Zootaxa 1687: 35–50 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Taxonomic studies on three marine species of *Frontonia* from northern China: *F. didieri* n. sp., *F. multinucleata* n. sp. and *F. tchibisovae* Burkovsky, 1970 (Ciliophora: Peniculida)

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Abstract

The morphology and infraciliature of three marine ciliates, *Frontonia didieri* **n. sp.**, *F. multinucleata* **n. sp.** and *F. tchibisovae*, isolated from northern China seas, were investigated using living observation and silver impregnation methods. *Frontonia didieri* **n. sp.** is recognized by: *in vivo ca.* 100–150 × 45–80 µm; 61–71 somatic, 3 vestibular and 3–5 postoral kineties; 4-rowed peniculus 1 and 2; peniculus 3 three-rowed; contractile vacuole centrally-located, with about eight conspicuous collecting canals. *Frontonia multinucleata* **n. sp.** is characterized by: 70–120 × 40–75 µm *in vivo*, dorsoventrally flattened about 2:1; 58–67 somatic, 3 vestibular and 4–5 postoral kineties; 3 peniculi each with 4 kineties; 2–4 globular macronuclear nodules; contractile vacuole located in posterior 1/3 of cell length. Based on both original and the Qingdao population, the poorly-defined *Frontonia tchibisovae* is redefined and a new diagnosis is supplied.

Key words: Peniculid ciliates; *Frontonia*; morphology and redescription; new species

Introduction

Species belonging to the peniculid genus *Frontonia* are commonly seen in marine and freshwater habitats (Bullington 1939; Carey 1992; Dragesco 1960; Dragesco 1972; Dragesco & Dragesco-Kernéis 1986; Fokin *et al.* 2006; Foissner 1987; Foissner *et al.* 1994; Kahl 1931; Roque 1961; Song 1995). However, only some of them have been well-described, based upon both live observations and silver impregnations, while many marine forms remain unknown or insufficiently described (Alekperov 2005; Burkovsky 1970a, b; Long *et al.* 2005; Petz *et al.* 1995; Roque & Puytorac 1972). These organisms should be identified morphologically by the combination of features like the buccal and somatic ciliature, position and character of contractile vacuole, size and shape of body, as well as the habitat (Foissner 1987; Foissner *et al.* 1994; Foissner & Song 2002).
During the recent investigations on the ciliate biodiversity in the northern China seas, three *Frontonia* species were sampled and morphological data were collected, revealing that two forms are new to science and one belongs to a poorly-defined species, *Frontonia tchibisovae*.

Materials and methods

Frontonia didieri* n. sp.** and ***F. multinucleata* n. sp.** were sampled from sandy beaches at Qingdao, northern China (36°08’ N, 120°43’ E) on November 24, 2005 and June 1, 2006, respectively. ***Frontonia tchibisovae

Accepted by A. Wright: 15 Nov. 2007; published: 23 Jan. 2008

was sampled from a scallop-larvae-rearing pond near Yantai, northern China (37°33' N, 121°20' E) on April 22, 2006.

Ciliates were isolated and stained several days after sampling and kept for up to two weeks at room temperature (20–22 ºC) for further studies. Cells were observed *in vivo* first using a stereomicroscope, and then with high magnification under a compound microscope (×100–1250). The infraciliature was revealed by silver carbonate (Ma *et al.* 2003), protargol (Wilbert 1975) and silver nitrate impregnation methods (Song & Wilbert 1995). Drawings of living cells were based on free-hand sketches and photomicrographs, and those of impregnated cells were made with a camera lucida. Terminology is mainly according to Corliss (1979).

Genomic DNA extraction, PCR amplification and sequencing of the 18S rRNA genes from *Frontonia didieri* n. sp. (GenBank/EMBL accession number: DQ885986), *F. lynni* Long *et al.*, 2005 (DQ190463) and *F. tchibisovae* (DQ883820) were performed according to Shang *et al.* (2003).

Results

Frontonia didieri n. sp.

(Figs. 1–3; Table 1)

Diagnosis: Marine *Frontonia in vivo ca.* 100–150 × 45–80 μm, body dorsoventrally slightly flattened. 61–71 somatic, consistently 3 vestibular while 3–5 postoral kineties. Both peniculus 1 and 2 consisting of 4 kinety rows; peniculus 3 three-rowed, extremely different in lengths. One oval macronucleus. Single contractile vacuole centrally-located, with about eight conspicuous collecting canals.

Type location: A mesotrophic sandy beach near Qingdao, salinity *ca.* 12‰.

Type slides: One holotype with protargol impregnated specimens (slide number: 2007:5:17:1) is deposited in the Natural History Museum, London, UK, and one paratype with silver nitrate impregnated specimens (slide number: 2005110701-2) is deposited in the Laboratory of Protozoology, Ocean University of China.

Etymology: We dedicate this species to Dr. Pierre Didier, a famous French protozoologist, who has greatly contributed to the ciliate taxonomy and systematics.

Description: Size *in vivo* mostly about 120 × 60 μm, with ratio of length: width about 2:1. Body shape rather constant, elliptical in outline with both anterior and posterior ends slightly narrow; dorsoventrally flattened about 5:4 (Figs. 1A, 1E; 3A). Extrusomes spindle-shaped, about 4 μm long, densely arranged (Figs. 1A; 3B). Somatic cilia about 7 μm long. Cytoplasm transparent and colourless, usually filled with many large diatoms (up to 50 μm long) (Figs. 1A, 1E; 3E). Macronucleus ellipsoidal, centrally positioned (Figs. 1C, 1E, 1F; 3H). One contractile vacuole (CV), about 15 μm in diameter, positioned equatorially, with *ca.* eight long and conspicuous collecting canals (Figs. 1A; 3J). CV-pores (CVP) mid-dorsally positioned (Fig. 2A–D).

Movement mostly by gliding back and forth on substrate; when swimming, moderately fast with rotation about the long axis of the cell.

Buccal cavity shallow and small, triangular in outline, occupying about 1/6 of body length (Figs. 1A; 3A–C, 3G). Buccal apparatus as shown in Figs. 1C, 1G and 3F, 3K: consistently 3 long vestibular kineties (VK) with densely arranged kinetosomes, extending from anterior level of buccal cavity to about middle level of cell. 3 peniculi (P1–3) located on left wall of cavity: P1 and 2 about equally long, positioned close to each other, and each composed of 4 rows of kinetosomes, whereas the posterior ends of those rows in P1 are conspicuously shortened; peniculus 3 (P3) consisting of only 3 kineties, of which only the rightmost one is complete, the middle row is about half length and the leftmost one is extremely shortened, i.e. about 1/10 length of rightmost one. The paroral membrane (PM) double-rowed, located on right edge of the buccal cavity (Figs. 1G; 3F).

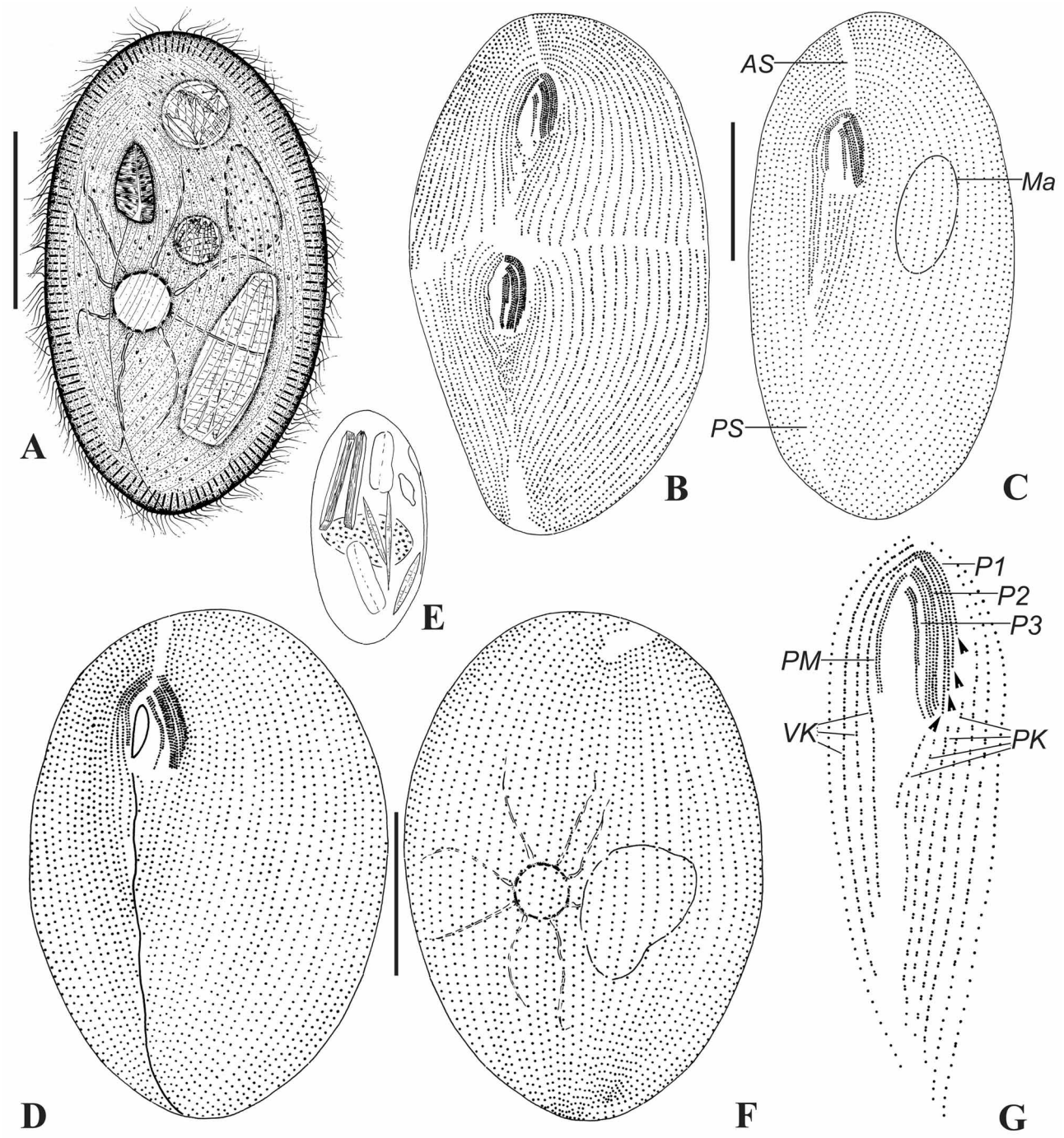

FIGURE 1. *Frontonia didieri* n. sp. from living cells (**A**), after protargol (**B, C, E, G**) and silver carbonate impregnations (**D, F**). (**A**) Ventral view of a typical specimen. (**B**) Ventral view of an individual in division. (**C, D, F**) To show the general infraciliature. (**G**) To show the oral apparatus, note the long vestibular kineties (VK) and two-rowed paroral membrane (PM). **AS** = anterior suture; **Ma** = macronucleus; **P1–3** = peniculus 1–3; **PK** = postoral kineties; **PS** = postoral suture. Scale bars = 40 μm.

On average 66 somatic kineties. Both anterior and postoral sutures conspicuously long and extending onto dorsal side (Figs. 1C, 1F; 2A–D). 3–5 postoral kineties (PK) locating posterior to the buccal cavity and ending at the postoral suture (PS) (Fig. 1C, 1D, 1G).

Silverline system as in other congeners: quadrangular cortical meshes after silver nitrate impregnation (Fig. 3D).

TABLE 1. Morphometric data of *Frontonia didieri* n. sp., *F. multinucleata* n. sp. and *F. tchibisovae* Burkovsky, 1970. Data are based on silver carbonate and silver nitrate impregnated specimens. All measurements in μm. CV = coefficient of variation in %; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of cells measured; P1–3 = peniculus 1–3; SD = standard deviation.

Characters	Min	Max	Mean	SD	CV	n
Body length						
F. didieri **n.sp.**	113	148	130.10	9.85	7.57	22
F. multinucleata **n.sp.**	72	112	94.20	10.42	11.06	20
F. tchibisovae	132	240	180.56	26.20	14.51	36
Body width						
F. didieri **n.sp.**	48	73	61.32	6.97	11.37	22
F. multinucleata **n.sp.**	44	72	57.40	7.82	13.62	20
F. tchibisovae	88	186	135.50	22.93	16.92	36
Number of somatic kineties						
F. didieri **n.sp.**	61	71	65.89	2.64	4.01	19
F. multinucleata **n.sp.**	58	67	63.39	2.28	3.60	18
F. tchibisovae	127	149	139.89	7.49	5.35	9
Number of postoral kineties						
F. didieri **n.sp.**	3	5	4.76	0.56	11.74	17
F. multinucleata **n.sp.**	4	5	4.24	0.44	10.38	17
F. tchibisovae	5	7	5.82	0.67	11.51	28
Number of vestibular kineties						
F. didieri **n.sp.**	3	3	3.00	0	0	18
F. multinucleata **n.sp.**	3	3	3.00	0	0	21
F. tchibisovae	3	4	3.07	0.27	8.79	27
Number of macronuclei/us						
F. didieri **n.sp.**	1	1	1.00	0	0	15
F. multinucleata **n.sp.**	2	4	3.76	0.66	17.55	17
F. tchibisovae	1	1	1.00	0	0	20
Number of ciliary rows in P1						
F. didieri **n.sp.**	4	4	4.00	0	0	18
F. multinucleata **n.sp.**	4	4	4.00	0	0	15
F. tchibisovae	4	4	4.00	0	0	20
Number of ciliary rows in P2						
F. didieri **n.sp.**	4	4	4.00	0	0	18
F. multinucleata **n.sp.**	4	4	4.00	0	0	15
F. tchibisovae	4	4	4.00	0	0	20
Number of ciliary rows in P3						
F. didieri **n.sp.**	3	3	3.00	0	0	17
F. multinucleata **n.sp.**	4	4	4.00	0	0	15
F. tchibisovae	4	4	4.00	0	0	20
Ratio of buccal/body length						
F. didieri **n.sp.**	0.14	0.21	0.17	0.02	11.76	17

to be continued.

TABLE 1. (continued)

Characters	Min	Max	Mean	SD	CV	n
Body length						
F. multinucleata **n.sp.**	0.21	0.28	0.24	0.02	8.33	14
F. tchibisovae	0.15	0.21	0.18	0.02	11.11	17
Number of CVP						
F. didieri **n.sp.**	1	4	-	-	-	4
F. multinucleata **n.sp.**	1	1	1.00	0	0	14
F. tchibisovae	1	3	2.17	0.58	26.73	23

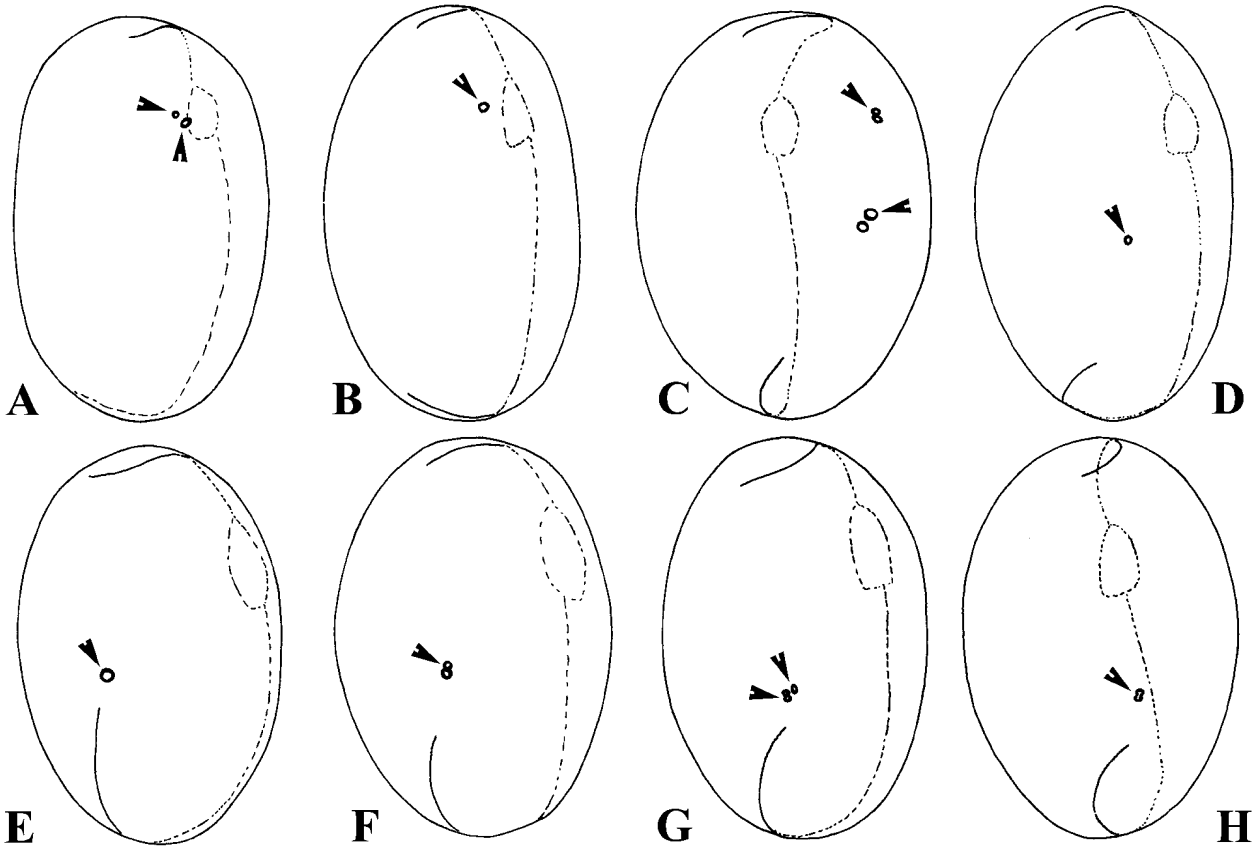

FIGURE 2. Dorsal-lateral views of *Frontonia didieri* n. sp. (**A–D**) and *F. tchibisovae* (**E–H**) (after silver nitrate impregnation), to show the CVP (arrowheads).

Comparison: Currently, over 40 morphotypes have been included in the genus *Frontonia* (Bullington 1939; Carey 1992; Dragesco 1960; Dragesco 1972; Dragesco & Dragesco-Kernéis 1986; Foissner *et al.* 1994; Kahl 1931; Long *et al.* 2005; Petz *et al.* 1995; Roque 1961; Roque & Puytorac 1972). Among those, about 27 were reported from fresh water biotopes, whereas *F. didieri* n. sp. is diagnosed by the unique, conspicuous contractile vacuole collecting canals from living cells (Alekperov 2005; Burkovsky 1970a, b; Foissner *et al.* 1994; Long *et al.* 2005; Petz *et al.* 1995; Roque 1961; Roque and Puytorac 1972).

Considering the body shape, size and the marine habitat, *Frontonia didieri* n. sp. is similar to *F. caneti* Dragesco, 1960 and *F. vacuolata* Dragesco, 1960 though in both forms the detailed structure of the oral apparatus is lacking (Table 2; Dragesco 1960). Nevertheless, *F. didieri* n. sp. can be clearly distinguished *in vivo* from the latter two by its centrally located contractile vacuole with the prominent collecting canals (vs. no collecting canals, CV left and located subcaudally in *F. caneti*, or located caudally in *F. vacuolata*) (Fig. 10D and 10Q).

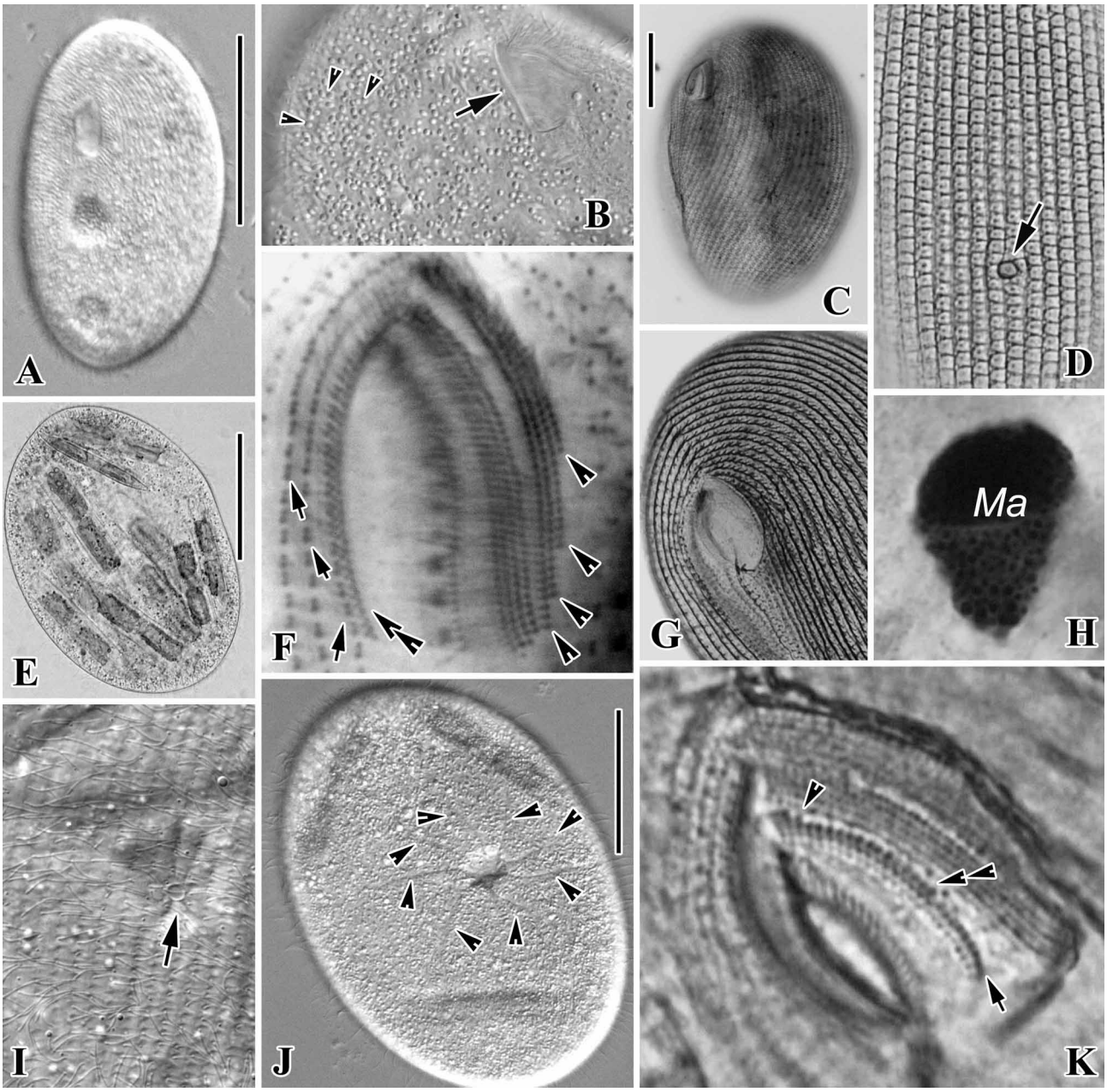

FIGURE 3. *Frontonia didieri* n. sp. from living cells (**A, B, E, I, J**), after silver carbonate (**G, H**), silver nitrate (**C, D, K**) and protargol (**F**) impregnations. (**A**) Ventral view, to show the typical body shape. (**B, G**) Anterior ventral part. Note the buccal cavity (arrow) and the extrusomes (arrowheads) in **B**. (**C**) Ventral view of a specimen. (**D**) To show the silverline system, arrow marks the CVP. (**E**) A pressed cell filled with diatoms. (**F**) Oral apparatus. Arrows indicate the vestibular kineties, arrowheads mark the posterior ends of kinety rows in P1, while double-arrowheads refer to the paroral membrane. (**H**) The macronucleus. (**I**) Arrow marks the CVP. (**J**) To show the contractile vacuole and the collecting canals (arrowheads). (**K**) To show the detail of the structure of P3. Note the shortest (arrowhead), the median-long (double-arrowheads) and the longest rows (arrow). Scale bars in (C) = 30 µm; in (J) = 40 µm; in (E) = 50 µm; in (A) = 70 µm.

Frontonia lynni and *F. salmastra* Dragesco and Dragesco-Kernéis, 1986 also resemble *F. didieri* n. sp. with reference to the general morphology (i.e. living cells) and the infraciliature of the buccal apparatus (Fig.10I, 10J, 10O, 10P). The new species can be recognized, however, in the presence of the collecting canals (vs. no collecting canals in the latter species), lower number of somatic kineties (61–71 vs. 71–83 in *F. lynni*, 90–100 in *F. salmastra*) and fewer kinety rows in peniculus 3 (3 vs. 4 in *F. lynni* and *F. salmastra*) (Table 2).

In addition, the dissimilarity of both forms is firmly supported by 18S rRNA gene sequence data as the sequence of *F. didieri* differs significantly in 143 nucleotides from that of *F. lynni* (structural similarity 91.8%) (Fig. 11).

***Frontonia multinucleata* n. sp.**
(Figs. 4–6; Table 1)

Diagnosis: marine *Frontonia in vivo* 70–120 × 40–75 µm, dorsoventrally flattened about 2:1. 58–67 somatic, 3 vestibular and 4–5 postoral kineties. 3 peniculi each with 4 kineties. 2–4 globular macronuclear nodules. Single contractile vacuole located in posterior 1/3 of cell length.

Type location: A clear sandy beach (salinity 30 ‰) of Qingdao, China.

Type slides: One holotype with silver nitrate impregnated specimens (slide number: 2007:5:17:2) is deposited in the Natural History Museum, London, UK, and one paratype (slide number: 2006060101-2) is deposited in the Laboratory of Protozoology, Ocean University of China.

Etymology: This species is the first one in *Frontonia* which has more than one macronucleus (2–4). Thus, it is named according to this character: *multi-* (many), *nucleata*.

Description: Size *in vivo* about 70–120 × 40–75 µm. Cell shape rather constant, ellipsoidal in outline when viewed from ventral side (Figs. 4A, 4B; 5A, 5D). Dorsoventrally flattened about 2:1. Extrusomes spindle-shaped, about 8 µm long (20–25 µm long after being ejected, Fig. 5B), densely arranged in cortex (Figs. 4A, 4B; 6C). Somatic cilia about 8 µm long. Cytoplasm colorless or grayish, often full of tiny granules, especially in caudal part (Figs. 4A, 4B; 5A, 5D). In all specimens observed from the culture which maintained for about 2 weeks, macronuclear nodules always in 2–4, yet mostly 4, generally globular in shape and about the same size, grouped or sparsely distributed (Figs. 4A, 4B, 4G, 4I; 6B, 6E, 6F). One large contractile vacuole located in posterior 1/3 of cell length, *ca.* 15 µm in diameter (Figs. 4A, 4B, 4E, 4J; 5E).

Movement mainly by gliding back and forth on substrate; when swimming, moderately rapid with rotation around the long axis of the cell.

Infraciliature as shown in Figs. 4I–K. Both anterior and postoral sutures conspicuous, which extend onto dorsal side (Fig. 4C, 4I, 4J). On average 63 somatic kineties; 4–5 postoral kineties (PK, Figs. 4H, 4I, 4K; 6N); 3 vestibular kineties, conspicuously short (Figs. 4H, 4I, 4K; 6J, 6L). Single CVP right dorsally located, at approximately posterior 1/3 of cell length (Figs. 4C; 6G, 6K).

Triangular buccal cavity occupying about 1/5 of body length. Peniculus 1–3 about equally long, all with 4 kineties; among them, P3 curved to right whose length becomes conspicuously shorter from right to left (Figs. 4F, 4H, 4I, 4K; 6D, 6H). Double-rowed paroral membrane on right side of buccal cavity (Figs. 4H, 4I, 4K; 6L). Argentophilic line positioned parallel to paroral membrane (Fig. 4H). Silverline system as quadrangular cortical meshes (Fig. 6I).

Comparison: Frontonia multinucleata n. sp. is the only one among members of the genus, which has consistently several macronuclear nodules (vs. single in all other known congeners). Hence, the new species is easily recognizable (Fig. 10C, 10D, 10I, 10J, 10K, 10M, 10N, 10R, 10S; Table 2).

***Frontonia tchibisovae* Burkovsky, 1970**
(Figs. 7–9; Table 1)

Neither details of living morphology, nor a clearly-outlined diagnosis of this species was given (Burkovsky

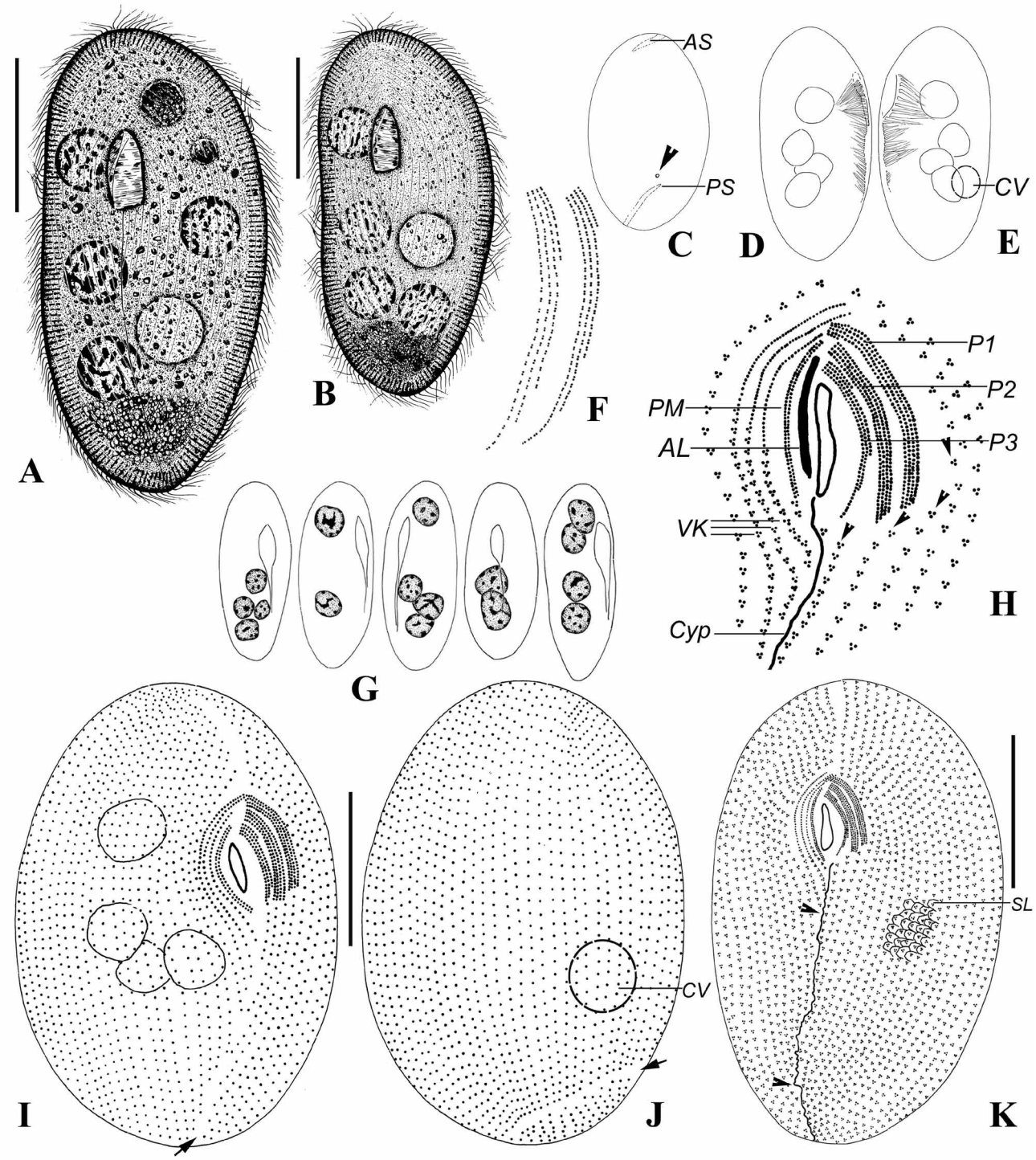

FIGURE 4. *Frontonia multinucleata* n. sp. from living cells (**A, B**), after silver nitrate (**C, F, H, K**), silver carbonate (**I, J**) and protargol (**D, E, G**) impregnations. (**A, B**) Ventral views of typical specimens. (**C**) Dorsal view, to show the contractile vacuole pore (arrowhead). (**D, E**) Side views, to show the fibers connected with the buccal and postoral suture area. Note the contractile vacuole is near the dorsal side. (**F**) Detailed structure of the peniculus 3. (**G**) To show the different situation of the macronuclear nodules. (**H**) The oral apparatus, note the two-rowed paroral membrane (PM) and the vestibular kineties (VK), in which the anterior part consists of monokinetids. (**I, J**) The infraciliature of ventral and dorsal (ventral-to-dorsal) side of the same specimen, to show the postoral suture (arrows in **I** and **J**), and the contractile vacuole. (**K**) Ventral view, note the long cytopyge (arrowheads). **SL** = silver line. Scale bars = 30 µm.

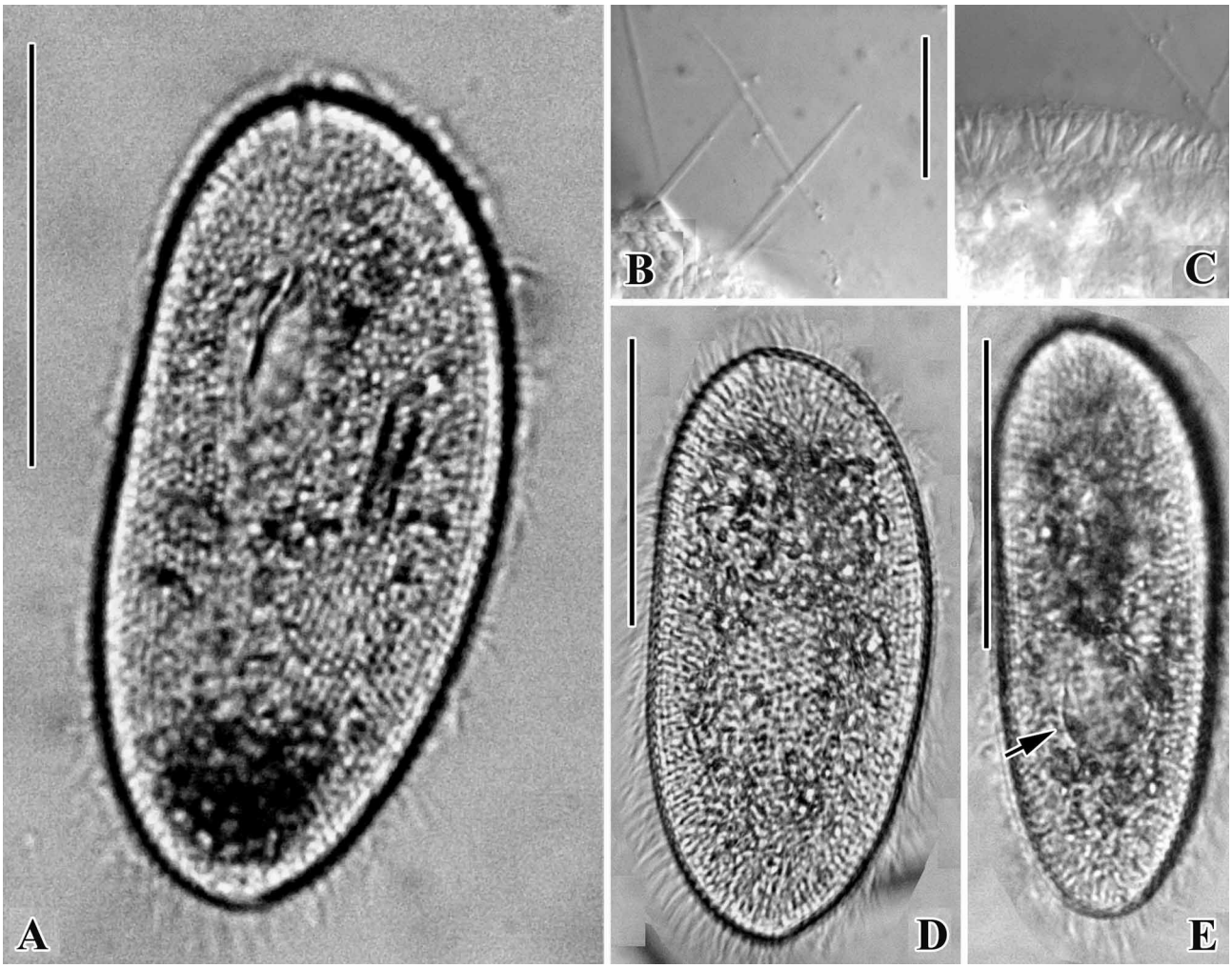

FIGURE 5. *Frontonia multinucleata* n. sp. from life (**A–E**). (**A, D**) Typical individuals. (**B**) To show the ejected extrusomes. (**C**) Extrusomes beneath the pellicle. (**E**) Lateral view, to show the contractile vacuole (arrow). Scale bars in (B) = 15 µm; in (D) = 45 µm; in (A, E) = 50 µm.

1970a). Hence a redescription and a redefined diagnosis based on both previous and present studies are supplied here.

Improved diagnosis: Marine *Frontonia*, *in vivo ca.* 130–250 × 80–190 µm, outline elliptical, dorsoventrally slightly flattened. 110–149 somatic, mostly 3 vestibular and 5–7 postoral kineties. Peniculus 1–3 each with 4 rows. One oval macronucleus. Single contractile vacuole centrally-located.

Sampling site: A scallop-larvae-rearing pond near Yantai, China, salinity *ca.* 27‰.

Voucher slides: One voucher slide with silver nitrate impregnated specimens (slide number: 2007:5:17:3) is deposited in the Natural History Museum, London, UK.

Description: Size highly variable, *ca.* 130–250 × 80–190 µm *in vivo*, but mostly about 200 × 140 µm. Body shape rather consistent, asymmetrical when viewed ventrally, slender elliptical in outline with narrowed posterior end; dorsoventrally slightly flattened (Figs. 7A; 8A, 8B). Somatic cilia *ca.* 8 µm long. Buccal cavity triangular in shape, about 1/5–1/6 of body length, positioned in anterior 1/3 of cell length (Figs. 7A; 8A, 8C, 8G; 9D). Cytoplasm hyaline and colourless, often with numerous tiny granules (< 6 µm long), especially in frontal or caudal portion (Figs. 7A; 8A, 8B). Macronucleus ellipsoid (Fig. 7A). Extrusomes spindle-like, 5 µm long, densely distributing in the cortex (Figs. 7A; 8A, 8C–E, 8G, 8H). Food vacuoles large, mainly containing algae and organic debris (Figs. 7A; 8F).

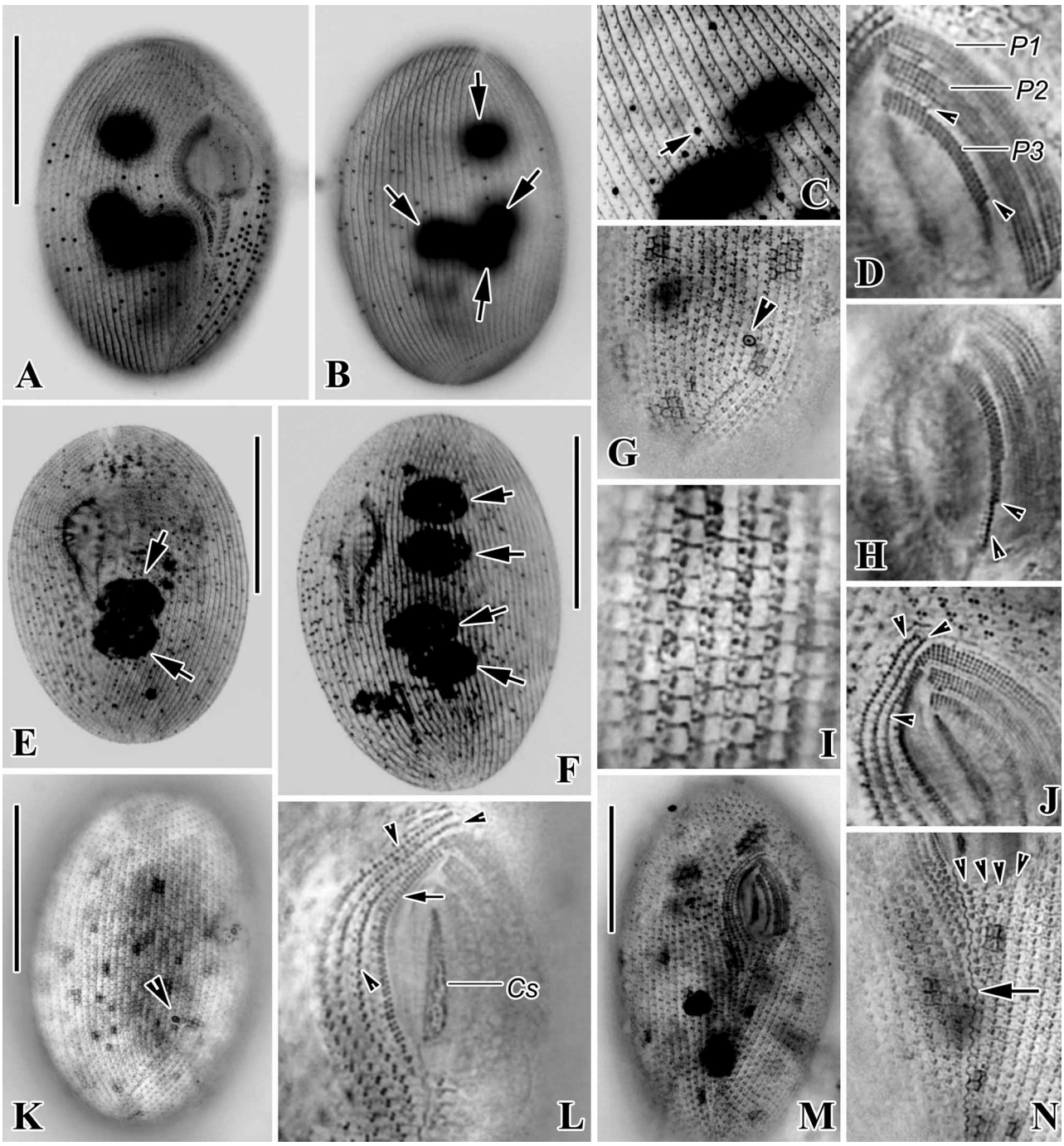

FIGURE 6. *Frontonia multinucleata* n. sp. after silver carbonate (**A–C, E, F**) and silver nitrate (**D, G, H–N**) impregnations. (**A, B, E, F, M**) Ventral and dorsal views of different cells. Note the macronuclear nodules (arrows). (**C**) Part of pellicle, arrow marks the extrusome. (**D, H**) Oral apparatus. Arrowheads mark the posterior ends of kinety rows in P3, which are in different lengths. (**G, K**) To point the CVP (arrowheads). (**I**) Detail of the silverline system. (**J, L**) Buccal area, to show the vestibular kineties (arrowheads) and the paroral membrane (arrow in **L**). (**N**) Structures posterior to the oral apparatus. Note the postoral kineties (arrowheads) and the cytopyge (arrow). Scale bars in (M) = 30 µm; in (A, E, F) = 45 µm; in (K) = 50 µm.

Movement by rotating about the long axis of the cell, slightly thigmotactic, sometimes attaching to the bottom of the Petri dish and circling.

Infraciliature as shown in Figs. 7C, 7D, 7G and 9H. 127–149 somatic kineties (Figs. 7C, 7G; 9E, 9H). Anterior and postoral sutures conspicuous (Figs. 7B, 7C, 7G; 9A). 5–7 postoral kineties located posterior to the buccal apparatus and ending successively beside the cytopyge (Fig. 7F).

Buccal apparatus typical of the genus. Mostly 3 vestibular kineties (at least two cells found with 4 VK, see Table 1) relatively short, starting from the anterior level of buccal cavity and ending before mid body level (Figs. 7E, 7F; 9C, 9H). Peniculus 1 and 2 each with 4 (about equally long) rows of kinetosomes; P3 slightly shorter, also composed of 4 rows, with their length gradually lengthened from left to right (Figs. 7F; 9B). Paroral membrane with an argentophilic line beside (Figs. 7C, 7F; 9I).

Silverline system typical of the genus (Fig. 9J). 1–3, on average two CVP-like pore(s) right mid-dorsally located, near right margin of the cell (n=23; Figs. 2E–H; 9F, 9G).

Remarks: With reference to the morphology of living cells, size, especially the features of the somatic and buccal ciliature, the Qingdao isolate corresponds very well with the original report by Burkovsky (1970a) (Fig. 7D, 7E). The only difference, as indicated originally, is the number of kineties in P3 (3 vs. 4 in the Qingdao population). This difference could be, in our opinion, a population-dependent diverse feature. Hence, we believe that the identification of our isolate is correct.

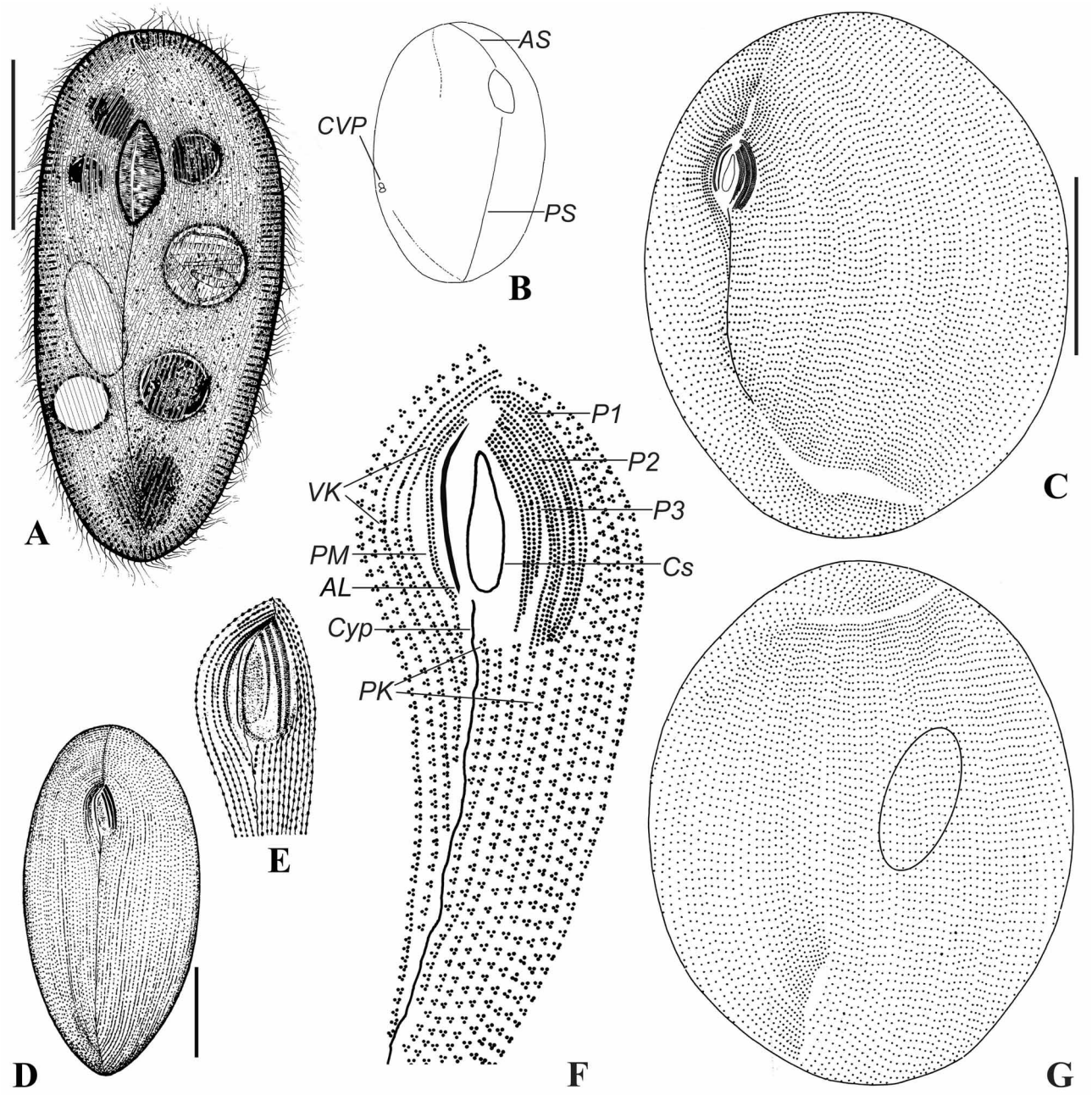

FIGURE 7. The Chinese population (except **D** and **E**) of *Frontonia tchibisovae* from living cells (**A**), after silver nitrate

(**B, D–F**) and silver carbonate (**C, G**) impregnations. (**A**) Ventral view of a typical specimen. (**B**) To show the position of the CVP. (**C, G**) Ventral and dorsal sides of the infraciliature. (**D, E**) General view of the ventral side and the oral apparatus (after Burkovsky 1970a). (**F**) Detail of the oral apparatus, note the 3 vestibular kineties (VK), of which the anterior part consists of monokinetids. **AL** = argentophilic line; **AS** = anterior suture; **Cs** = cytostome; **Cyp** = cytopyge; **P1–3** = peniculus 1–3; **PK** = postoral kinety; **PM** = paroral membrane; **PS** = postoral suture. Scale bars in (D) = 30 µm; in (A, C) = 60 µm.

The dorsally-located CVP-like pore(s) may probably derive from the CVP, as their location corresponds well with that of other known *Frontonia* species, or they are just structures, which were created by over staining.

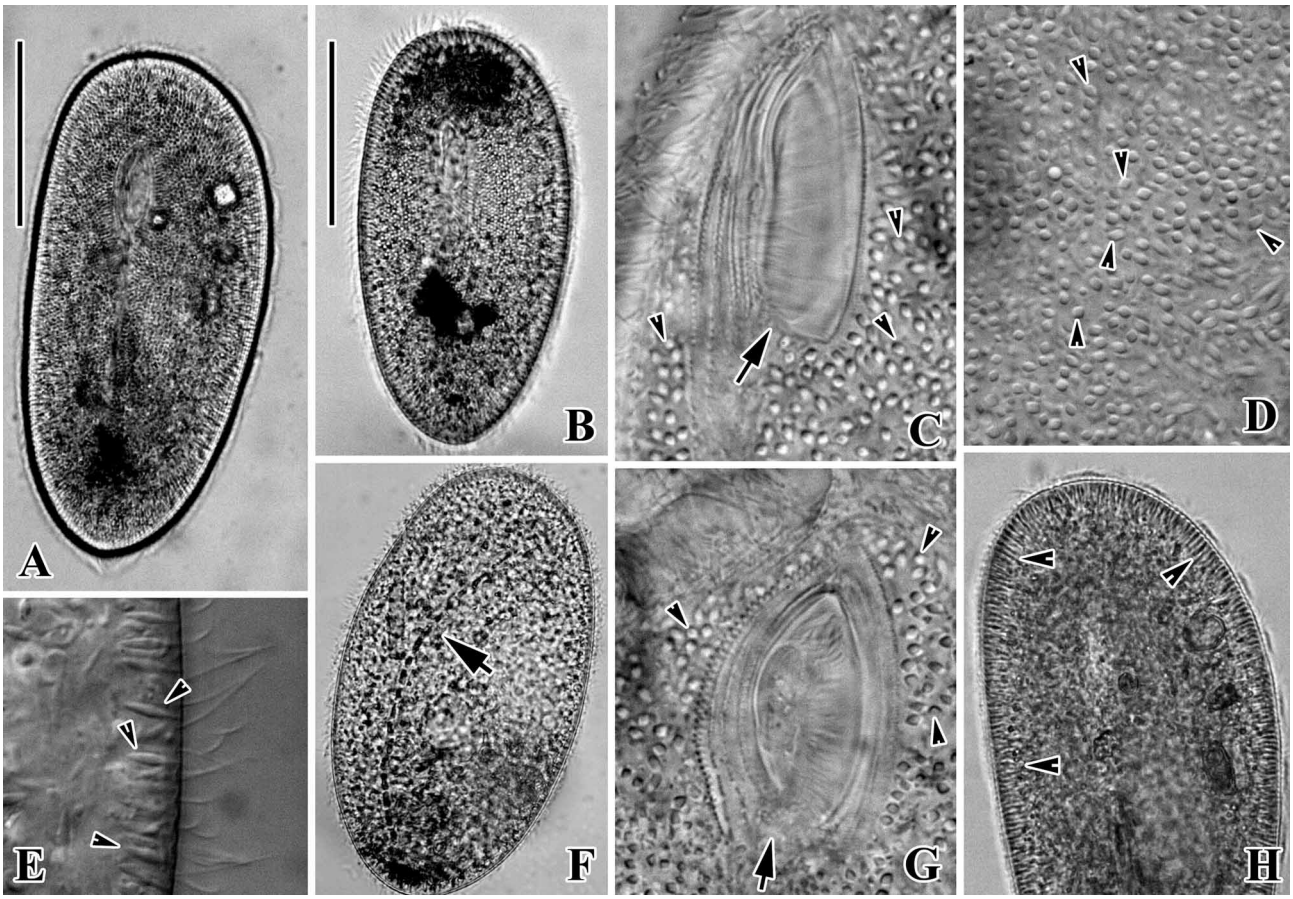

FIGURE 8. The Chinese population of *Frontonia tchibisovae* from life (**A–H**). (**A, B**) Ventral views of two typical specimens. (**C–E, G, H**) To show the densely-arranged extrusomes (arrowheads). Arrows in **C, G** mark the buccal field. (**F**) Arrow indicates an ingested large alga in the cytoplasm. Scale bars in (A) = 65 µm; in (B) = 80 µm.

With reference to the general morphology and the marine habitat, *Frontonia tchibisovae* is similar to the following morphospecies: *F. elongata* Burkovsky, 1970, *F. frigida* Petz *et al.*, 1995, *F. lynni, F. marina* Fabre-Domergue, 1891 and. *F. marisalbi* Burkovsky, 1970.

Frontonia elongata differs from *F. tchibisovae* in having fewer somatic kineties (45–50 vs. 110–149 in the latter) and a smaller buccal cavity (1/14–1/11 of body length vs. 1/7–1/5) (Fig. 10E, 10F; Table 2). *F. marina* has more vestibular kineties (6 vs. 3–4), kinety rows in P1 (6 vs. 4) and P2 (5 vs. 4), hence it can be clearly distinguished from *F. tchibisovae* (Fig. 10K, 10L; Table 2).

Compared with *Frontonia tchibisovae*, *F. frigida* has a different cell shape (lanceolate with both ends conspicuously narrowed vs. elliptical with wide ends in *F. tchibisovae*), more vestibular kineties (5 vs. 3–4 in *F. tchibisovae*) and a smaller buccal cavity (1/10–1/7 vs. 1/7–1/5 in *F. tchibisovae*) (Fig. 10G, 10H; Table 2)

(Petz *et al.* 1995). *F. marisalbi* can be easily distinguished from *F. tchibisovae* by its reniform body shape (vs. elliptical in the latter) and fewer rows in P3 (3 vs. 4 in the Qingdao population) (Fig. 10M, 10N; Table 2) (Burkovsky 1970a).

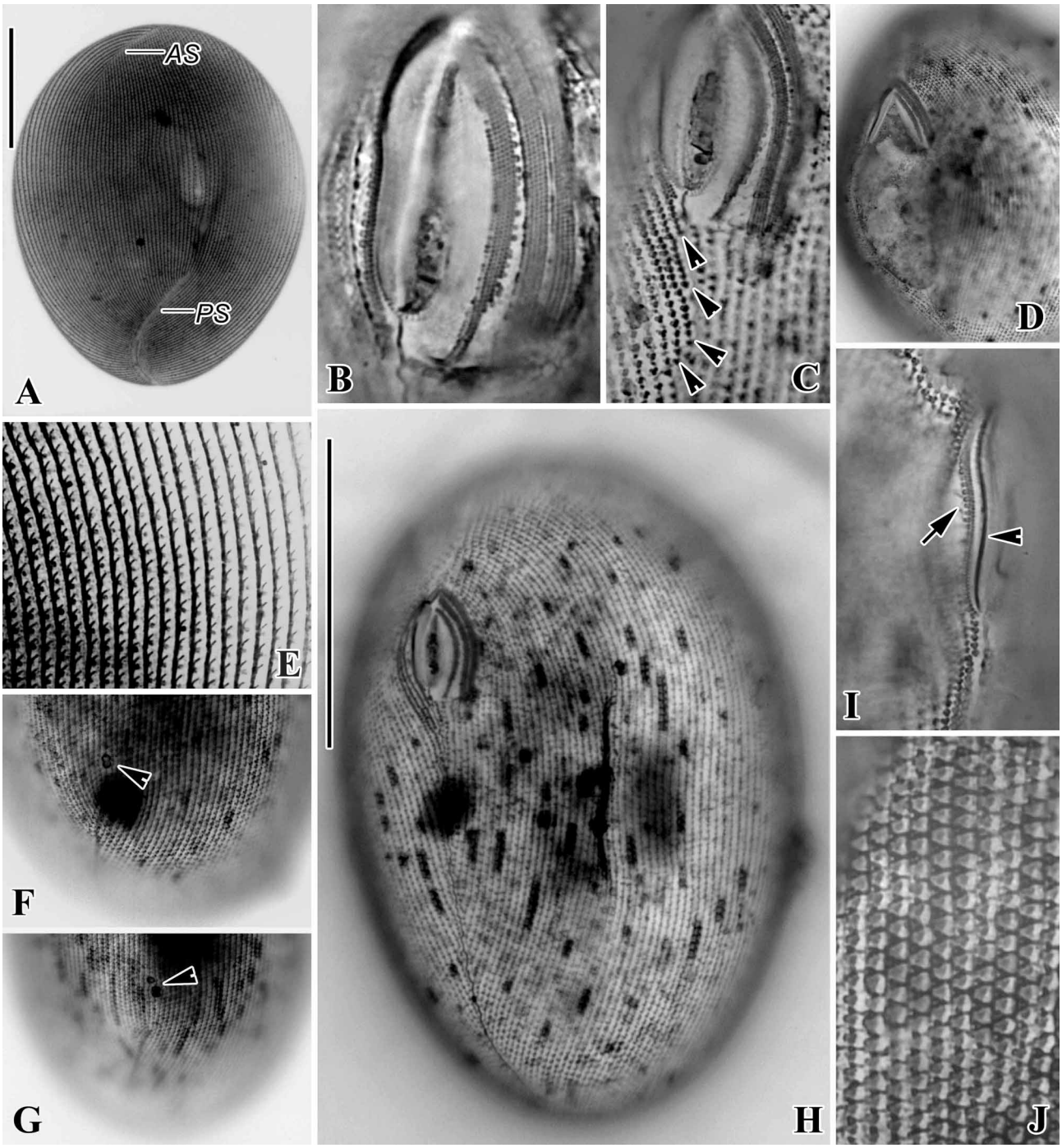

FIGURE 9. The population of *Frontonia tchibisovae* in China after silver carbonate (**A, E**) and silver nitrate (**B–D, F–J**) impregnations. (**A, F, G**) Dorsal views, to show the CVP (arrowheads). (**B, C**) Buccal area, to show the oral apparatus, arrowheads in **B** mark the vestibular kineties. (**D**) Ventral view of a cell ingesting food. (**E, J**) Pellicle and silverline system structure. (**H**) Ventral view of a whole cell. (**I**) To show the paroral membrane (arrow) and the argentophilic line (arrowhead). Scale bars in (A) = 60 µm; in (H) = 80 µm.

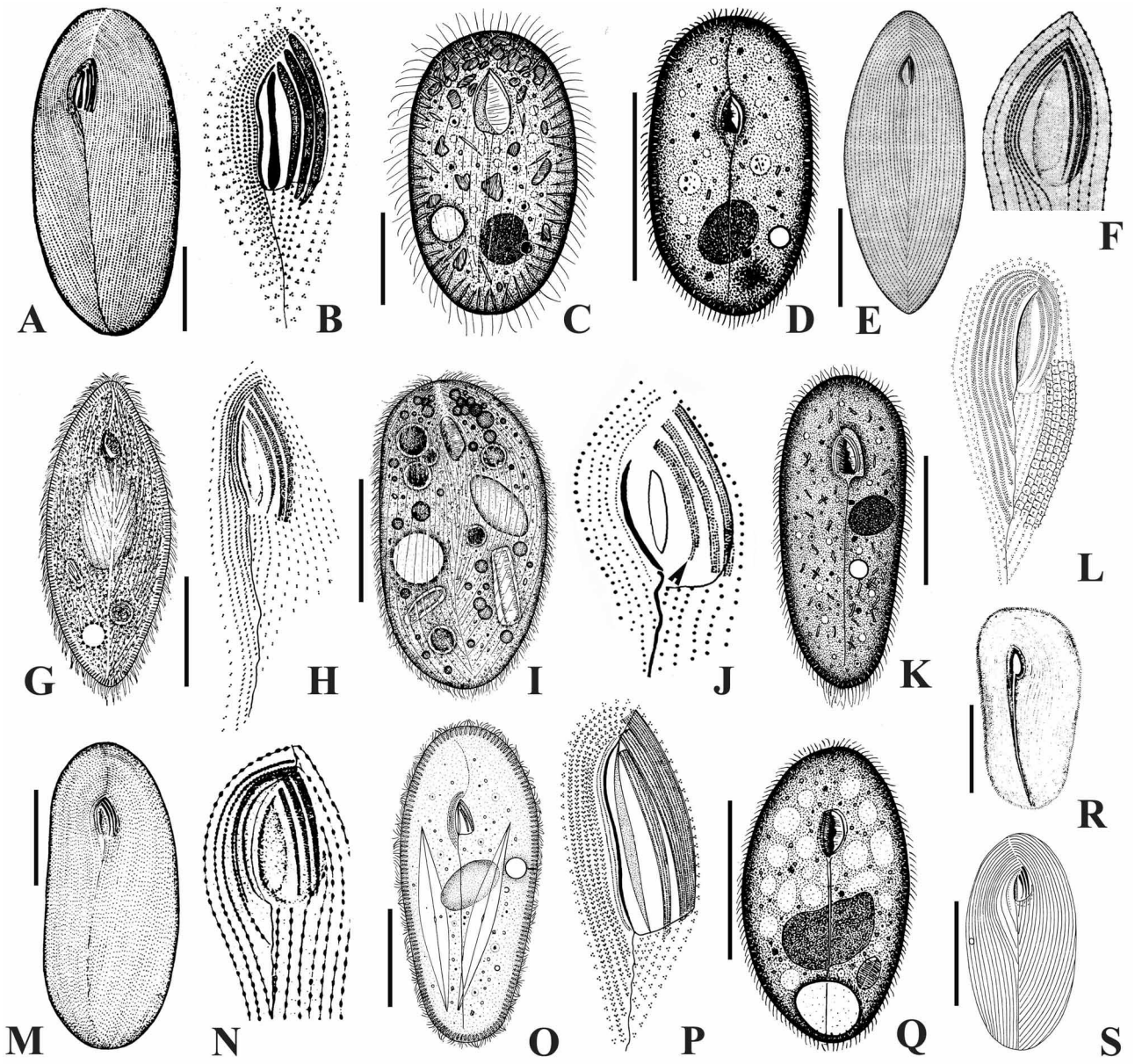

FIGURE 10. *Frontonia* species from living cells (**C, D, G, I, K, O, Q**) and after silver nitrate impregnations (**B, E, F, H, J, L, N, P**). (**A, B)** *F. azerbaijanica* Alekperov, 2005 (after Alekperov 2005). (C) *F. bullingtoni* Dragesco, 1960 (after Dragesco 1960). (**D)** *F. caneti* Dragesco, 1960 (after Dragesco 1960). **(E, F)** *F. elongata* Burkovsky, 1970 (after Burkovsky 1970b). (**G, H)** *F. frigida* Petz *et al.*, 1995 (after Petz *et al.* 1995). (**I, J)** *F. lynni* Long *et al.* 2005 (after Long *et al.* 2005). (**K, L)** *F. marina* Fabre-Domergue, 1891 (**K** after Dragesco 1960; **L** after Roque 1961). (**M, N)** *F. marisalbi* Burkovsky, 1970 (after Burkovsky 1970a). (**O, P)** *F. salmastra* Dragesco and Dragesco-Kernéis, 1986 (after Dragesco & Dragesco-Kernéis 1986). (**Q)** *F. vacuolata* Dragesco, 1960 (after Dragesco 1960). (**R)** *F. vernalis* Bullington, 1939 (after Bullington 1939). (**S)** *F. canadensis* Roque and Puytorac, 1972 (after Roque & Puytorac 1972). Scale bars in (C) = 25 µm; in (M) = 30 µm; in (S) = 50 µm; in (E) = 60 µm; in (G) = 90 µm; in (D, K, Q, R) = 100 µm; in (A) = 150 µm.

After rechecking the silver-nitrate impregnated specimens, it is confirmed that the P3 structure of *Frontonia lynni* was previously misinterpreted (Long *et al.* 2005): there should be 4 kineties in P3 rather than 5 as described in the original report, of which the rightmost "kinety" is in fact not composed of kinetosomes, but argentophilic dots along the right-most kinety (Fig. 10J, arrowhead). *F. tchibisovae* differs from *F. lynni* in having much more somatic kineties (110–149 vs. 71–83; Table 2). Besides, the dissimilarity of the two forms is firmly supported by 18S rRNA gene sequence data as the sequence of *F. lynni* differs in 30 nucleotides from that of *F. tchibisovae* (structural similarity 98.3%) (Fig. 11).

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F.lynni     : AACCTGGTTGATCCTGCCAGTTGTCATATGCTTGTCTTAAAGATTAAGCCATGCATGTCTAAGTTTAAATAGCATACAGTGAAACTGCGAATGGCTCATTAAAAC : 105
F.didieri   : .....................A.-.............C.........................A.......T.............................. : 104
F.tchibiso  : .....................A.....................................A.............................................. : 105

F.lynni     : AGTTATAGTTTATTTGATAGTATT-TTACATGGATAACCGTTGTAACTG-AGGGCTAATACATGCGCAAAGACCTGACTCACGAAAGGTTGTATTTATTAGATTT : 208
F.didieri   : ........................AA.................G....T.CT..A.................G..C.TT.-----....C.............. : 204
F.tchibiso  : .....................................G....T.CT.............................................................. : 209

F.lynni     : AACCATCACTTGGTGAATCATAGTAACTGATCGGATCGTGCGACTGCACGATAAATCATTCAAGTTTCTGCCCTATCAGCTT-CGATGGTAGTGTATTGGACTAC : 312
F.didieri   : ........T...................T.........C...TAA..GT..C.............................T..................... : 309
F.tchibiso  : ..........................................................................T..................... : 314

F.lynni     : CATGGCAGTCACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCACAAGAAGGCAGCAGGCGCGTAAATTACCCAAT : 417
F.didieri   : ..................................................................A..TA.G.........G..................... : 414
F.tchibiso  : ..................................................................A..TA.G.............................. : 419

F.lynni     : TCCGATTCGGAGAGGTAGTGACAAGAAATAATAACTTTAGAGGTTCGCCT-CTAGAAGATTGCAATGAGAACAATATAAATCATTTAACGAGTAACAATTGGAGG : 521
F.didieri   : C.........G.....................C....CG..T......T.CA...CGG.................C......T.........G.......... : 519
F.tchibiso  : C.........G.....................C.....CG...........-.....................................G.......... : 523

F.lynni     : GTAAGCCTGGTGATCAGCATCCGCAACAATTCCAGCTCCAATAGTGTATACATAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACTTCTGGTTGGGCACAGTCG : 626
F.didieri   : .C...T......-C.....G....GGT.............G...C......T.................................A...GAT....T.GAT.GTA : 623
F.tchibiso  : .C...T......-C.....G....GGT.............................................................................. : 627

F.lynni     : CGGCTTCC-GCCAGGCTGCTGTCTAATCATCCGTCTGCAACCCTACATCGGCCTTCACTGGTTCGAGTAGGTGAGCAGACAATTTACCTTGAAAAAATTAGAGTG : 730
F.didieri   : .....C.TT.T.T.C.AT...G.C..GTC.......AT..A....T....A....A.........GA....A...T.......................... : 728
F.tchibiso  : ........-.............................................................................................. : 731

F.lynni     : TTTCAGGCAGGTACTCGCCGGAATACATTAGCATGGAATAATGGAATAGGACTCCGATCCTTTGTTGGTTTCGGGATCAGAGTAATGGTTAATAGGAACAGATGG : 835
F.didieri   : ............CA.....................................TT.G...A...........T...C.C.........A........G........ : 833
F.tchibiso  : ..........................................................................A..................... : 836

F.lynni     : GGGCATTAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTATTAAAGACTAACTAATGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGT : 940
F.didieri   : ..................................................T.............................................. : 938
F.tchibiso  : .............................................................................................. : 941

F.lynni     : TAGGGGATCAAAGACGATCAGATACCGTCGTAGTCTTAACTATAAACTATACCGACTCGGGATCGGAGGGGTTTTCGTTTTTGCCCCTTCGGCACCGTATGAGAA : 1045
F.didieri   : ..........................................................A....A..TTA..CG....T..........C........ : 1043
F.tchibiso  : .............................................................................................. : 1046

F.lynni     : ATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAACTTGACTC : 1150
F.didieri   : ..........................................................................T....... : 1148
F.tchibiso  : ..........................................................................T....... : 1151

F.lynni     : AACACGGGGAAACTTACCAGGTCAAAACATGGATGGGATTGACAGATTGAAAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCCTAGTTGGTGGAG : 1255
F.didieri   : ..............................................................................T............ : 1253
F.tchibiso  : .............................................................................................. : 1256

F.lynni     : TGATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAACCTGCTAACTAGTTCGTTCCTGAATAAGGGGCGTAACTTCTTAGAGGGACTATGCGTGTAAACGC : 1360
F.didieri   : ..........................................G.............................................-C...... : 1357
F.tchibiso  : ..................................................A.............T.............................. : 1361

F.lynni     : ATGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCCTAGACGTCCTGGGCCGCACGCGCGCTACACTGACACGTTCAGCGAGCAAATTCACCTGGCCCGAAAGG : 1465
F.didieri   : ..........A.............................T.....................................TTC...T.............G.. : 1462
F.tchibiso  : ..........................................................................A.......... : 1466

F.lynni     : GTTCGGGAAATCTTGTTAGGACGTGTCGTGCTGGGGATCGATCTTTGCAATTATAGATCTTGAACGAGGAATTCCTTGTAAGCACAAGTCATCAGCCTGTGCTGA : 1570
F.didieri   : ........................................A.....................................CG..........A....... : 1567
F.tchibiso  : .............................................................................................. : 1571

F.lynni     : ATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTTCGAGTGATGTGGTGAACATTCTGGACTGCGCTAAGACTTGAGCTTGGTGCAGGAAGTT : 1675
F.didieri   : ..................................................................C.............T.TG.........CAAA...G.A..... : 1672
F.tchibiso  : .............................................................................................. : 1676

F.lynni     : ATGTAAACCTTATCACTTAGAGGAAGGAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA : 1747
F.didieri   : ............................G.................................................. : 1744
F.tchibiso  : .............................................................................. : 1748
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FIGURE 11. Small subunit rRNA gene sequence of *Frontonia lynni* (*F. lynni*) aligned with the sequence of *F. didieri* (*F. didieri*) and *F. tchibisovae* (*F. tchibiso*.). Numbers at the end of lines indicate the number of nucleotides. The differences in sequence length were compensated for by introducing alignment gaps (-) in the sequences. Matched sites are marked with dots.

Acknowledgements

This work was supported by the National Science Foundation of China (Project No. 40676076; 40506033; 30430090), and the grant from the Center of Excellence in Biodiversity, King Saud University. Many thanks are also due to Prof. Xiaozhong Hu, the Laboratory of Protozoology, OUC, for kindly reading the first draft of the manuscript and to Ms. Shan Gao and Mr. Hongbo Pan, for their technical help in sampling, staining and drawing. We really appreciate the kind help and great patience of Dr. Andre-Denis G. Wright, Zootaxa editor, in revising the manuscript.

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