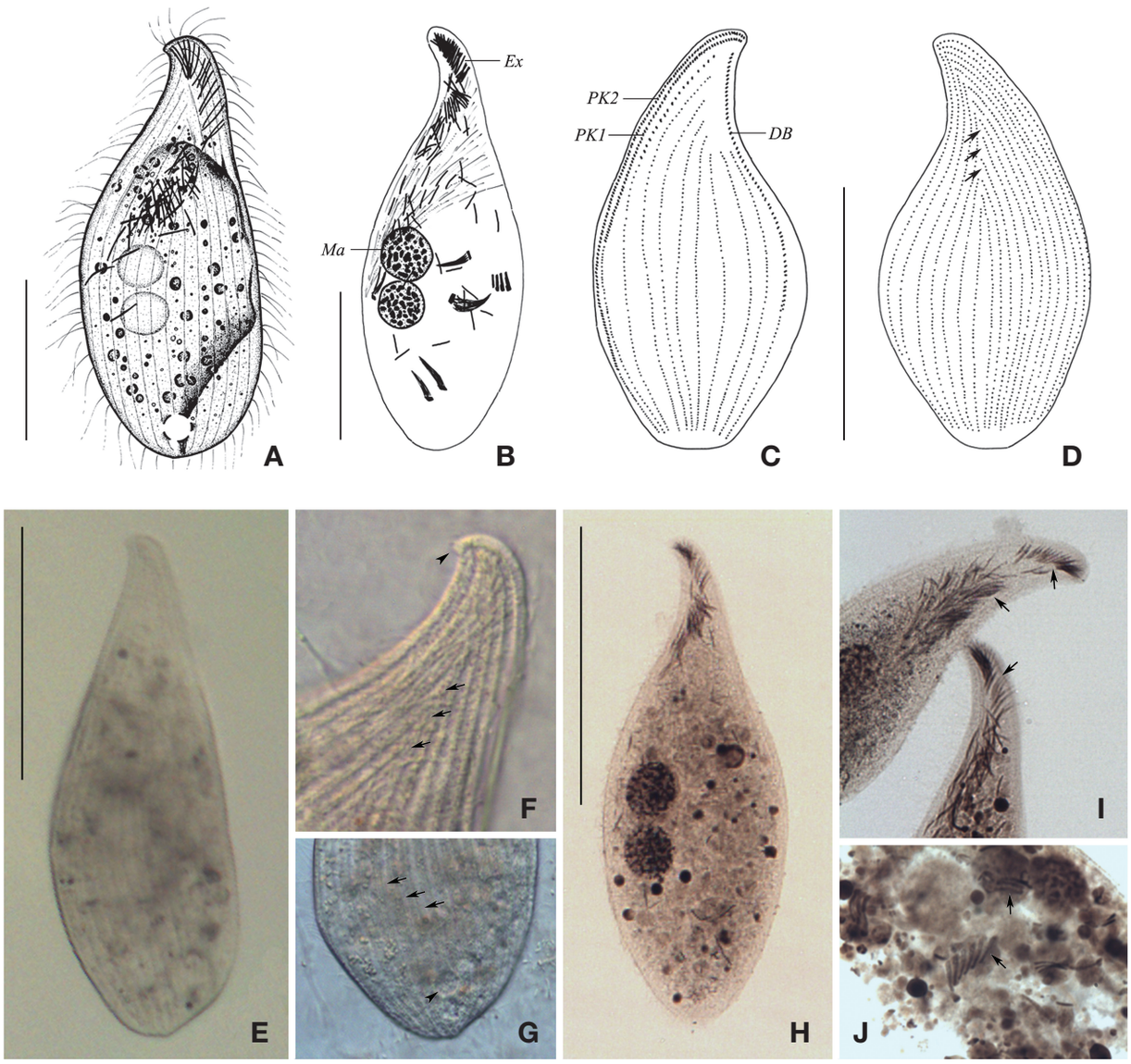

Fig. 1. Morphology of *Amphileptus litonotiformis* from live (A, E-G) and protargol-impregnated (B-D, H-J) specimens. A, E, Appearance of a typical live individual; B, H, Nuclei, extrusomes, and nematodesmata with protargol-impregnation. C, D, Ciliary pattern after protargol-impregnation, left (C) and right (D) sides; F, Right view of live specimen, beak-like anterior region (arrowhead) and suture (arrows); G, Posterior region, contractile vacuole (arrowhead) and somatic kineties (arrows); I, Anterior region, extrusomes (arrows); J, Inside body, membranelles of feeding hypotrichs (arrows). Scale bars: 100 μm.

***Kentrophyllum setigerum* (Quennerstedt, 1867)**
강모가시나뭇잎섬모충 (Table 1, Fig. 2)

Loxophyllum setigerum Quennerstedt, 1867, p. 6, Pl. I 4.

Loxophyllum verrucosum Dragesco, 1965, p. 373, Fig. 12.

Kentrophyllum setigerum Petz *et al*., 1995, p. 55; Wu *et al*., 2015, p. 11.

Material examined. Specimens were collected from the Bongpo-port (38°17′N, 128°33′E), Gangwon-do in the East Sea on 12-13 Mar 2008. Environmental conditions of the sampling site were 10.6°C, *ca* 14.8 psu, and pH *ca* 8.55.

Description. Live cell size 150-200 × 60-80 μm, wide and flat leaf-shaped in outline; laterally compressed; many longitudinal shallow grooves appeared (Fig. 2A, E-G). Spines thin, needle-shaped, usually detectable in

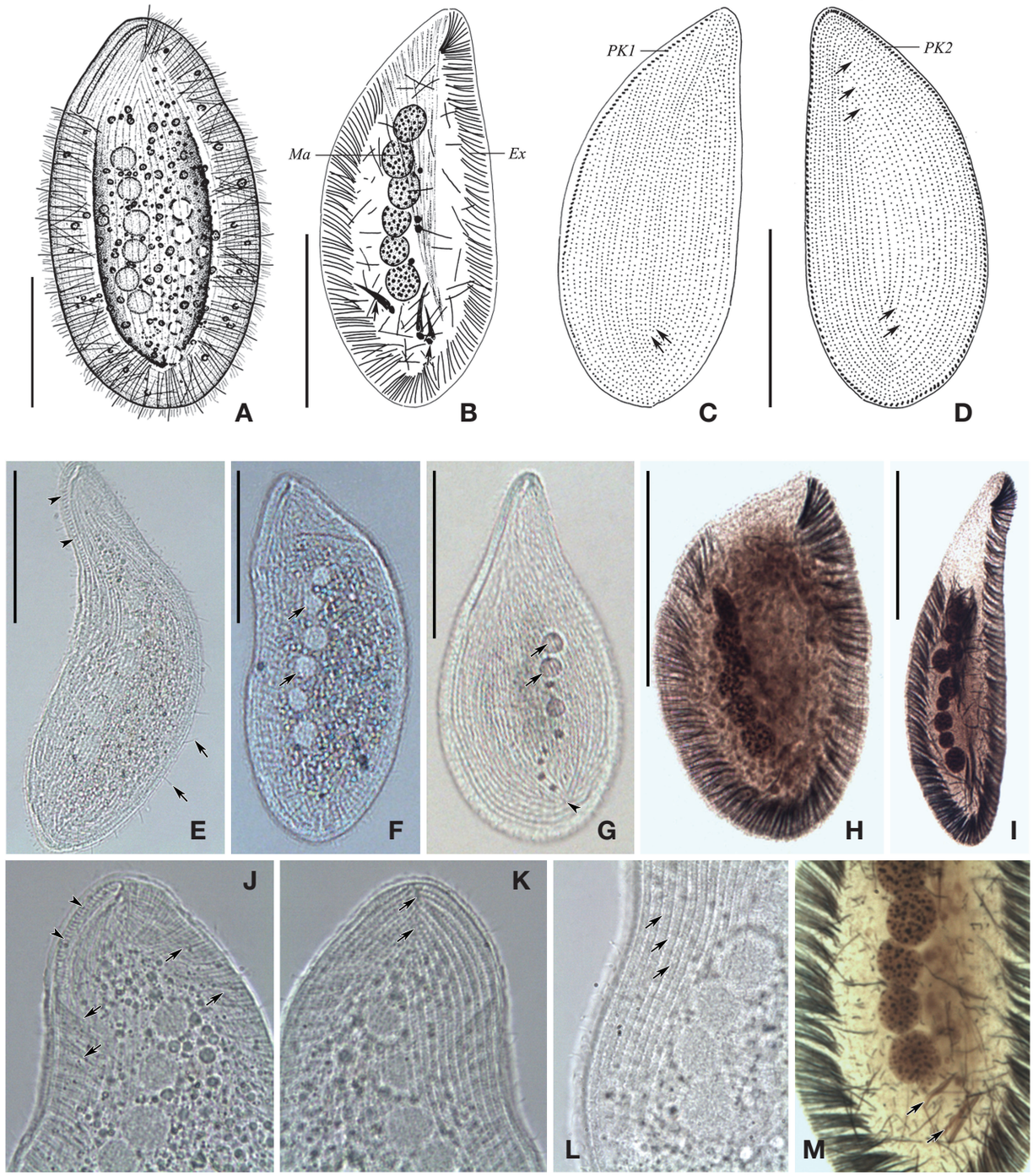

Fig. 2. Morphology of *Kentrophyllum setigerum* from live (A, E-G, J-L) and protargol-impregnated (B-D, H, I, M) specimens. A, E-G, Appearance of live individuals, peribuccal papillae (arrowheads in E), spines (arrows in E), nuclei (arrows in F), suture (arrowhead in G), contractile vacuoles (arrows in G); B, H, I, Nuclei, extrusomes, and nematodesmata with protargol-impregnation. C, D, Ciliary pattern after protargol-impregnation, left (C) and right (D) sides; J, K, Anterior region, peribuccal papillae (arrowheads in J), extrusomes (arrows in J), suture (arrows in K); L, somatic kineties (arrows); M, Inside body, cirri of feeding hypotrichs (arrows). Scale bars = 50 μm (A-D), 100 μm (E-I).

live specimens, evenly spaced along body margin, except cytostome region; invisible after protargol staining (Fig. 2E). Peribuccal papillae placed along cytostome, conspicuous, 40-56 μm long in live specimens (Fig. 2E, J).

Four to eleven Ma connected by thread-like funiculus, leaned on ventral side of body center, usually detectable in live specimens; each nodule egg-shaped, about 10 × 7 μm after fixation; Mi not observed (Fig. 1F, H, I, M). 6-9 CVs recognized along dorsal margin, usually 10-13 μm in diameter (Fig. 1G).

Ex bar-shaped, curved, about 12 μm long; recognizable in live cells; densely distributed along whole body, except cytostome region; some scattered inside body (Fig. 2H-J, M).

Cytoplasm slightly grayish colored, with many large and shining globules (Fig. 2E, F, J). Sutures formed on right and left sides (Fig. 2C, D, G, K). Cirri and membranelles of feeding hypotrichs detected after fixation (Fig. 2M). Movement by slow gliding, or swimming.

Ciliary pattern typical of *Kentrophyllum* (Fig. 2C, D, L). Two PK placed around oral slit, PK1 on left, and PK2 on right; kinetid rows more tightly packed than somatic kineties; PK1 contains dense dikinetids in anterior 2/3, and a continuous row of monokinetids, reaches to posterior end; PK2 composed of dikinetids, enclosed full-body, formed a circle. DB not detected. All somatic kineties composed of monokinetids; 29-32 LSK, with PK1; 34-40 RSK, including PK2. Nd stretches to 4/5 of the cytoplasm region.

Distribution. USA, Japan, Africa, and Korea (This study).

Deposition. A voucher slide with protargol-impregnated specimens was deposited in the National Institute of Biological Resources in Korea (NIBRPR0000107166).

Accession number of SSU rDNA. KX581243-KX 581245.

Remarks. *Kentrophyllum setigerum* was originally described by Quennerstedt (1867). After that, it had been an ambiguous status with its congeners, *K. pseudosetigerum*, *K. qingdaoense*, *K. verrucosum*. Recently, the genus *Kentrophyllum* was revised by Wu *et al.* (2015) and then it have been improved the definition of *K. setigerum*. Characteristics of the Korean population correspond very well with the African population (Dragesco, 1965) synonymized by Wu *et al.* (2015) in most respects, such as habitat, body shape and size, status of spines and peribuccal papillae, and the number of nuclei, contractile vacuoles and somatic kineties.

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