Taxonomic Redescription of *Loxophyllum perihoplophorum* and *L. rostratum* (Ciliophora: Pleurostomatida) from Korea

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ABSTRACT

Two pleurostomatid ciliates, *Loxophyllum perihoplophorum* Buddenbrock, 1920 and *L. rostratum* Cohn, 1866, were collected from the coastal waters of the East Sea, Korea. Their morphologies are described based on live observation and protargol staining, and morphometrics are provided. *Loxophyllum perihoplophorum* is characterized by the following features: 200–650 μm long *in vivo*; body slender leaf-shaped, flexible and contractile, with thin and wide extrusome-belted zone; 2 macronuclear nodules (Ma) and 1 micronucleus (Mi); 7–9 contractile vacuoles (CV) positioned along dorsal margin; extrusomes (Ex) evenly distributed along edge of entire body, with about 10 dorsal warts (Wa); 9–11 left (LSK) and 19–22 right somatic kineties (RSK), 4–5 furrows (Fu) on left side. *Loxophyllum rostratum* is about 100–130 μm long *in vivo*; body oblate leaf-shaped, contractile, convex ventral side and S-shaped dorsal side, beak-like anterior end; 2 Ma and 1 Mi; 1 CV terminally located; Ex distributed along edge of entire body, with about 9–10 dorsal Wa; 7–8 LSK and 15–19 RSK, ca. 5 Fu on left body side. In addition, sequences of small subunit ribosomal DNA were determined from these two *Loxophyllum* species and compared with the known *Loxophyllum* sequences.

Keywords: Haptoria, Pleurostomatida, Litonotidae, *Loxophyllum perihoplophorum*, *Loxophyllum rostratum*, East Sea, Korea

INTRODUCTION

Pleurostomatids are bilaterally compressed ciliates with a slit-like cytostome on the ventral side and commonly found in various habitats all over the world (Foissner, 1984; Fenchel, 1987; Lynn 2008). They have been accepted as a monophyletic taxon based on morphological and molecular studies (Corliss, 1979; Lipscomb and Riordan, 1990; Strüder-Kypke et al., 2006; Lynn, 2008; Pan et al., 2010). *Loxophyllum* spp. are leaf-shaped pleurostomatids, and about 50 species have been described (Warren, 2015). It can be distinguished from other pleurostomatid genera by differences in perioral kineties, somatic ciliation, distribution of extrusomes, status of suture, etc. (Lynn and Small, 2002; Song et al., 2009). The genus was considered a monophyletic group in phylogenetic analyses using small subunit ribosomal DNA (SSU rDNA) sequences (Pan et al., 2013; Wu et al., 2014, 2015; Vdacný et al., 2015). However, it is not easy to identify *Loxophyllum* species due to relatively similar morphologies *in vivo* and a lack of full infraciliature description in early morphological studies. The SSU rDNA sequences of 12 species have been currently registered in GenBank database (30 Sep 2015). In order to further assess the taxonomic status of the genus *Loxophyllum*, additional morphological and molecular studies are required for more species. To date, two *Loxophyllum* species, *L. meleagris* and *L. chaetonotum*, have only been reported in Korea (Lee et al., 2006; Gong et al., 2007). In the present study, two *Loxophyllum* species were collected from the East Sea, Korea. We described and illustrated morphological characteristics based on live observation and protargol staining. Additionally, an SSU rDNA analysis was conducted with known congener sequences.

MATERIALS AND METHODS

Morphological taxonomy procedures

Samples were collected from coastal areas in the East Sea, Korea, using PEF-S (Xu et al., 2009) and then transferred to...
a laboratory. They were continually maintained in petri dishes at 17°C (light-dark 12:12 cycles) with rice grains as a food source to enhance bacterial growth. Living specimens were observed using phase contrast and differential interference microscopes at different magnifications. The protargol staining (Foissner, 1991) was performed to reveal ciliary pattern, nuclear apparatus, and extrusomes. Enumeration and measurements of stained specimens were performed under ×1,000 magnification (Leica DM2500; Wetzlar, Germany). Drawings of specimens were made using a lucida camera. The classification scheme used here was based on Lynn (2008). Terminology followed Corliss (1979), Lin et al. (2005), and Lynn (2008). Abbreviations were as follows: CV, contractile vacuole; DB, dorsal brush kinety; Ex, extrusome; Fu, furrow; FV, food vacuole; LSK, left somatic kinety; Ma, macronuclear nodule; Mi, micronucleus; Nd, nematodesmata; PK, perioral kinety; RSK, right somatic kinety; Wa, wart.

**Molecular taxonomy procedures**

Each living individual was isolated using a micropipette under a dissecting microscope (Leica MZ 12.5), and 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 7.0.9 (Hall, 1999). Nucleotide diversity within the species was calculated using MEGA 6.06 (Tamura et al., 2013) with the p-distance value.

**RESULTS**

**Phylum Ciliophora Doflein, 1901**

Class Litostomatea Small and Lynn, 1981

Subclass Haptoria Corliss, 1974

Order Pleurostomatida Schewiakoff, 1896

Family Litonotidae Kent, 1882

*Genus Loxophyllum* Dujardin, 1841

**Loxophyllum perihoplophorum** Buddenbrock, 1920

(1st line) and **Loxophyllum rostratum** (2nd line) from protargol stained specimens

| Characters | Min. | Max. | Mean | SD | SE | CV | n |
|------------|------|------|------|----|----|----|---|
| Body length (μm) | 115 | 350 | 231.50 | 50.66 | 11.33 | 21.88 | 20 |
| Body width (μm) | 87.5 | 145 | 119.85 | 15.47 | 3.75 | 12.91 | 17 |
| No. of RSK<sup>a</sup> | 19 | 22 | 20.15 | 0.81 | 0.18 | 4.03 | 20 |
| No. of LSK<sup>b</sup> | 15 | 19 | 16.24 | 1.15 | 0.28 | 7.07 | 17 |
| No. of Ma | 9 | 11 | 10.20 | 0.52 | 0.12 | 5.13 | 20 |
| Length of Ma | 2 | 2 | 2.00 | 0.00 | 0.00 | 0.00 | 17 |
| Width of Ma | 19.2 | 41.6 | 29.84 | 5.01 | 1.12 | 16.80 | 20 |
| No. of Mi | 19.2 | 41.6 | 29.84 | 5.01 | 1.12 | 16.80 | 20 |
| No. of Mi | 10 | 22 | 16.18 | 3.63 | 0.88 | 22.42 | 17 |
| No. of Mi | 5 | 11 | 7.76 | 1.48 | 0.36 | 19.06 | 17 |
| No. of Mi | 1 | 1 | 1.00 | 0.00 | 0.00 | 0.00 | 17 |
| Length of Mi | 1 | 1 | 1.00 | 0.00 | 0.00 | 0.00 | 17 |
| Length of Mi | 1.92 | 4.8 | 3.17 | 0.78 | 0.18 | 24.72 | 20 |
| Length of Ex | 6.4 | 9.6 | 6.64 | 0.78 | 0.18 | 11.79 | 20 |
| Length of Ex | 4 | 7 | 4.89 | 0.68 | 0.16 | 13.84 | 18 |

All measurement in μm. Min., minimum; Max., maximum; SD, standard deviation; SE, standard error; CV, coefficient of variation in %; n, sample size; RSK, right somatic kinety; LSK, left somatic kinety; Ma, macronuclear nodule; Mi, micronucleus; Ex, extrusome.

<sup>a</sup> PK2 and PK3 included.

<sup>b</sup> PK1 and DB included.

Korean name: 1<sup>st</sup>나뭇잎섬모충 (신칭), 2<sup>nd</sup>넓은테두리나뭇잎섬모충 (신칭)

Material examined. Specimens were collected from the coastal waters of Hajeo-ri (36°23′N and 129°24′E), Gyeongsangbuk-do in the East Sea on 28 Apr–24 Jun 2008. Description. Live cell size 200–650 × 50–100 μm, slender leaf-shaped in outline, anterior end hooked toward dorsal side; body flexible and contractile, length ratio of fully extended to most contracted bodies about 2–3:1, a thin and wide Ex-belted zone enclosed; highly shrunken after protargol staining.
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Protargol staining, about 115–350 μm in length (Figs. 1A, B, 2A–D, F). Laterally compressed at a ratio of about 2–3 : 1, right side flat, left slightly vaulted; 4–5 longitudinal Fu on left side (Fig. 1A).

Two Ma connected by thread-like funiculus, dumbbell-shaped, located in body center, usually detectable in live specimens; each nodule egg-shaped, about 30×15 μm after fixation (Fig. 2C, I). One Mi positioned between two Ma; globule-shaped, ca. 2–5 μm in length (Fig. 2I). Approximately 7–9 CV settled along dorsal region, size variable; a

Fig. 1. Morphology of Loxophyllum perihoplophorum (A–D) and L. rostratum (E–H) drawn from live and protargol stained specimens. A, E. Left view of a typical live individual; B, F. Nuclei, extrusomes, warts and nematodesmata with protargol staining; C, D, G, H. Ciliary pattern after protargol staining, left (C, G) and right (D, H) sides. DB, dorsal brush kinety; Ex, extrusome; Ma, macronuclear nodule; Mi, micronucleus; PK, perioral kinety; Wa, wart. Scale bars = 100 μm (A–D), 50 μm (E–H).
few FV recognized in vivo (Fig. 2E).

Ex thin, bar-shaped, about 6–10 μm long, recognizable in live cells under optimal conditions; evenly distributed along outline of entire body, and especially clustered into ca. 10 Wa along anterior 2/3 of dorsal margin; some scattered in cytoplasm (Figs. 1A, B, 2F, J).

Cytoplasm grayish; central portion of the body opaque, with numerous tiny shiny globules; cortical granules not observed (Fig. 2C). Movement by crawling along substrates or swimming with fast rotation and twisting.

Ciliary pattern typical of *Loxophyllum* (Figs. 1C, D, 2G, H, K, L). Three PK placed around oral slit, PK1 on left, and PK2-3 on right; kinetid rows more tightly packed than somatic kineties; PK1 contains dense dikinetids in anterior 1/3, and a continuous row of monokinetids extending to posterior end; PK2 and PK3 entirely composed of monokinetids. DB situated on dorsolateral area, with dense dikinetids in anterior 2/5, and a continuous row of monokinetids extending to posterior end. All somatic kineties composed of monokinetids; 19–22 kineties on right side, including PK2 and PK3; 9–11 kineties on left side, with PK1 and DB.

Nd stretches to 2/3 of the cytoplasm region (Fig. 1B).

**Distribution.** Germany (North Sea), China (Mangrove wetland in Techeng Island), and Korea (East Sea, this study).

**Remarks.** *Loxophyllum perihoplophorum* was originally described by Buddenbrock (1920) and redescribed by Kahl (1931) and Wu et al. (2014). Characteristics of the Korean population correspond very well with Buddenbrock (1920) and Kahl (1931) in most respects, such as habitat, body shape and size, the distribution of extrusomes, the number of nuclei, contractile vacuoles, somatic kineties and warts (Table 2). However, the Korean population differs from the Chinese population in the distribution of extrusomes along the dorsal margin (both warts and not wart area vs. only in warts) and habitat (marine vs. brackish) (Table 2). Nevertheless, the two populations show 99.6% similarity in SSU rDNA sequences (Korean population, accession No. KT880227–KT880228; Chinese population, KC493570).

**16** *Loxophyllum rostratum* Cohn, 1866

(Table 1, Figs. 1E–H, 3)

*Loxophyllum rostratum* Cohn, 1866: 280, figs. 8–11; Song,
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1993: 44, figs. 1, 2; Petz et al., 1995: 55, fig. 17; Lin et al., 2008: 331, figs. 1-16.

Material examined. Specimens were collected from the coastal waters of Munam 2-ri (38°17′ N, 128°33′ E), Gangwon-do in the East Sea on 12-19 Mar 2008. Environmental conditions of the sampling site were 9.2°C, 34.2 psu, and pH 8.3.

Description. Live cell size 100-130×45-65 μm; oblate leaf-shaped in outline; convex ventral side and S-shaped dorsal side; beak-like anterior end hooked toward dorsal side; winding dorsal-neck region, about 15-25 μm in length; body contractile, slightly shrunk after protargol staining (Figs. 1E, 3A, B). Laterally compressed at a ratio of about 2-3:1, right side flat, left side notably vaulted; 4-5 conspicuous longitudinal Fu on left side (Fig. 3A, F).

Two Ma connected by thread-like funiculus located in body center, usually detectable in live specimens; each nodule ovoid to elongate, about 10-22×5-11 μm after fixation (Fig. 3D, H). One Mi positioned between two Ma; globule-shaped, ca. 2-5 μm in length. One CV recognized at terminal region.

Ex thin, bar-shaped, about 4-7 μm long, recognizable in live cells under optimal conditions; evenly distributed along entire ventral margin, and clustered into 9-10 Wa on dorsal margin; some scattered in cytoplasm (Figs. 1E, F, 3C, E, J).

Cytoplasm slightly grayish colored, with numerous shining lipid globules and particles; dot-shaped cortical granules

Table 2. Comparison among populations of Loxophyllum perihoplophorum and L. rostratum

| Species            | Body length (μm in vivo) | No. of CV | No. of LSK and RSK | Distribution of Ex in margin | No. of Wa | Habitat | Reference       |
|--------------------|-------------------------|-----------|--------------------|-------------------------------|-----------|---------|-----------------|
| L. perihoplophorum | 200-650                 | 7-9       | 9-11/19-22         | Entire                        | ca. 10    | Marine  | This study      |
|                    | ca. 400                 | Several   | –/20               | Entire                        | 8b        | Marine  | Buddenbrock (1920) |
|                    | ca. 400                 | Several   | –/20               | Entire                        | 9b        | Marine  | Kahl (1931)      |
| L. rostratum       | 100-130                | 1         | 7-8/15-19          | Entire                        | 9-10      | Marine  | This study      |
|                    | 100-180                | 1         | –                  | Entire                        | 10b       | Marine  | Cohn (1866)     |
|                    | 150-250                | 1         | 6-7/16-18          | Entire                        | 9-11      | Marine  | Song (1993)     |
|                    | 150-250                | 1         | 8-10/13-23         | Entire                        | 12-15     | Brackish | Wu et al. (2014) |
|                    | 100-300                | 1         | 6-9/16-19          | Entire                        | 6-13      | Marine  | Lin et al. (2008) |

CV, contractile vacuole; LSK, left somatic kinety; RSK, right somatic kinety; Ex, extrusome; Wa, wart.

*Entire region of ventral margin and only in warts along dorsal margin.

No. counted in drawings.
detected on pellicle, densely placed between rows of RSK (Fig. 3A–G). Movement by slow gliding, flexible crawling along substrates, or swimming with fast rotation and twisting.

Ciliary pattern typical of *Loxophyllum* (Figs. 1G, H, 2G, I, K, L). Three PK placed around oral slit, PK1 on left, and PK2-3 on right; kinetid rows more tightly packed than somatic kineties; PK1 contains dense dikinetids in anterior 2/3, and a continuous row of monokinetids reaching to posterior end; PK2 composed of dikinetids reaching to posterior end; PK3 entirely formed of monokinetid. DB situated on dorsolateral area, with dense dikinetids in anterior 5/6, and a continuous row of monokinetids reaching to posterior end. All somatic kineties composed of monokinetids; 15–19 kineties on right side, including PK2 and PK3; 7–8 kineties on left side, with PK1 and DB. Nd not detected.

**Distribution.** Germany (North Sea), Antarctica (Weddell Sea), China (Yellow Sea) and Korea (East Sea, this study).

**Remarks.** *Loxophyllum rostratum* was originally described from an aquarium population based on live observation by Cohn (1866). Several redescriptions based on protargol staining have improved the definition of *L. rostratum* (Song, 1993; Petz et al., 1995; Lin et al., 2008). The Korean population corresponds well with the characteristics of the original description and the subsequent studies. However, the Korean population shows a small difference from the Chinese population (Song, 1993) in body size (100–130 μm vs. 150–250 μm) and the Weddell Sea population (Petz et al., 1995) in the number of warts (9–10 vs. ca. 12) (Table 2).

SSU rDNA sequences of the Korean population were deposited in Genbank under accession number KT880229-KT880230. The sequences are identical, and are 1,592 bp in length. They show 99.7% similarity with known *L. rostratum* (DQ190465).

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