

Taxonomic Study of Poorly-known Marine Pleurostomatid Ciliates of *Litonotus paracygnus* and *L. pictus* (Ciliophora: Pleurostomatida) from Korea

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ABSTRACT

Two poorly known and often confused pleurostomatid ciliates, *Litonotus paracygnus* Song, 1994 and *L. pictus* Gruber, 1884, were collected from the coastal waters of Yeonggeumjeong and Bongpo-port, Gangwon-do in the East Sea and from the Iwon tide embankment near Ganwol-do, Chungcheongnam-do in the Yellow Sea, Korea. These species were described based on live observations, the protargol-impregnation and morphometrics of the species. Also provided are their diagnoses. The small subunit ribosomal DNA (SSU rDNA) sequences of these species were compared with previously known sequences of related species. The diagnostics of the two *Litonotus* species are as follows. *L. paracygnus*: 150-300 μm long in vivo, strongly contractile neck region, two ellipsoid macronuclei (Ma) and one micronucleus (Mi), 7 left (LSK) and 11-14 right somatic kineties (RSK), 2-4 contractile vacuoles (CV) located on the posterior end, extrusomes (Ex) distributed on the anterior region of the ventral margin only. *L. pictus*: about 200-600 μm long in vivo, extremely contractile, beautiful body color with rows of yellow to yellow-brownish cortical pigment granules, 12-21 Ma arranged in moniliform pattern, infrequently vermiform, 7-11 LSK and 18-26 RSK, several CV located on both margins, Ex distributed on the anterior region of the ventral margin only. In this study, this genus was firstly recorded in Korea.

Key words: pleurostomatid ciliates, *Litonotus paracygnus*, *Litonotus pictus*, SSU rDNA, Korea

INTRODUCTION

Free living pleurostomatids are ciliates of medium to large size that are commonly distributed in various habitats around the world (Kahl, 1931; Borror, 1963; Dragesco, 1966; Fryd-Versavel et al., 1975; Foissner, 1984; Li, 1990; Song, 1994; Lin and Song, 2004; Lin et al., 2005). Marine pleurostomatids are characterized as voracious predators of flagellates, other ciliates, and even small metazoans. However, previous studies of these organisms have led to many misidentifications due to their simple and similar morphological characteristics (Song and Wilbert, 1989; Song, 1993; Foissner and Leipe, 1995; Petz et al., 1995; Lin et al., 2005; Lin et al. 2008). Recently, to resolve taxonomic limitations dependent on morphological characteristics, taxonomists have come to accept the notion that DNA sequences may represent the valuable taxonomic tools (Prescott, 1994; Tautz et al., 2003; DeSalle et al., 2005; Barth et al., 2006; Chantangsi et al., 2007; Kim et al., 2007). So far in Korea, taxonomic study of pleurostomatids has only been performed on three species: *Loxophyllum meleagris* Müller, 1773 and *Siroloxo-*

phyllum utriculariae Penard, 1922 by Lee et al. (2006); and *Loxophyllum chaetonotum* Borror, 1965 by Gong et al. (2007a).

Litonotus is one of the largest genera of pleurostomatids, with over 50 morphotypes (Khal, 1931; Foissner, 1984; Song and Wilbert, 1989; Song, 1994; Lin et al., 2008). However, *Litonotus* has not been recorded in Korea. In this study, we redescribe two *Litonotus* species in an effort to mitigate the confusion of their identification as caused by the absence of literature describing staining methods that show the infraciliature. In order to solve taxonomic ambiguities within these ciliates, the necessities of morphological reinvestigations and the use of molecular data are recognized. Detailed morphological descriptions and genetic characteristics down to the small subunit ribosomal DNA (SSU rDNA) sequences of these organisms isolated from coastal areas in Korea were carried out.

MATERIALS AND METHODS

Morphological taxonomy

Samples were collected from three sites of costal areas in Korea using PEF-S (Xu et al., 2009) and the natural slides.

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They were then transferred to a laboratory. In successive steps, cultures were maintained at 17°C in a chamber at a light and dark photoperiod of 12 : 12 h. Rice grains were used to enrich bacterial growth in the cultures. Specimens were observed using phase contrast and differential interference microscopes at different magnifications. The protargol-impregnation technique (Foissner, 1991) was applied to reveal the infraciliature of the ciliates. Enumeration and measurements of silvered specimens were performed under 1000× magnification (Leica DM2500). Drawing of the impregnated specimens was made with a camera lucida. The classification scheme used here was based on Lynn (2008). Terminologies followed Corliss (1979) and Lynn (2008). In addition, new terminology for pleurostomatid ciliates followed Lin et al. (2005). Abbreviations were CV, contractile vacuole; DB, dorsal brush kinety; DK, dikinetosomes; Ex, extrusomes; LSK, left somatic kinety; Ma, macronuclear nodules; Mi, micronucleus; Nd, nematodesmata; PK, perioral kinety; and RSK, right somatic kinety.

Molecular taxonomy

Each living individual ciliate was isolated using a micropipette under a dissecting microscope (Leica MZ 12.5) and was then transferred to a sterile seawater droplet on a slide glass. The cleaned single cell was transferred into 1.5 mL microcentrifuge tubes. After three freeze-thaw cycles (−20 to 20°C), DNA extraction, amplification of the complete SSU rDNA and sequencing were performed according to Gong et al. (2007b). Nucleotide diversity among the species was calculated by the MEGA 4.0 program (Tamura et al., 2007) using the Kimura 2-parameter model.

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Class Litostomatea Small and Lynn, 1981
Subclass Haptoria Corliss, 1974
Order Pleurostomatida Schewiakoff, 1896
Family Litonotidae Kent, 1882
Genus ^{1*}*Litonotus* Wresniowski, 1870

1. ^{2*}*Litonotus paracygnus* Song, 1994 (Figs. 1, 2 and Table 1)

Litonotus paracygnus Song, 1994, p. 131, Figs. 1-11.

Material examined. Ciliates collected from the coastal waters of Yeonggeumjeong (38°12'N and 128°36'E) and Bongpo-port (38°17'N and 128°33'E), Gangwon-do in the East Sea

of the Republic of Korea on 18 Apr. 2008 (Yeonggeumjeong, 15.2°C, ca 23.2 psu, pH ca 8.5; Bongpo-port, 14.1°C, ca 23.0 psu, pH ca 8.3).

Diagnosis. *Litonotus* about 150-300 µm long in vivo, spindle-like body, strongly contractile neck region; 2 ellipsoid macronuclei and 1 micronucleus; 7 left and 11-14 right somatic kineties; 2-4 contractile vacuoles located on the posterior end; extrusomes bar-shaped, distributed on the anterior region of the ventral margin only.

Redescription. Body size variable, about 150-300 × 40-60 µm in vivo. Cells spindle-shaped; high contraction and elongation of the anterior region resembling the neck of a swan; 6 longitudinal furrows on the left side with a conspicuous hump; laterally compressed about 3 : 1 (Figs. 1A, B, 2A-C, H). Somatic cilia of the right side visually developed, left somatic cilia difficult to observe in vivo (Fig. 2E-G).

Cytoplasm gray to bright yellow, with numerous tiny cortical granules. After examining raw culture during several days in the laboratory, body color often appeared to be faint (Fig. 2A-G).

Extrusomes bar-shaped, about 4-6 µm, distributed on the anterior region of the ventral margin only, some scattered in the cytoplasm (Figs. 1A, 2J).

Two macronuclear nodules, spherical to ovoid, about 8-18 × 8-13 µm after fixation, located near equatorial region of body, usually detected in vivo (Figs. 1C, 2D, H, J-L). Single micronucleus, ca 2 µm in length, situated between the macronuclear nodules (Figs. 1C, 2J-L). 2-4 contractile vacuoles located on the posterior (Figs. 1A, 2G). Usually gliding slowly on the substrate or swimming with rapid rotation in water.

Infraciliature shown in Figures 1C-D and 2F, I, J-L. Three perioral kineties (PK1-3): PK1, the left of the oral slit, consisted of dikinetids in the anterior 1/2 region, subsequently monokinetids to the posterior (Fig. 1C); PK2, 3 on the right of the cytostome, PK2 consisting of dikinetids, PK3 composed of monokinetids, terminated at the posterior end of the cell (Figs. 1D, 2I). Dorsal brush kinety (DB) extended to nearly the posterior end, composed of dikinetids in the anterior 1/2, after monokinetids to the posterior (Fig. 1C). About 11-14 right kineties including PK2, 3 (mean 12.15) anteriorly shortened along the perioral kineties (Figs. 1D, 2K). Left side, 7 kineties including PK1 and DB (Figs. 1C, 2L). Nematodesmata not observed.

SSU rDNA of sequences *L. paracygnus* were deposited in Genbank under the accession number of GQ351697-GQ-351698. Two sequences are identical and 1634 bp in length. They show 99.6% similarity with known *L. paracygnus* (EU242509).

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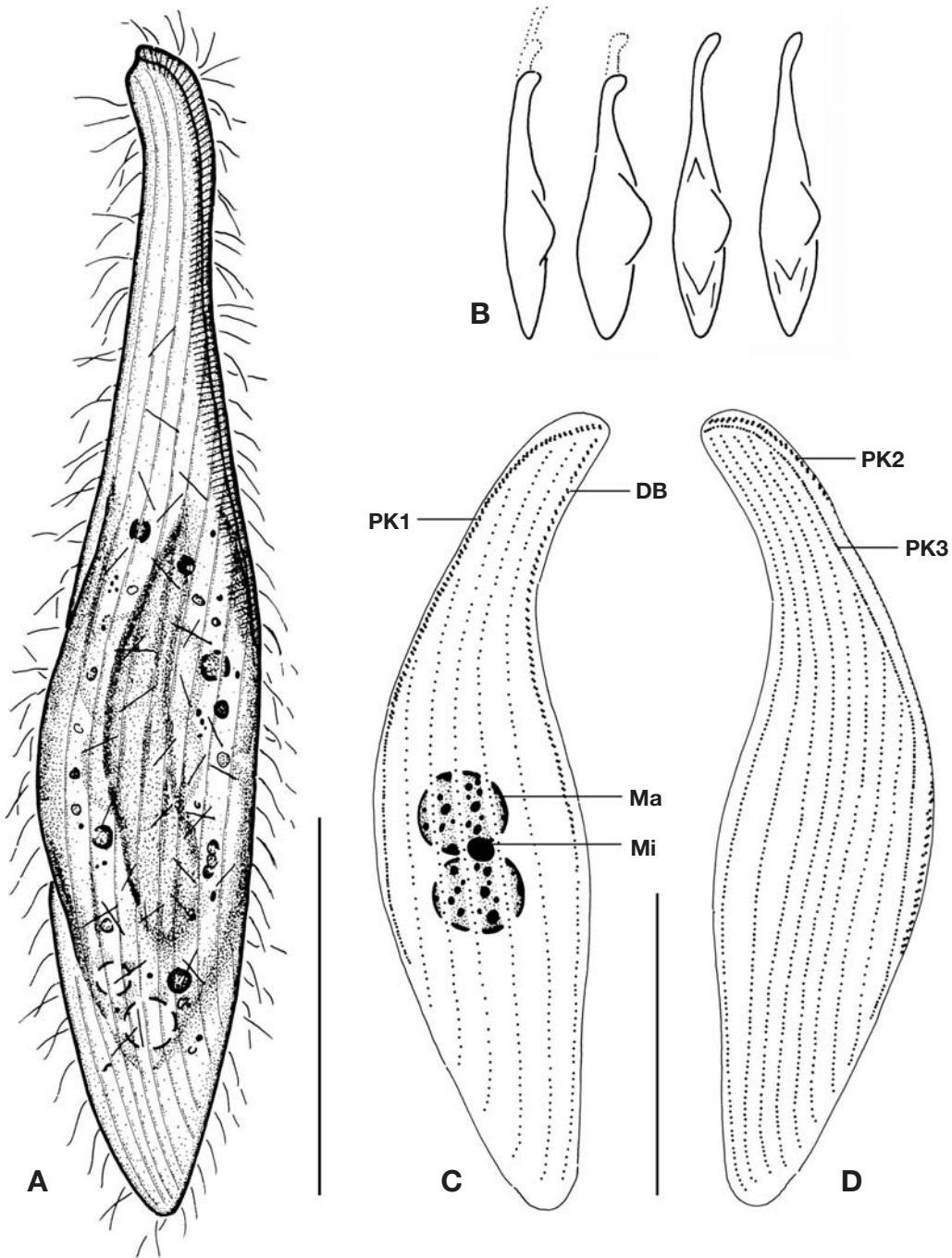


Fig. 1. Morphology of *Litonotus paracygnus* drawn from life (A, B) and after protargol impregnation (C, D). A, Right view of a typical individual; B, Contractile anterior region; C, D, The infraciliature of the left (C) and right (D) sides. Abbreviations are described in the subsection of materials and methods. Scale bars=70 μ m (A), 50 μ m (C, D).

Remarks. *Litonotus paracygnus* was originally reported from the Yellow Sea, China (Song, 1994). Characteristics of this population correspond well with the original description by Song (1994) in most respects, such as the body shape, size, the number and shape of nuclei, the distribution of extru-

somes, the existence of furrows and the marine habitat (Figs. 1-2, 6A and Table 2). This population, however, differs slightly from that of China in the number of left (7 vs. 8-9) somatic kineties, and the number (1-4 vs. 1) of contractile vacuoles (Table 2). Usually, the shape of contractile vacuole

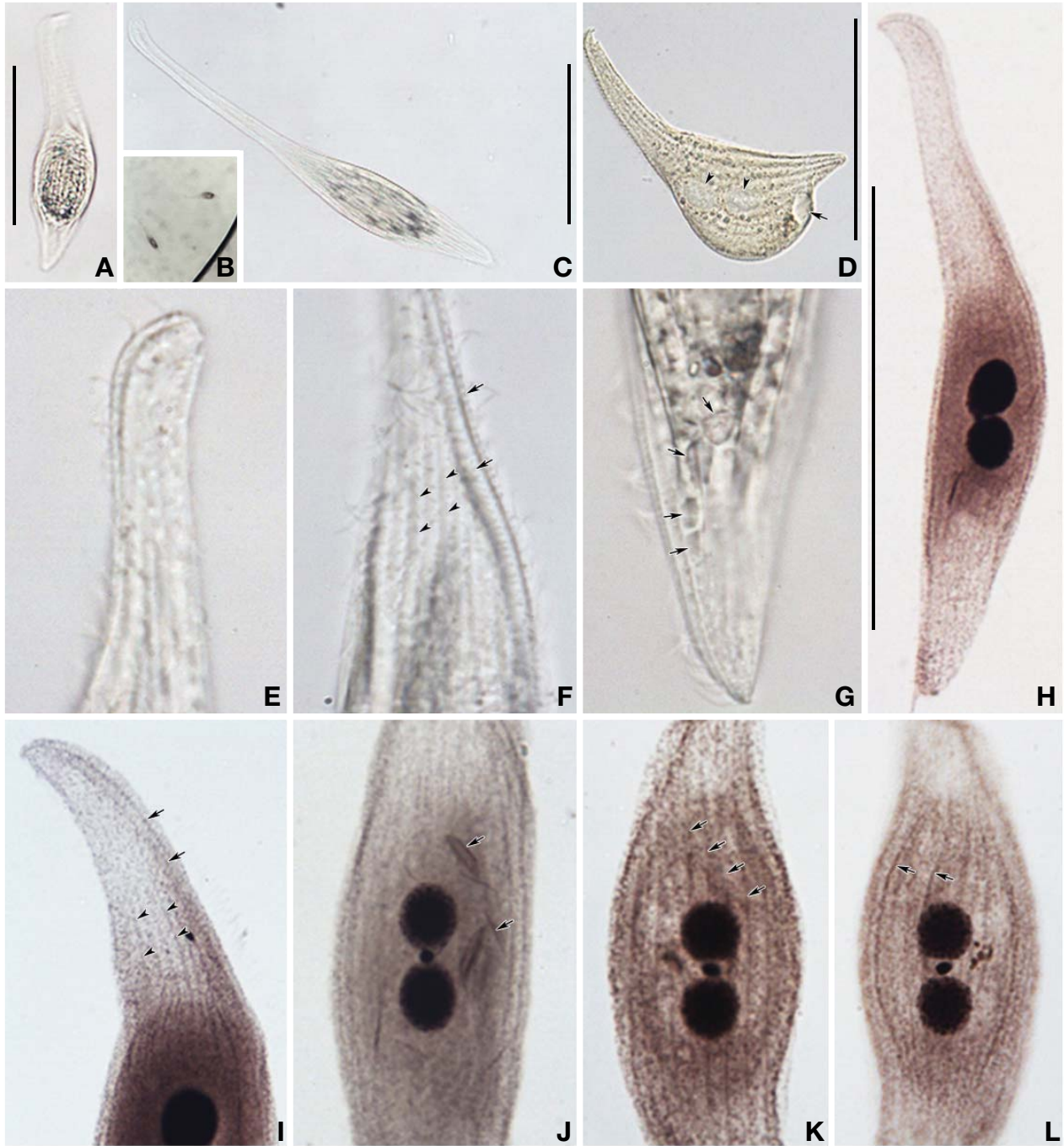


Fig. 2. Photographs of *Litonotus paracygnus* from life (A-G) and after protargol impregnation (H-L). A-D, H, Shape variants; E, Left of anterior region; F, I, Arrows mark perial kineties and arrowheads kineties on the right side; G, Arrows indicate contractile vacuoles on the posterior; J, The middle region of the body shows the extrusomes (arrow) and nuclei; K, L, View right (K) and left (L) side, arrows indicate somatic kineties. Scale bars=100 μ m.

frequently changes in vivo and is sometimes difficult to observe. These are considered the main point of population differences. In addition to newly determined SSU rDNA sequences of *L. paracygnus* showed high similarity (99.6%)

to that of known *L. paracygnus* (EU242509).

With reference to the extraction and contraction of the neck region, the possession of nuclei and the distribution of extrusomes, *L. paracygnus* is most similar to *L. cygnus* (Mül-

Table 1. Morphometric characteristics of *Litonotus paracygnus* (above) and *L. pictus* (below) from protargol-impregnated specimens: Abbreviations are described in the subsection of materials and methods

	Min	Max	Mean	SD	SE	CV	n
Body length	120	224	157.76	32.07	7.17	20.33	20
	175	600	342.50	115.31	25.78	33.67	20
Body width	24	32	27.84	2.67	0.60	9.58	20
	20	90	49.63	20.81	4.65	41.94	20
Length of Nd	—	—	—	—	—	—	—
	112	320	173.50	50.72	11.34	29.23	20
Number of RSK ^a	11	14	12.15	0.59	0.13	4.83	20
	18	26	22.45	2.26	0.51	10.06	20
Number of LSK ^b	7	7	7.00	0.00	0.00	0.00	20
	7	11	7.30	0.98	0.22	13.41	20
Number of Ma	2	2	2.00	0.00	0.00	0.00	20
	12	21	16.55	2.39	0.54	14.47	20
Length of Ma	8	17.6	11.90	2.49	0.56	20.91	20
	8.75	25	15.94	4.57	1.02	28.66	20
Width of Ma	8	12.8	10.16	1.62	0.36	15.97	20
	5	17.5	9.06	2.95	0.66	32.54	20
Number of Mi	1	1	1.00	0.00	0.00	0.00	20
Length of Mi	—	—	—	—	—	—	—
	1.6	3.2	2.22	0.56	0.13	25.34	20
Number of CV	2	4	2.7	0.82	0.26	30.49	10
	3	23	10.60	6.45	2.04	60.85	10
Length of Ex	4	6	5.04	0.78	0.17	15.44	20
	3.2	9.6	6.68	1.69	0.38	25.26	20

All measurement in μm . Abbreviations not described in the text are as follows: CV, coefficient of variation in %; Max., maximum; Min., minimum; n, sample size; SD, standard deviation; SE, standard error of mean.

^aPK 2 and 3 included.

^bPK 1 and DB included.

ler, 1776), in that its name originated from the constellation derived from the Latin for a swan. Thus, it is difficult to distinguish from *L. cygnus* due to the existence of slight differences in the body size, the number of right kineties and the habitats (Table 2). However, *L. paracygnus* can be distinguished from *L. cygnus* by the number of dikinetosomes in dorsal brush (many vs. 6-13) (Song, 1994; Foissner et al., 1995) and furrows (Table 2). In spite of that, *L. cygnus* is only clearly described in terms of the infraciliature by Foissner (1984); the accuracy of the infraciliature data could not be justified from other descriptions (Khal, 1931, 1933; Dragesco and Dragesco-Kernéis, 1986) (Fig. 6B, C, F and Table 2). For these reasons, distinguishing between two species is still difficult. A reinvestigation of the infraciliature and molecular studies from *L. cygnus* is necessary.

Distribution. China (Song, 1994) and Korea (this study).

2. ¹*Litonotus pictus* Gruber, 1884 (Figs. 3-5 and Table 1)

Litonotus pictus Gruber, 1884, p. 521, Figs. 52-53; Khal,

1931, p. 190, Fig. S. 187, 22; Khal, 1933, p. c 62, Figs. 5.16, 5.19.

Material examined. Ciliates were collected from the Iwon tide embankment near Ganwol-do (36°54'N and 126°16'E) of Chungcheongnam-do in the Yellow Sea of the Republic of Korea on 11 Apr. 2008.

Diagnosis. Large marine *Litonotus* about 200-600 μm long in vivo, lanceolate body, extremely contractile; beautiful body color with rows of yellow to yellow-brownish cortical pigment granules; 12-21 macronuclear nodules arranged in a moniliform pattern, infrequently vermiform; 7-11 left and 18-26 right kineties; several contractile vacuoles located on both margins; extrusomes bar-shaped, distributed on the anterior region of the ventral margin only.

Redescription. Body size extremely variable, about 200-600 μm long in vivo (Figs. 3B; 4A, B). Cells slender lanceolate-shaped, highly contractile, rounded posterior end; many longitudinal ridges within the ciliary rows on the right side; left side with an inconspicuous weak hump; laterally com-

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Table 2. Comparison of two *Litonotus* species in present and previous specimens

Species	Body length in vivo (μm)	Position of CV (No.)	No. of LSK and RSK	Distribution of Ex	Furrow	No. of DK in DB	Biotope	No. of Ma	Body color	Reference
<i>L. paracygnus</i>	150-250	Subterminal (1)	8-9/12-13	AVM	6	Many	Marine	2	–	Song (1994)
<i>L. paracygnus</i>	150-300	Subterminal (2-4)	7/11-14	AVM	6	Many	Marine	2	Gray to bright yellow	This study
<i>L. cygnus</i>	500	Subterminal (1)	/11?	AVM	4*	–	Marine & fresh water	2	–	Khal (1931)
<i>L. cygnus</i>	500	Subterminal (1)*	–	AVM*	4*	–	Marine	2*	–	Khal (1933)
<i>L. cygnus</i>	90-132	Subterminal (1)*	-/8-9	AVM*	–	–	Fresh water	2	–	Foissner (1984)
<i>L. cygnus</i>	500	Subterminal (1)	-/15?	AVM	–	–	Marine	2	–	Dragesco and Dragesco-Kernéis (1986)
<i>L. cygnus</i>	100-500	Subterminal (1)	/6-13	AVM	–	6-13	Marine & brackish water	2	–	Foissner et al. (1995)
<i>L. pictus</i>	500	Subterminal (1)	–	Unrecognized	–	–	Marine	2-Numerous	Dark-yellow/orange	Gruber (1884)
<i>L. pictus</i>	250-500	Subterminal (1) with satellites	-/15?	AVM	–	–	Marine	Numerous	Orange-yellow	Khal (1931)
<i>L. pictus</i>	200-500	Subterminal (3) with satellites*	–	AVM*	5-6	–	Marine	Numerous	Yellow	Khal (1933)
<i>L. pictus</i>	200-600	Both sides (several)	7-11/18-26	AVM	–	–	Marine	12-21 or vermiform	Yellow to yellow brownish	This study

AVM, anterior of the ventral margin; *, data according to illustration; ?, data not justified to include perioral kineties and dorsal brush; –, data not described.

pressed about 3 : 1 (Figs. 3A, B; 4A-C, F). Somatic cilia, 6-8 μm long, of the right side visually developed (Fig. 4J); left somatic cilia difficult to detect in vivo.

Cytoplasm yellow to yellow-brownish (Fig. 4), beautiful body color, with numerous tiny cortical pigment granules. Pigment arrangement of right side comparatively regular, dot-like cortical granules along both sides of ciliary rows (Figs. 3G, H, 4N); left side irregularly arranged between ciliary rows (Figs. 3I, 4O).

Extrusomes bar-shaped, about 3-10 μm , distributed on the anterior region of the ventral margin only, some scattered in the cytoplasm (Figs. 3A, C, 4M, 5J, K).

Macronucleus consisted of two types: Type 1, moniliform with 12-21 nodules, elongated to ovoid macronuclear nodules, each about 9-25 \times 5-18 μm after fixation (Figs. 3A, D, E, 4K, 5A, B, E); Type 2, a long vermiform (Figs. 3D, 5C, D), lean to the ventral region, usually detectable in vivo using differential interference contrast microscopy (Fig. 4K). Micronucleus not observed. Typically one large contractile vacuole, subterminally positioned, several satellites, ca 9.6, located along both boundaries of a hump on the left side (Figs. 3A; 4L), frequently, exhausted individuals exhibit several large vacuoles all over the body (Fig. 4H, I). Numerous large lumps of prey existed in the cytoplasm (Fig. 5D, F).

Usually gliding slowly on the substrate, swimming with rapid rotation or twisting in the water (Fig. 4A-G).

Infraciliature as shown in Figs. 3C, E, F and 5G-I, L. Three perioral kineties (PK1-3): PK1, the left of the oral slit, consisting of dikinetids in the anterior 2/3 region and subsequently monokinetids to the posterior (Fig. 3E); PK2, 3 on the right of the cytostome, PK2 consisting of dikinetids, PK3 composed of monokinetids, terminated at the posterior end of the cell (Fig. 3F). Dorsal brush kinety (DB) extends to nearly the posterior end, composed of regularly spaced dikinetids (Figs. 3E; 5I, arrowheads). About 18-26 right kineties including PK2, 3 (mean 22.5) closely spaced, anteriorly shortened along the perioral kineties (Figs. 3F; 4N; 5G). Left side, about 7-11 (mean 7.3) kineties including PK1 and DB (Figs. 3E; 4O; 5H-I). Nematodesmata highly prominent and extending along the cytopharynx into the cytoplasm, about 110-320 μm long (Figs. 3C; 5L, arrowheads).

SSU rDNA sequences *Litonotus pictus* were deposited in Genbank under the accession number of GQ351699-GQ-351701. Three sequences are identical and 1,635 bp in length. *Remarks.* Among the known nominal *Litonotus* morphotypes, *Litonotus pictus* is well distinguished by its unique body size, body color and presence of macronuclear nodules. In addition to the original description (Gruber, 1884),

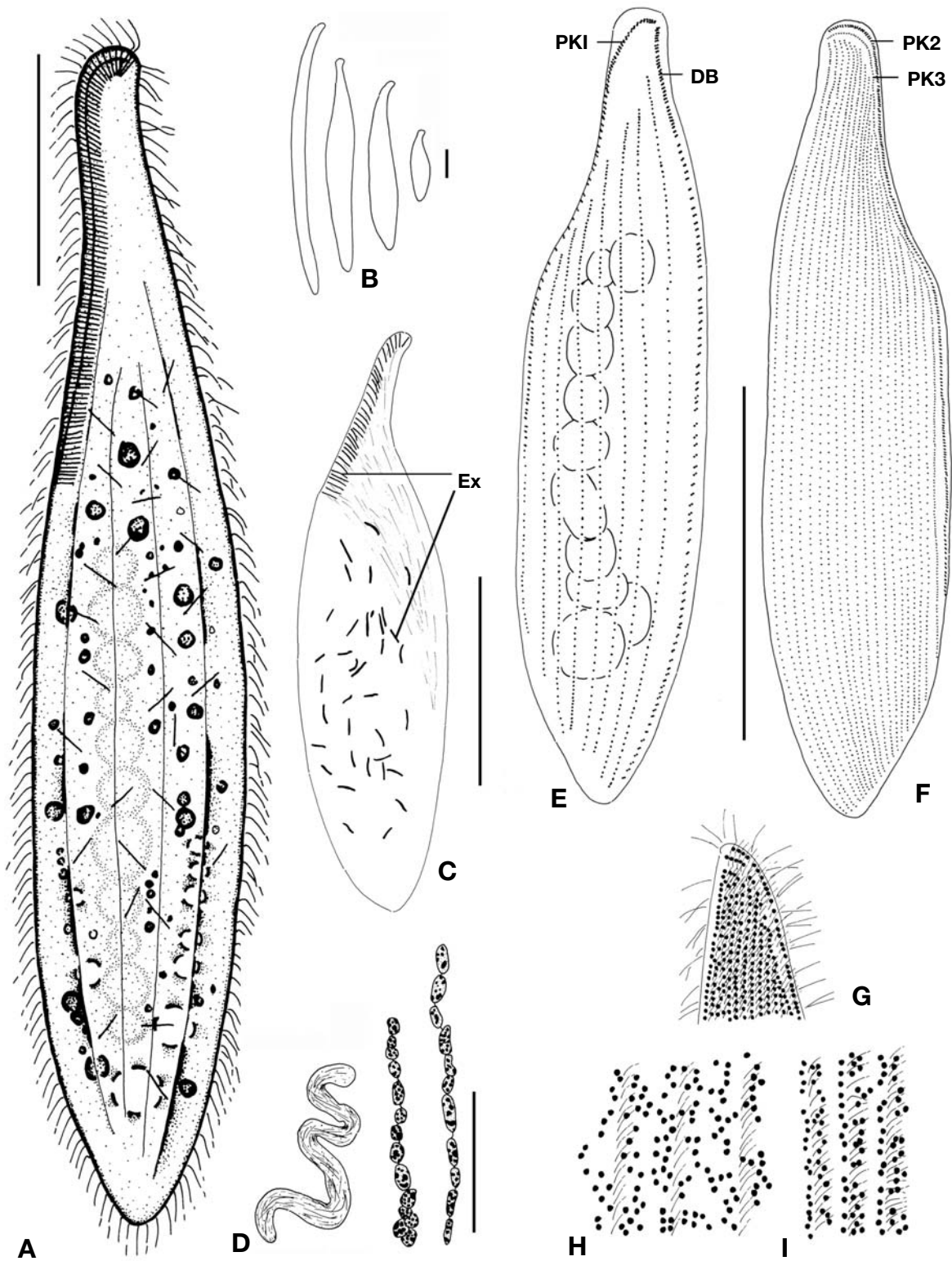


Fig. 3. Morphology of *Litonotus pictus* drawn from life (A, B, G-I) and after protargol impregnation (C-F). A, Left view of a typical individual; B, Shape variants; C, Extrusomes and nematodesmata; D, Variants of macronuclear nodules; E, F, The infraciliature of left (E) and right (F) sides; G-I, The arrangement of pigment granules on right (G, H) and left (I) sides. Abbreviations are described in the subsection of materials and methods. Scale bars=100 μ m.

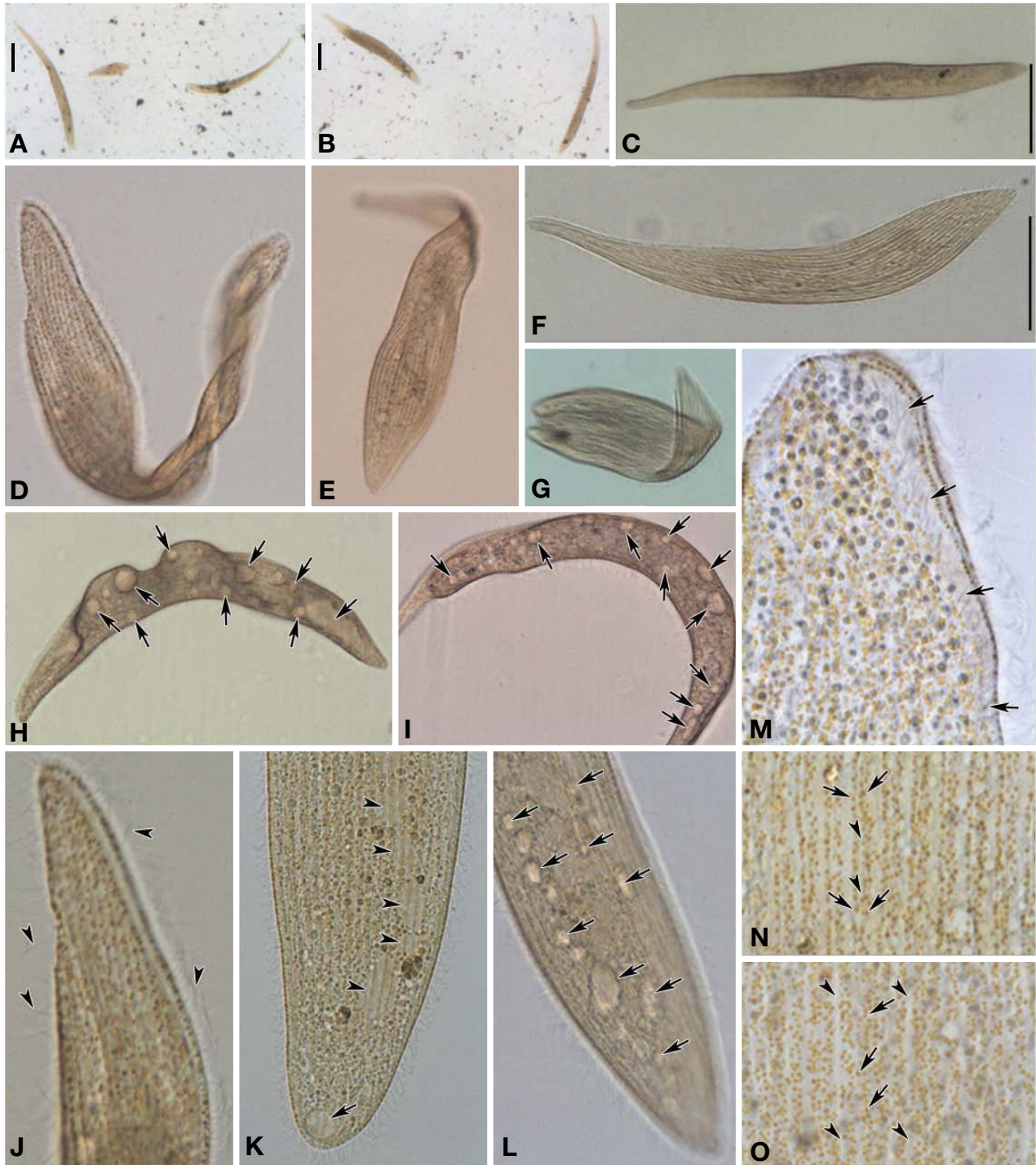


Fig. 4. Photographs of *Litonotus pictus* from life. A-G, Shape variants and movement; H, I, Arrows mark contractile vacuoles in exhausted individuals; J, Arrowheads indicate cilia; K, L, View of the posterior region showing macronuclear nodules (arrowheads) and contractile vacuoles (arrows); M, Left of anterior region, arrows indicate extrusomes; N, O, Right and left sides; arrows indicate pigment granules and arrowheads mark kineties. Scale bars=100 μ m.

subsequent redescriptions (Khal, 1931, 1933) have been published. In the present study, we described previously un-

known infraciliature using specific staining methods.

The population used in this study corresponds well with

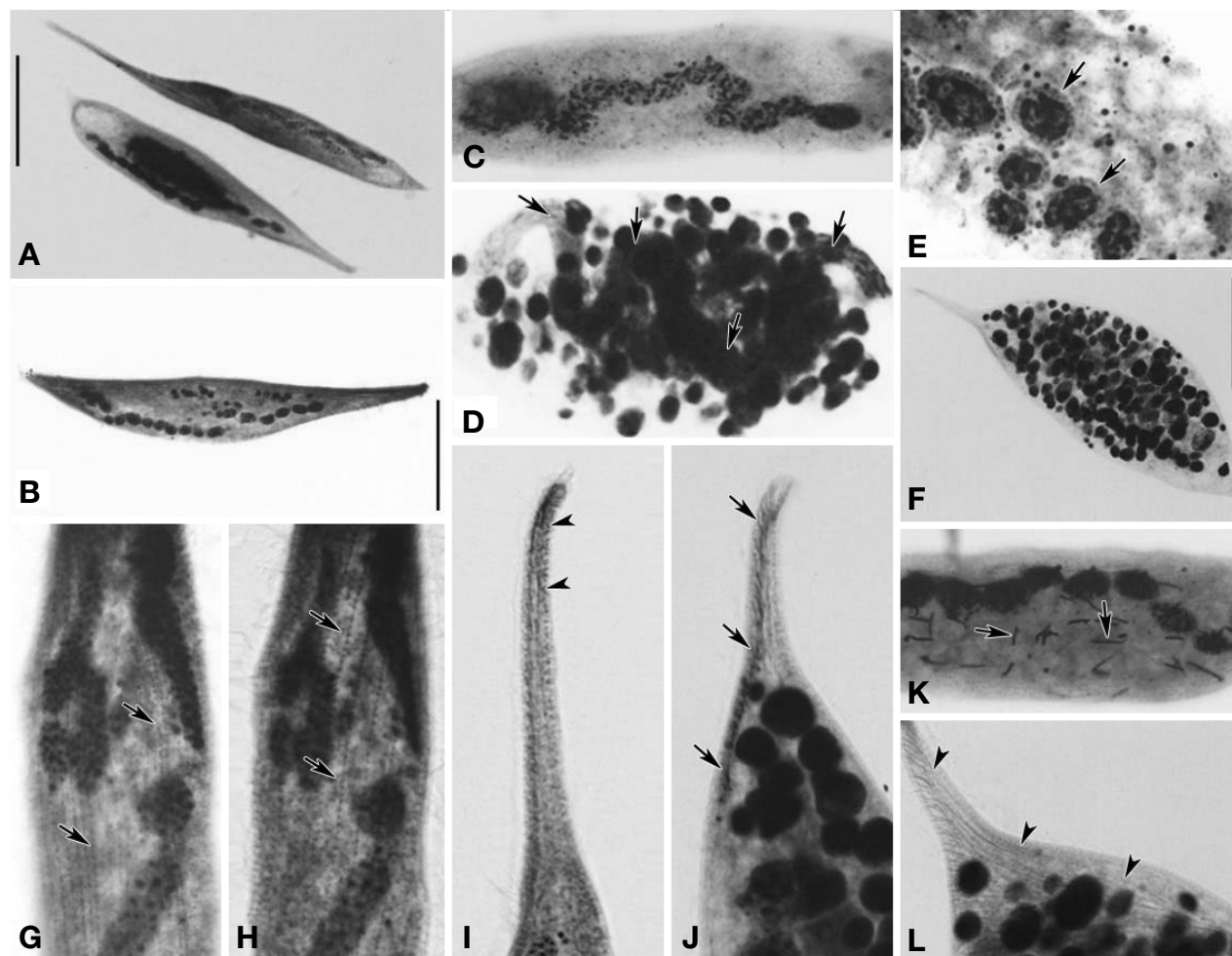


Fig. 5. Photographs of *Litonotus pictus* after protargol impregnation. A, B, Different body shape; C-E, Shape variants of macronuclear nodules, long vermiform (arrows in D) and ovoid (arrows in E); F, View numerous large lump of prey; G, H, View right (G) and left (H) side, arrows indicate somatic kineties; I, J, The anterior region, show the dorsal brush kinety (arrowheads) and extrusomes (arrows); K, Arrows mark extrusomes in the body; L, Arrowheads indicate nematodesmata. Scale bars=100 μ m.

the original description by Gruber (1884) and the subsequent description by Khal (1931, 1933) in most respects, i.e., the body size, body shape, distribution of extrusomes, body color, macronuclear nodules and habitat (Fig. 6G-K, Table 2). However, this population differs slightly in terms of the number of contractile vacuoles; *Litonotus pictus* by Gruber (1884) showed only one on subterminal region. Generally, shape of the contractile vacuoles frequently changes in vivo and contractile vacuoles, as satellites are sometimes especially difficult to observe. These are considered population differences.

On the other hand, Lin et al. (2008) inferred *Litonotus pictus* of Gruber (1884) and Khal (1931) to *Loxophyllum pictus* based on the distribution of extrusomes along both margins. In general, *Litonotus* and *Loxophyllum* can be dis-

tinguished by the distribution of extrusomes: the extrusomes of *Loxophyllum* are distributed along both margins, while those of *Litonotus* are restricted to ventral region. Although Lin (2008) described that *Litonotus pictus* of Gruber (1884) has extrusomes along both margins, Gruber (1884), in his original description, had clearly mentioned that he could not recognize the distribution of extrusomes in that species. The literature described by Khal (1931) seems rather controversial in this regard. The extrusomes distributed along sides of the both margins are well illustrated in the picture but in the text, it is mentioned that extrusomes were only found around the oral region. Since the illustration and text are not matching, it seems that the species was described discordantly.

In the subsequent literature, Khal (1933) described clear-

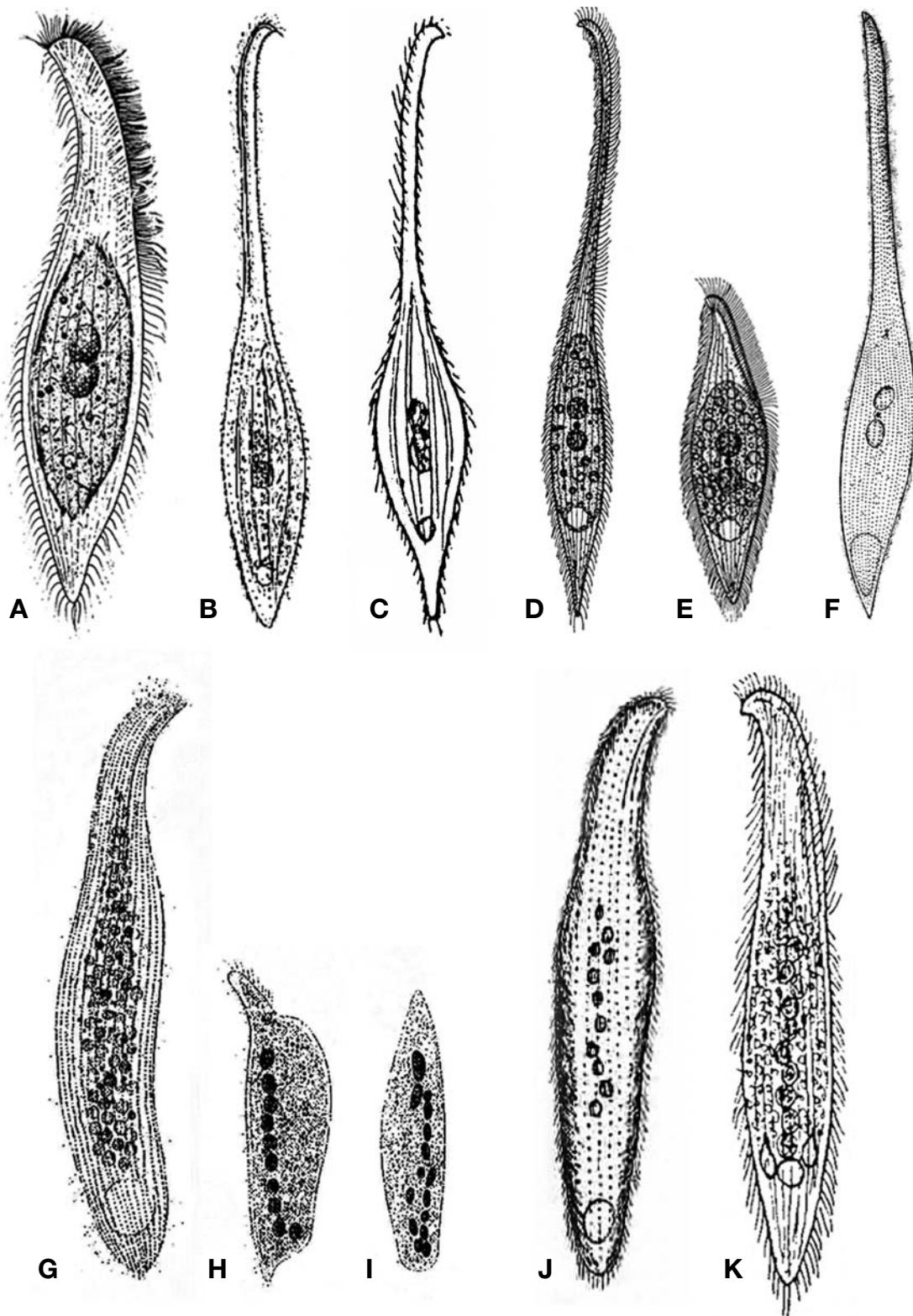


Fig. 6. Overview of the representative species related to this study. A, *Litonotus paracygnus* from Song (1994); B, *Litonotus cygnus* from Khal (1931); C, *Litonotus cygnus* from Khal (1933); D, E, *Litonotus cygnus* from Foissner (1984); F, *Litonotus cygnus* from Dragesco and Dragesco-Kernéis (1986); G-I, *Litonotus pictus* from Gruber (1884); J, *Litonotus pictus* from Khal (1931); K, *Litonotus pictus* from Khal (1933).

ly, both in text and illustration, that the extrusomes of *Litonotus pictus* were distributed only on oral region. Therefore we could conclude that the drawing showing extrusomes on both sides of the margins were improperly presented by Khal (1931). At this moment, we can also draw this inference from the sequence analysis because the newly determined three SSU rDNA sequences of *L. pictus* showed the closest relationship with that of *Litonotus paracygnus*, while they are not clustered with known *Loxophyllum* species (data not showed). Summarizing these evidences, the *pictus* species has extrusomes only around the oral region and it should be included in the genus *Litonotus*.

Distribution. Germany (Gruber, 1884; Khal, 1931; Khal, 1933) and Korea (this study).

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REFERENCES

- Barth, D., S. Krenek, S.I. Fokin and T.U. Berendonk, 2006. Intraspecific genetic variation in *Paramecium* revealed by mitochondrial cytochrome C oxidase I sequences. *J. Eukaryot. Microbiol.*, 53: 20-25.
- Borror, A.C., 1963. Morphology and ecology of the benthic ciliated protozoa of Alligator Habor, Florida. *Arch. Protistenkd.*, 106: 465-534.
- Chantangsi, C., D.H. Lynn, M.T. Brandl, J.C. Cole, N. Hetrick and P. Ikononi, 2007. Barcoding ciliates: a comprehensive study of 75 isolates of the genus *Tetrahymena*. *Int. J. Syst. Evol. Microbiol.*, 10: 2412-2425.
- Corliss, J.O., 1979. *The Ciliated Protozoa*. Pergaman Press, New York, pp. 1-455.
- DeSalle, R., M.G. Egan and M. Siddall, 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 360: 1905-1916.
- Dragesco, J., 1966. Observations sur quelques ciliés libres. *Arch. Protistenkd.*, 109: 155-206.
- Dragesco, J. and A. Dragesco-Kernéis, 1986. Ciliés libres de l'Afrique intertropicale. *Faune Trop.*, 26: 1-559.
- Foissner, W., 1984. Taxonomie und Ökologie einiger Ciliaten (Protozoa, Ciliophora) des Saprobien-systems. I: Genera *Litonotus*, *Amphileptus*, *Opisthodon*. *Hydrobiologia*, 119: 193-208.
- Foissner, W., 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Europ. J. Protistol.*, 27: 313-330.
- Foissner, W. and D. Leipe, 1995. Morphology and ecology of *Siroloxophyllum utriculariae* (Penard, 1922) n. g., n. comb. (Ciliophora, Pleurostomatida) and an improved classification of pleurostomatid ciliates. *J. Eukaryot. Microbiol.*, 42: 476-490.
- Foissner, W., H. Blatterer, H. Berger and F. Kohmann, 1995. Taxonomische und ökologische Revision der Ciliaten des Saprobien-systems. Band IV: Gymnostomatea, Loxodes, Suctoria. Bayerischen Landesamtes für Wasserwirtschaft, Deggendorf, pp. 318-325.
- Fryd-Versavel, G., F. Tftode and J. Dragesco, 1975. Contribution à la connaissance de quelques ciliés gymnostomes. II. Prostomiens, pleurostomiens: morphologie, stomatogénèse. *Protistologica*, 11: 509-530.
- Gong, J., J.K. Choi, D.M. Roberts, S.Y. Kim and G.S. Min, 2007a. Morphological descriptions of new and little-known benthic ciliates from ganghwa tidal flat, Korea. *J. Eukaryot. Microbiol.*, 54: 306-316.
- Gong, J., S.J. Kim, S.Y. Kim, G.S. Min, D.McL. Roberts, A. Warren and J.K. Choi, 2007b. Taxonomic Redescriptions of Two Ciliates, *Protogastrostyla pulchra* n. g., n. comb. and *Hemigastrostyla enigmatica* (Ciliophora: Spirotrichea, Stichotrichia), with Phylogenetic Analyses Based on 18S and 28S rRNA Gene Sequences. *J. Eukaryot. Microbiol.*, 54: 468-478.
- Gruber, A., 1884. Die Protozoen des Hafens von Genua. *Nova Acta Leop. Carol.* 46: 521-523.
- Kahl, A., 1931. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha. *Tierwelt Dtl.*, 21: 181-398.
- Kahl, A., 1933. Ciliata Libera et Ectocommensalia. In Grimpe, G. and E. Wagler, eds., *Die Tierwelt der Nord- und Ostsee*. Lief 23 (Teil II, c3). Leipzig, pp. 29-146.
- Kim, S.Y., S.J. Kim, G.S. Min, E.J. Yang, M.H. Yoo and J.K. Choi, 2007. Analysis of genetic variation in the small subunit ribosomal RNA gene of *Euplotes* ciliates for developing species diagnostic molecular marker. *The Sea*, 12: 225-233.
- Lee, J.M., J. Yoon and M.K. Shin, 2006. Two litonotid ciliates (Ciliophora: Litostomatea: Pleurostomatida) unknown from Korea. *Korean J. Syst. Zool.*, 22: 217-221.
- Li, L., 1990. A new species of ciliates, *Hemiphrys polymicronuclei* sp. nov. from Donghu Lake, Hubei Province. *Chin. J. Oceanol. Limnol.*, 8: 97-100.
- Lin, X. and W. Song, 2004. Establishment of a New *Amphileptid* Genus, *Apoamphileptus* nov. gen. (Ciliophora, Litostomatea, Pleurostomatida), with Description of a New Marine Species, *Apoamphileptus robertsi* nov. spec. from Qingdao, China. *J. Eukaryot. Microbiol.*, 51: 618-625.
- Lin, X.F., W. Song and A. Warren, 2005. Two new marine pleurostomatid ciliates from China, *Amphileptus gui* nov. spec. and *Amphileptus yuianus* nov. spec. (Ciliophora, Pleurostomatida). *Eur. J. Protistol.*, 41: 163-173.

- Lin, X.F., J.Q. Li, J. Gong, A. Warren and W. Song, 2008. Taxonomic studies on three marine pleurostomatid ciliates, *Litonotus bergeri* nov. spec., *L. blattereri* nov. spec. and *L. petzi* nov. spec. (Ciliophora, Pleurostomatida) from North China Sea. *Eur. J. Protist.*, 44: 91-102.
- Lynn, D.H., 2008. The ciliated protozoa: characterization, classification, and guide to the literature. Springer, New York, pp. 1-605.
- 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: 1-23.
- Prescott, D., 1994. The DNA of ciliated protozoa. *Microbiol. Rev.*, 58: 233-267.
- Song, W., 1993. Studies on the morphology and systematic status of *Loxophyllum rostratum* Cohn, 1866 (Ciliophora, Pleurostomatida). *J. Oceanogr. Huanghai Bohai Sea*, 11: 44-49 (in Chinese with English summary).
- Song, W., 1994. Morphology and infraciliature of a new marine ciliate, *Litonotus paracygnus* nov. sp. (Ciliophora, Pleurostomatida). *Acta Zool. Sin.*, 40: 131-136 (in Chinese with English summary).
- Song, W.B. and N. Wilbert, 1989. Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. *Lauterbornia*, 3: 2-221.
- Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24: 1596-1599.
- Tautz, D., P. Arctander, A. Minelli, R.H. Thomas and A.P. Vogler, 2003. A plea for DNA taxonomy. *Trends Ecol. Evol.*, 18: 70-74.
- Xu, H., G.S. Min, J.K. Choi, S.J. Kim, J.H. Jung and B.J. Lim, 2009. An investigation on periphytic ciliate colonization of an artificial substrate in Korean coastal waters. *J. Mar. Biol. Assoc. UK.*, 89: 669-679.

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