

***Frontonia lynni* n. sp., a new marine ciliate (Protozoa, Ciliophora, Hymenostomatida) from Qingdao, China**

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Abstract

The morphology, infraciliature and silverline system of a new marine ciliate, *Frontonia lynni* n. sp., isolated from a sandy beach at Qingdao, China, was investigated using live observation and silver staining methods. The new species is recognized by the combination of the following characters: body about 100–210 x 70–150 μm *in vivo*, elliptical in outline; dorsoventrally flattened (3:1); one large contractile vacuole equatorially located, right of median; 71–83 somatic kineties and three vestibular kineties; small oral cavity with peniculi 1 and 2 each having four ciliary rows and peniculus 3 possesses five gradually shortened rows.

Key words: Marine ciliate; *Frontonia lynni*; morphology; new species

Introduction

The peniculine ciliates *Frontonia* are commonly found as members of the pelagic and benthic fauna in both fresh and marine biotopes and most species have been well described using silver impregnation methods (Borror 1963; Burkovsky 1970; Dragesco 1972; Foissner 1987; Foissner et al. 1994; Gil & Perez-Silva 1964a, b, c; Kahl 1931; Petz et al. 1995; Roque 1961a, b, c; Roque & de Puytorac 1972; Song & Wilbert 1989). Taxa belonging to this genus are separated from each other by the combination of the following characters: the body shape and size, number of somatic kineties, morphology of the oral apparatus, the position of the contractile vacuole, and their habitats (Corliss 1979; Dragesco & Dragesco-Kernéis 1986; Foissner et al. 1994; Roque & de Puytorac 1972).

Recently, the authors isolated an unknown *Frontonia* from a sandy beach at Qingdao. After detailed investigation, it is believed to be a previously unknown member of this genus. The results are documented here.

Materials and methods

Organisms were sampled from a beach on the yellow sea coast at Qingdao, the Yellow Sea, north China on November 24, 2004. The upper 15 cm layer of sand was collected together with some water from site (temperature about 10 °C, salinity 26 ‰). Isolated ciliates were kept for up to two weeks at room temperature (20–24 °C) for further studies.

Cells were observed *in vivo* first using a stereomicroscope, and then with high magnification under a compound microscope ($\times 100$ –1250). The infraciliature was revealed by silver carbonate (Ma *et al.*, 2003), protargol and silver nitrate impregnation methods (Song & Wilbert 1995). Drawings of live cells were based on free-hand sketches and micrographs, and those of impregnated ones were made with a camera lucida. Terminology is mainly according to Corliss (1979).

Results

Order Hymenostomatida Delage & Herouard, 1896

Family Frontoniidae Kahl, 1926

Genus *Frontonia* Ehrenberg, 1838

Frontonia lynni n. sp. (Figs. 1-3; Table 1)

Diagnosis: Marine *Frontonia* about 100–210 \times 70–150 μm *in vivo*, body shape ellipsoidal, dorsoventrally strongly flattened of about 3:1 with conspicuously small buccal cavity. 71–83 somatic, three vestibular and five postoral kineties. Peniculi 1 and 2 each with four rows, whereas peniculus 3 has five ciliary rows. Macronucleus ellipsoidal. Single contractile vacuole, equatorially located on the right margin. Extrusomes spindle-shaped.

Type location: A mesotrophic sand beach (salinity 26 ‰) in Qingdao (36°08' N, 120°43'), China.

Type specimens: One holotype with silver nitrate impregnated specimens is deposited in the Natural History Museum, London, UK with registration number 2005:24:12. Two paratypes with silver nitrate impregnated specimens are deposited in the Laboratory of Protozoology, OUC (slides number: 2004111501-1, 2004111501-2).

Dedication: We dedicate this species to Prof. Dr. Denis H. Lynn, University of Guelph, Canada, a world-famous protozoologist, who has greatly contributed to the ciliate taxonomy and systematics.

Description: Size highly variable, *in vivo* about 100–210 \times 70–150 μm , but mostly 130–170 μm in length; ratio of length: width about 3:2. Body shape constant, ellipsoidal in outline when viewed from ventral or dorsal; right margin straight while left margin slightly convex (Figs. 1A-C; 3A, 3B). Both ends broadly rounded. Dorsoventrally conspicuously flattened about 3:1 (Fig. 1G). Macronucleus ellipsoidal, positioned at body center; micronucleus spherical, adjacent to macronucleus (Fig. 1I). One contractile vacuole, about 20

µm in diameter, positioned equatorially and conspicuously right of median. Extrusomes spindle-shaped, about 6 µm long, densely arranged in cortex (Figs. 1D; 3B, 3C, 3K). Cytoplasm transparent, colorless to slightly grey-yellowish, usually filled with many food vacuoles (> 10 µm in diameter), as well as dark granules and crystals (3-5 µm across) (Figs. 1C; 3B), containing bacteria, diatoms and other small ciliates (Figs. 1A; 3J). Somatic cilia about 8 µm long. Movement mostly by gliding back and forth on substrate; when swimming, moderately rapid with rotation around the long axis of the cell.

Buccal cavity small and shallow, triangular in outline, occupying about 1/7 of body length (Figs. 1A, 1B, 1J, 1K; 3C). Three short vestibular kineties (VK1–3) run from anterior vertex of cavity to postoral suture, with densely arranged kinetosomes (Figs. 1H, 1J; 3C, 3H). Three inconspicuous peniculi deeply located on the left wall of cavity: peniculi 1 and 2 (P1, 2) about equally long, positioned close to each other, parallel to the edge of the left vestibular wall and each composed of four rows of kinetosomes; peniculus 3 (P3) slightly curved to right, composed of five relatively shorter kineties, of which two are complete, another two are slightly shortened and the leftmost one is about half the length of the two on the right (Figs. 1J; 3E).

Single-rowed paroral membrane (PM) on the right edge of the buccal cavity runs from the anterior of the buccal overture to the posterior edge. Anterior portion of argentophilic line (AL) positioned parallel to paroral membrane (Figs. 1J; 3E).

As with its congeners, silverline system as quadrangular cortical meshes that can be observed after silver nitrate impregnation (Figs. 1E; 3I).

TABLE 1. Morphometric data of *Frontonia lynni* n. sp. Data from protargol impregnated specimens. All measurements in µm.

Characteristics	Min	Max	Mean	SD	SE	CV	n
Body length	128	208	163.7	16.98	3.13	10.4	29
Body width	64	144	93.3	21.02	3.95	22.5	29
Number of somatic kineties	71	83	74.5	5.66	1.24	7.6	17
Length of peniculus 1	24	32	26.8	2.14	0.40	8.0	29
Number of postoral kineties	5	5	5	0	0	0	10
Number of kineties in peniculus 1	4	4	4	0	0	0	14
Number of kineties in peniculus 2	4	4	4	0	0	0	14
Number of kineties in peniculus 3	5	5	5	0	0	0	9
Number of vestibular kineties	3	3	3	0	0	0	15

Abbreviations: CV = coefficient of variation in %, Max = maximum, Mean = arithmetic mean, Min = minimum, n = number of individuals examined, SD = standard deviation, SE = standard error of arithmetic mean.

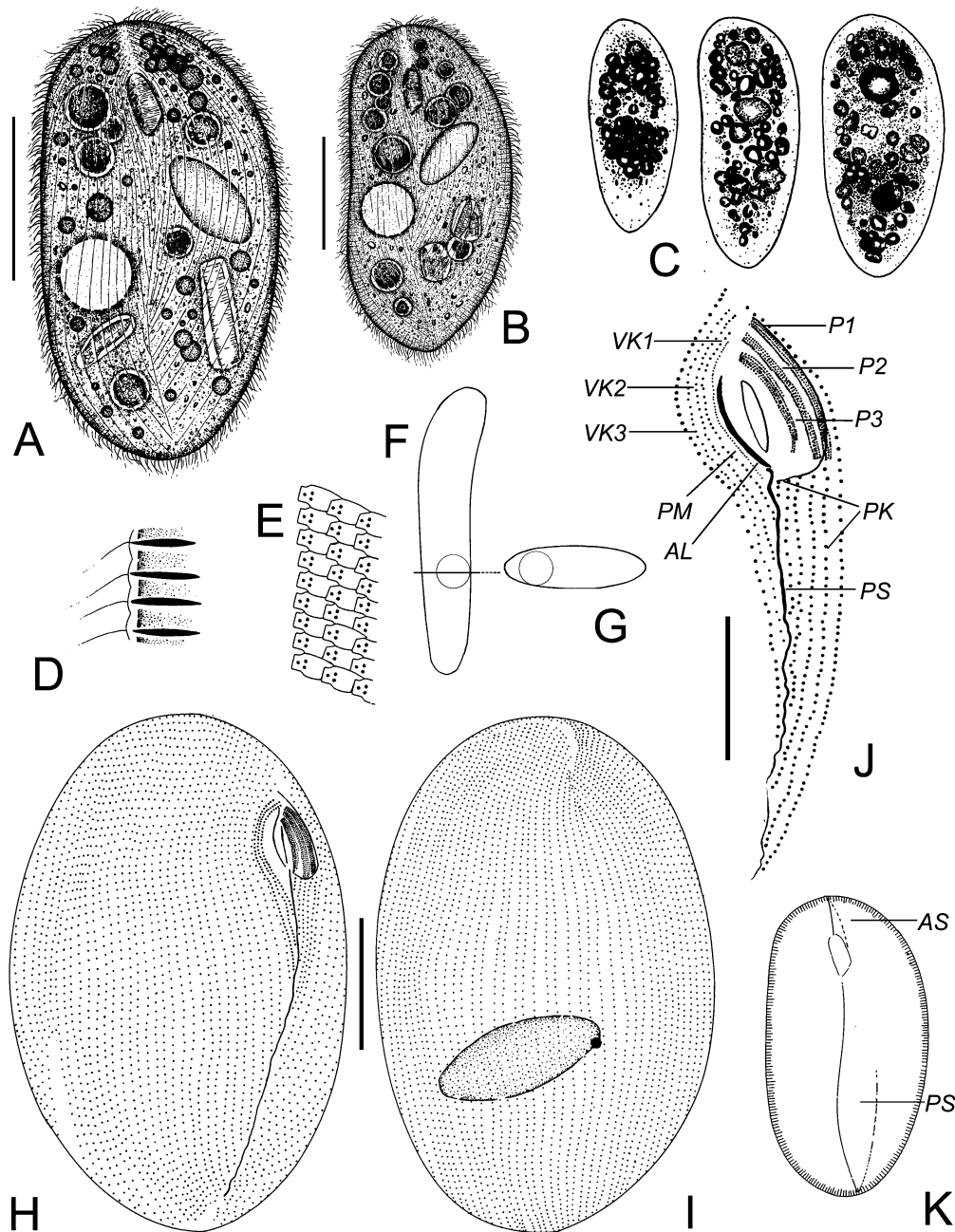


FIGURE 1. *Frontonia lynni* n. sp. live (**A–D**, **F**, **G**), after silver carbonate (**H**, **I**, **K**) and silver nitrate impregnation (**E**, **J**). (**A**) Ventral view of a typical specimen. (**B**) A slightly deformed individual. (**C**) Showing variations in body shape. (**D**) Part of pellicle, to show extrusomes. (**E**) Silver-line pattern. (**F**, **G**) Left and apical views of the same specimen, to show the position of the contractile vacuole. Note that the body is strongly (!) flattened. (**H**, **I**) The infraciliature of the same cell, note the small oral apparatus. (**J**) Oral apparatus. (**K**) To show the anterior and postoral sutures. **AL** = argentophilic line; **AS** = anterior suture; **P1–3** = peniculi 1–3; **PK** = postoral kineties; **PM** = paraoral membrane; **PS** = postoral suture; **VK1–3** = vestibular kineties 1–3. Scale bars in (**A**, **B**) = 50 μ m, in (**H**, **I**) = 40 μ m, in (**J**) = 20 μ m.

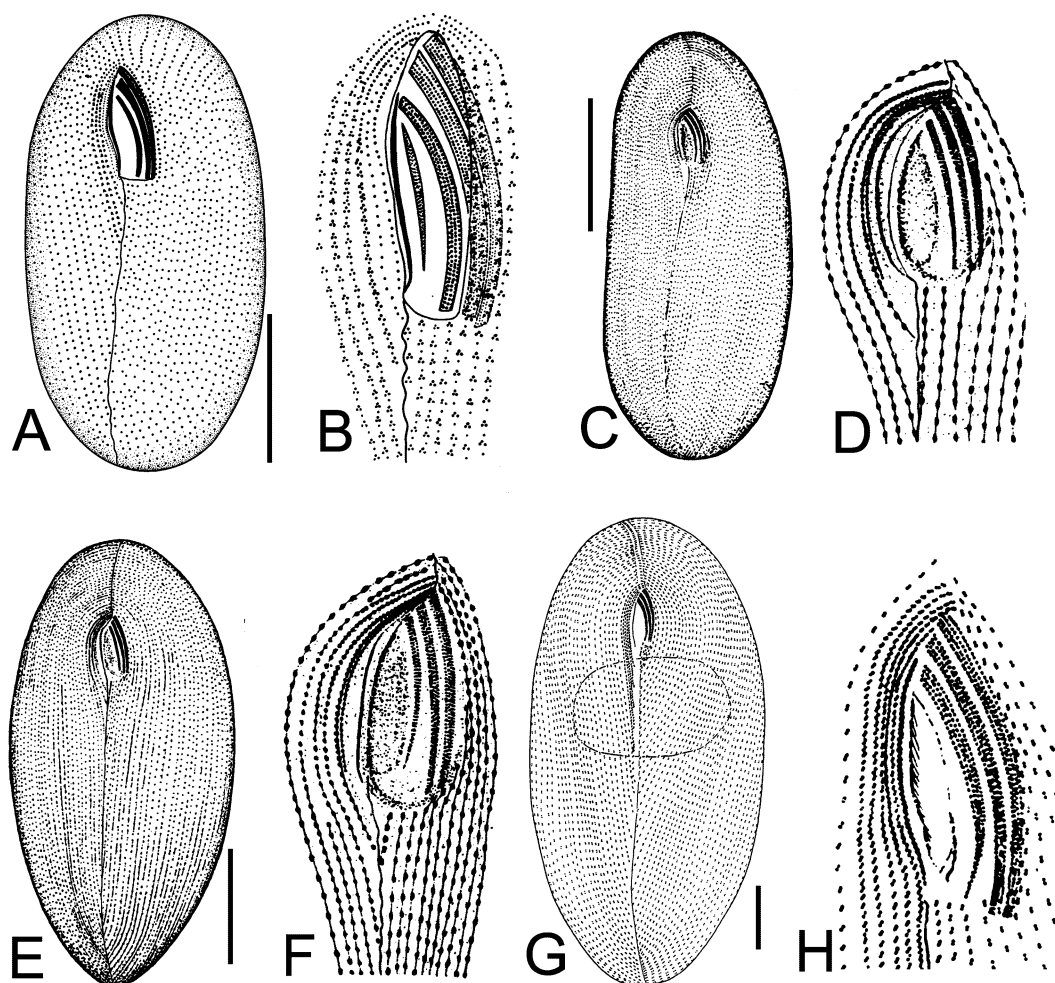


FIGURE 2. Ventral views (A, C, E, G) and oral field (B, D, F, H) of *Frontonia ambigua* (A, B, from Dragesco & Dragesco-Kernéis 1986), *Frontonia marisalbi* (C, D from Burkovsky, 1970) and *Frontonia tchibisovae* (E, F, from Burkovsky, 1970) after silver nitrate impregnation. *Frontonia frigida* (G, H, from Petz *et al.*, 1995; G after protargol impregnation, H after silver nitrate impregnation). Scale bars = 30 μ m.

Discussion

Since the genus *Frontonia* was established (Ehrenberg 1838), about 30 nominal species have been recognized and described (Al-Rasheid 1999; Burkovsky 1970; Carey 1992; Dragesco 1960, 1972; Dragesco & Dragesco-Kernéis 1986; Foissner 1987; Gil & Perez-Silva 1964a, b, c; Kahl 1931; Roque 1961a, b, c; Roque & Puytorac 1972). Recent investigations using silver staining methods have demonstrated that the location of contractile vacuole, infraciliature, and especially the structure of oral apparatus are the most reliable criteria for species separation (Foissner 1994; Petz *et al.* 1995; Song & Wilbert 1995).

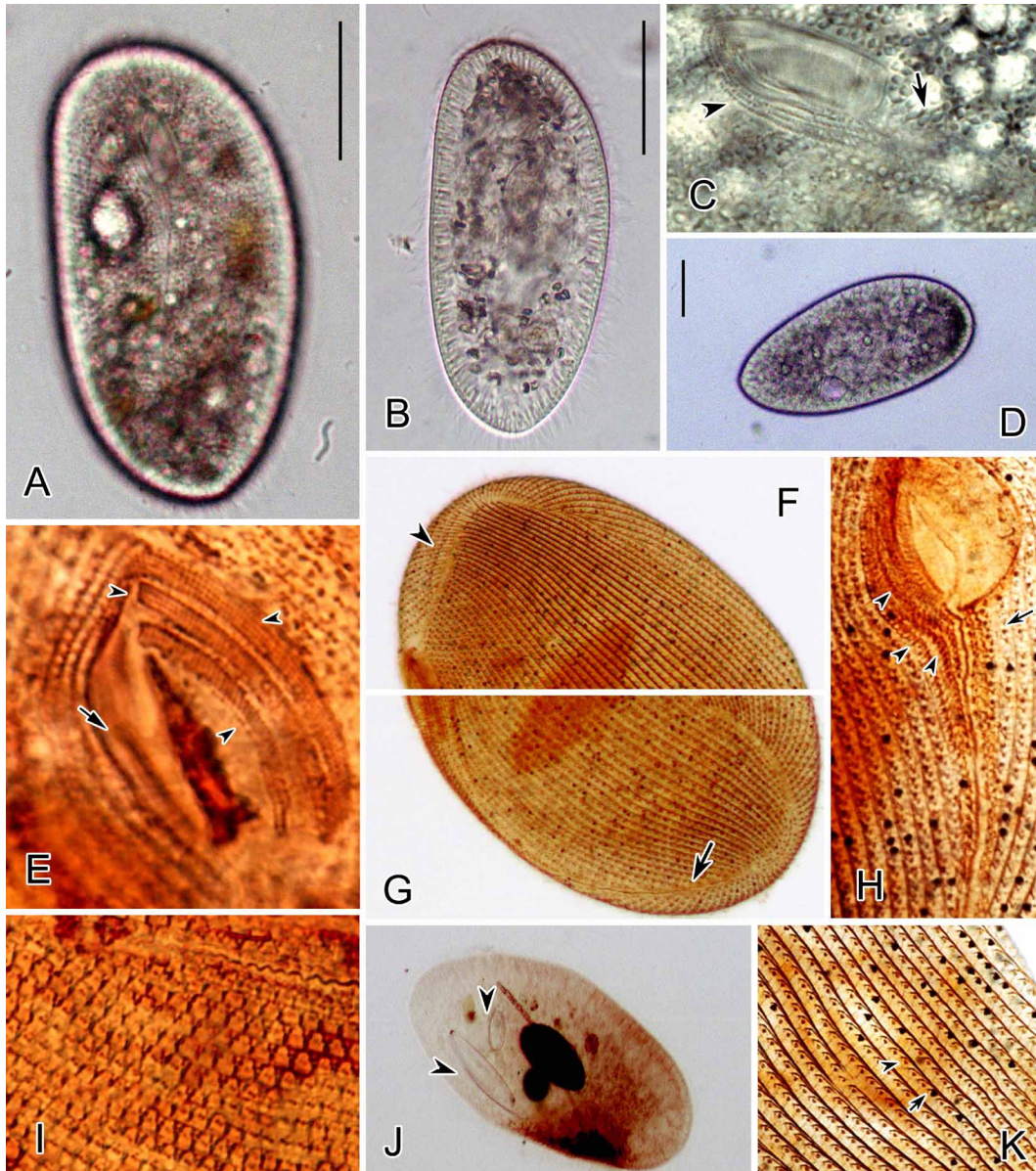


FIGURE 3. *Frontonia lynni* n. sp. live (A–D), after protargol (J), silver carbonate (F, G, H, K) and silver nitrate impregnation (E, I). (A) Dorsal view. (B) Arrangements of extrusomes and cilia. (C) Buccal area, showing vestibular kineties (arrowhead) and extrusomes (arrow). (D) Dorsal view, showing the contractile vacuole. (E) Buccal area, arrowheads refer to peniculi, arrow marks argen-tophilic line. (F) Lateral view, showing anterior suture (arrowhead). (G) Lateral view, showing posterior suture (arrow). (H) Infraciliature of oral field, arrow marks postoral kineties, arrowheads indicate vestibular kineties. (I) Silverline system. (J) Lateral view, arrowheads mark ingested diatoms. (K) A part of pellicle, arrowhead refers to a single kinetosome, arrow shows a rest extrusome. Scale bars = 50 μ m.

Considering the body shape and size, and marine habitat, at least four species namely *Frontonia marisalbi* Burkovsky, 1970, *F. tchibisovae* Burkovsky, 1970, *F. ambigua* Dragesco, 1972 and *F. frigida*, Petz *et al.*, 1995 should be compared with our new species.

Frontonia marisalbi differs from *F. lynni* in having reniform (vs. elliptic) body shape, many more somatic kineties (120–140 vs. 71–83) and fewer kineties (3 vs. 5) in peniculus 3 (Figs. 1J; 2C, 2D) (Burkovsky 1970).

Frontonia tchibisovae has more somatic (110–130 vs. 71–83), vestibular (4 vs. 3) and postoral kineties (7 vs. 5), and fewer ciliary rows in peniculus 3 (3 vs. 5) (Figs. 1J; 2E, 2F) (Burkovsky 1970), and hence can be clearly distinguished from *F. lynni*.

With reference to the shape of the cell, numbers of vestibular, and the postoral kineties, *Frontonia lynni* also resembles *F. ambigua* (Figs. 1A, 1B, 1K; 2A). However, the latter differs in having three (vs. 5 in *F. lynni*) kineties in peniculus 3 (Figs. 1J; 2A, 2B). Furthermore, *F. ambigua* is much smaller (78–100 μm in length), and has a relatively larger buccal field (ca. 1/4 of body length vs. ca. 1/7) (Dragesco & Dragesco-Kernéis 1986).

Frontonia lynni is also similar to *F. frigida* in terms of body size and the location of the contractile vacuole. However, the former can be recognized by possessing: 1) elliptical body shape (vs. spindle-shaped or lanceolate); 2) fewer somatic kineties (71–83 vs. 93–129); 3) fewer vestibular kineties (3 vs. 5); 4) one spherical micronucleus (vs. 2 bean-shaped) and more (5 vs. 4) ciliary rows in peniculus 3 (Figs. 1A, 1I; 2G, 2H) (Petz *et al.* 1995).

In addition, the living individuals of this new species are extremely flattened dorsoventrally, which distinguishes it from most other known congeners and therefore could be an easily observable feature used for identification of this taxon in ecological studies.

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