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Morphology of Two Novel Species of *Chaenea* (Ciliophora, Litostomatea): *Chaenea paucistriata* spec. nov. and *C. sinica* spec. nov.

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Abstract. During faunistic studies of ciliates in coastal waters of Daya Bay and Bohai Bay, China, two previously unknown ciliates were discovered and investigated using standard taxonomic methods. Morphological comparative analyses revealed that they represent two novel species in the genus *Chaenea*. *Chaenea paucistriata* spec. nov. can be distinguished from its congeners by the following traits: body length *in vivo* about 180–250 μm; eight somatic kineties; dorsal brush rows 1–4 consisting of three, five, seven, and two dikinetids, respectively; rod-like extrusomes, 8 μm long; 63–94 macronuclei; cortical granules minute and colourless. *Chaenea sinica* spec. nov. differs from its congeners in having: body length *in vivo* about 140–240 μm; 17–21 somatic kineties; dorsal brush rows 1–4 consisting of 3–7, 10 or 11, 11–13, and 3–6 dikinetids, respectively; rod-like extrusomes about 6–8 μm long; 71–164 macronuclei. A key is presented to assist the identification of all *Chaenea* species.

Key words: *Chaenea*, ciliary pattern, identification key, marine ciliates, new species, taxonomy.

INTRODUCTION

The widespread haptorid genus *Chaenea* Quennerstedt, 1867 has been found in marine sand, freshwater, brackish water and moist soil (Borror 1963; Carey 1992; Dragesco 1960, 1966; Dragesco and Dragesco-Kernéis 1986; Fauré-Fremiet and Ganier 1969; Foissner 1984; Fryd-Versavel *et al.* 1975; Gao *et al.* 2008; Kahl 1926, 1927, 1928, 1933; Kwon *et al.* 2014; Lipscomb and Riordan 1990; Song *et al.* 2009; Wang 1934). Its members are characterized by the following features: cell elongate and contractile; cytostome apically located and surrounded by dikinetid circumoral kinety; somatic kineties which are slightly spiralled when contracted and mainly composed of monokinetids; dorsal brush consisting of four dikinetidal rows; one permanent contractile vacuole located at the posterior end of the body; extrusomes rod-like or thorn-like, attached to the oral

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bulge and scattered in the cell (Foissner *et al.* 1995, Petz *et al.* 1995, Song *et al.* 2009). Since being established, 14 nominal species have been assigned to this genus, namely, *C. crassa* Maskell, 1887, *C. gigas* Kahl, 1933, *C. limicola* Lauterborn, 1901, *C. minor* Kahl, 1926, *C. mirabilis* Kwon *et al.*, 2014, *C. psammophila* Dragesco, 1960, *C. robusta* Kahl, 1930, *C. sapropelica* Kahl, 1930, *C. simulans* Kahl, 1930, *C. stricta* (Dujardin, 1841) Foissner *et al.*, 1995, *C. teres* (Dujardin, 1841) Kent, 1881, *C. tesselata* (Kahl, 1935) Dragesco and Dragesco-Kerneis, 1986, *C. torrenticola* Foissner, 1984, and *C. vorax* Quennerstedt, 1867. In 1995, Foissner *et al.* synonymized *C. torrenticola* with *C. stricta*. Consequently, 13 species and an unidentified species from Petz *et al.* (1995) remain in the genus. Among these species, most have only been reported once, mainly based on living observation. Data on the ciliary pattern, especially detailed information regarding the dorsal brush, is only available for *C. teres* and *C. mirabilis* (Kwon *et al.* 2014, Petz *et al.* 1995). For *C. vorax* and *C. stricta*, although no statistical data is available, the number of dikinetids in the dorsal brush is shown in illustrations (Foissner *et al.* 1995, Song and Packroff 1997). Since this genus shares a similar pattern of general ciliature and body shape, some morphological characters, such as the number of dikinetids in the dorsal brush as well as the length and shape of extrusomes, have been used to distinguish closely related species (Petz *et al.* 1995). When describing novel species of this genus, therefore, these characters should be paid close attention to.

During a faunistic survey of ciliates in the coastal waters of Daya Bay and Bohai Bay, China, two species of *Chaenea* were isolated. Investigation of the morphology of living cells and their ciliature revealed that they represent two new forms.

MATERIALS AND METHODS

Chaenea paucistriata spec. nov. was collected from coastal waters of Daya Bay, China (22°42′N, 114°32′E), on December 12, 2007 when the water temperature was 21°C and the salinity was 30‰. *Chaenea sinica* spec. nov. was collected from coastal waters of Bohai Bay, China (37°37′N, 121°22′E), on March 27, 2006 when the water temperature was 8°C and the salinity was 34‰. Water samples were taken directly with a plastic jar, and then transported to the laboratory and maintained in Petri dishes. Rice grains were added to increase the amount of food for the bacteria (Chen *et al.* 2013). Large numbers of both species were present after about a week, when the rice granules had decomposed (Li *et al.* 2013). Living cells were observed using bright field and differential interference contrast microscopy (100 × to 1,000 × magnifications). Protargol staining was used to reveal the ciliature (Wilbert 1975). Counts and measurements of stained specimens were performed at a magnification of 1,000 ×. Drawings were made with the help of a camera lucida. Terminology mainly follows Kwon *et al.* (2014).

RESULTS AND DISCUSSION

Chaenea paucistriata spec. nov. (Figs 1, 2; Table 1)

Diagnosis: Extended cell size *in vivo* usually about 220 × 18 μm. Eight somatic kineties. Dorsal brush rows 1–4 consisting of three, five, seven and two dikinetids, respectively. About 63–94 macronuclei. Cortical granules minute and colourless.

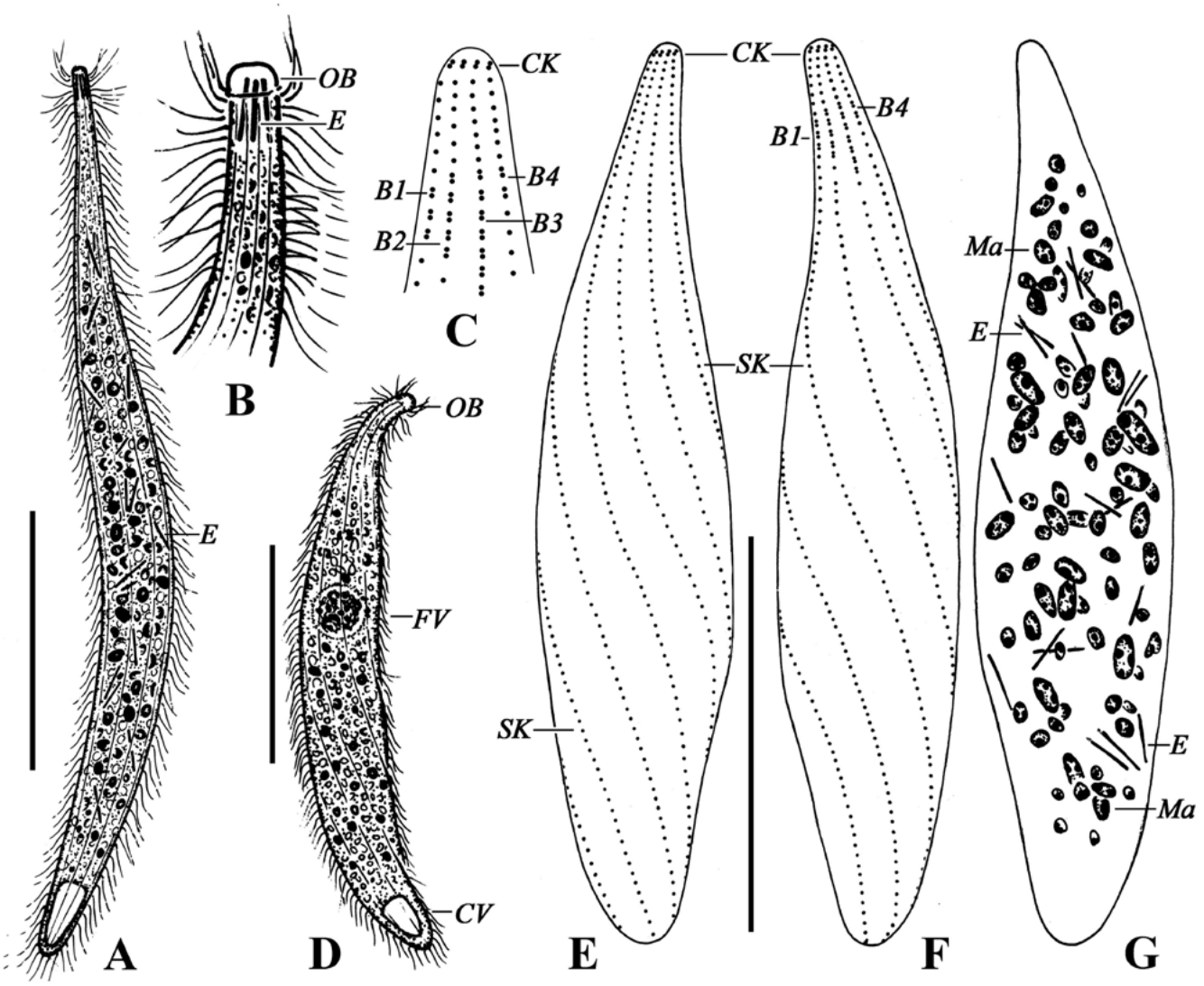
Type locality: Coastal water of Daya Bay (22°42′N, 114°32′E), China.

Type material: A protargol slide containing the holotype specimen marked with an ink circle is deposited in the Laboratory of Protozoology, Ocean University of China (Registry no. FXP2007122006).

Etymology: The species group name is a composite of the Latin prefix *pauci* (“few”), and the Latin adjective *striatus, -a, -um* [m, f, n] (“striated”), which reflects the fact that this species possesses fewer somatic kineties than its congeners.

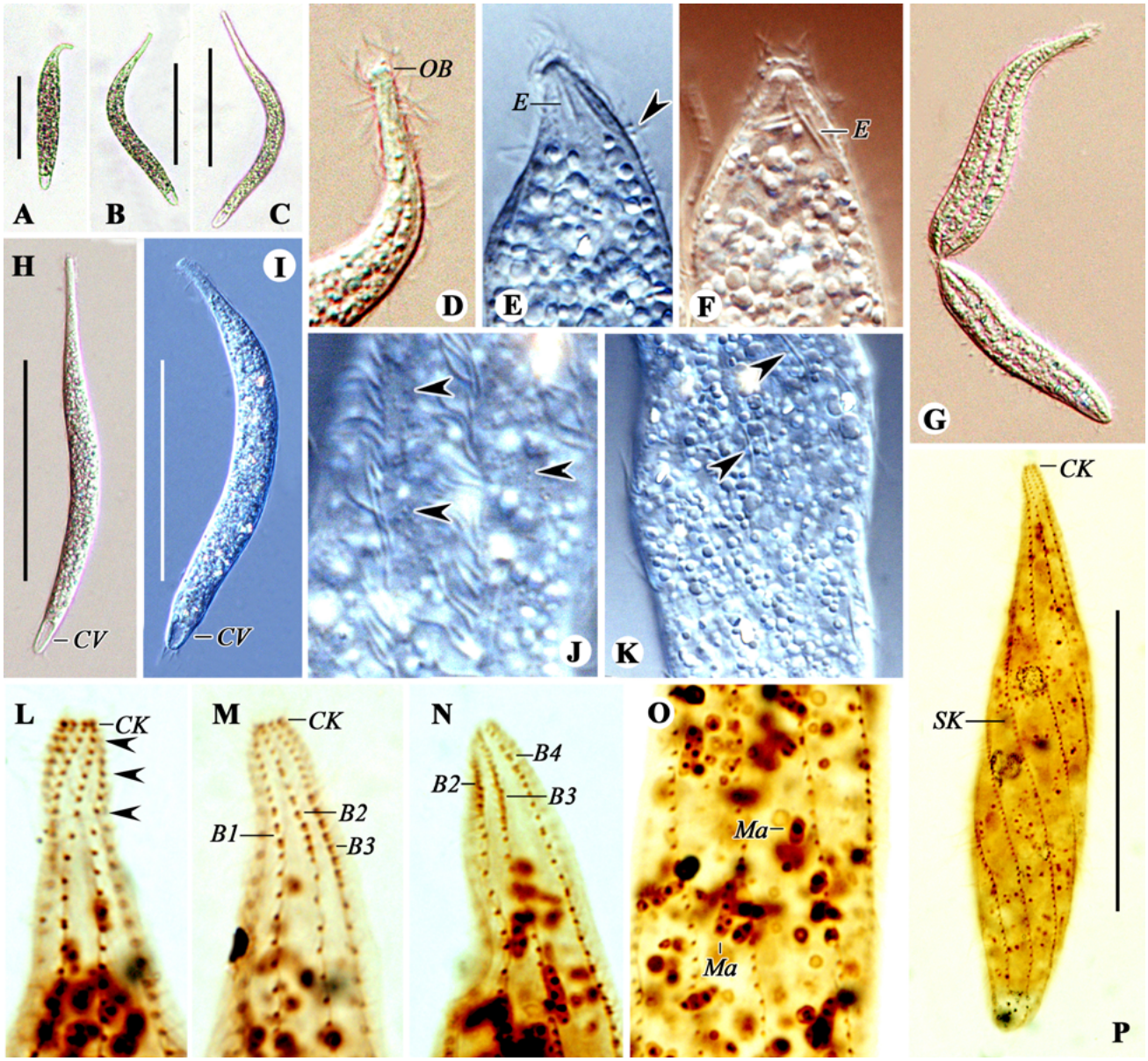
Gene sequence data: The small subunit rRNA gene sequence of *Chaenea paucistriata* spec. nov. was deposited in GenBank with accession number FJ876970 (Zhang *et al.* 2012).

Description: Extended cells measuring about 180–250 × 15–25 μm *in vivo*, with a length: width ratio of about 12–14:1; narrowly flask-shaped; cell very flexible and contractile; when contracted, cell measuring about 100–180 × 20–30 μm, with ratio of length to width about 4–7:1 (Figs 1A, D, 2A–C, H, I). Anterior portion of body distinctly narrowed, with an inconspicuous head; posterior part tapering to rounded (Figs 1A, D, 2A–C, H, I). Oral bulge, ca. 2 × 6 μm, on top of anterior body end, forming a short snout and usually bent (Figs 1A, B, D, 2A, D, H, I). 63–94 ellipsoid macronuclei, with size about 2–5 × 1–2 μm, scattered in the whole cell except for the anterior and posterior portion (Figs 1G, 2O). Single contractile vacuole located at the posterior end (Figs 1A, D, 2A–C, H, I). Extrusomes rod-like, about 8 μm long, usually in batches attached to oral bulge and scattered in cell (Figs 1A, B, G, 2E, F, K). Cortex flexible, and furrowed by somatic kineties (Fig. 2G). Cell



Figs 1A–G. *Chaenea paucistriata* spec. nov. *in vivo* (**A, B, D**) and after staining with protargol (**C, E–G**). **A** – a naturally extended individual, noting rod-shaped extrusomes scattered in cell; **B** – anterior body end to show oral bulge and the extrusomes attached to it; **C** – ciliary pattern of anterior end marking circumoral kinety and dorsal brush rows 1–4; **D** – a contracted individual, noting oral bulge, food vacuole and contractile vacuole; **E, F** – ciliary pattern of ventral (**E**) and dorsal (**F**) side of holotype specimen, indicating the circumoral kinety, dorsal brush rows 1–4, and somatic kineties; **G** – distribution of macronuclei and extrusomes. B1–4 – dorsal brush rows 1–4, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, FV – food vacuole, Ma – macronuclei, OB – oral bulge, SK – somatic kinety. Scale bars: 50 μm.

colour brownish in middle of body due to packed food vacuoles and cytoplasmic granules, while anterior portion and posterior end transparent (Figs 2A–C). Fine cortical granules colourless, with diameter less than 0.5 μm, distributed between somatic kineties (Fig. 2J). Cytoplasmic granules ellipsoid or round, with diameter about 2–5 μm (Figs 2E, F, K). Movement by slowly crawling on bottom of Petri dish. Typically, whole of somatic kineties consist of monokinetids (Figs 1E, F, 2L–N, P). Cilia about 7–8 μm long and arranged in longitudinal rows, although these become spiral in form in contracted specimens (Figs 1E, F, 2P). Consistently, eight somatic kineties, each of which consists of six or seven narrowly spaced oralized somatic monokinetids and 60–89 ordinarily spaced somatic monokinetids (Figs 1E, F, 2L, P). Four dorsal brush rows consistently



Figs 2A–P. *Chaenea paucistriata* spec. nov. *in vivo* (**A–K**) and after protargol impregnation (**L–P**). **A–C** – different body shapes; **D** – anterior body end to show the oral bulge; **E, F** – fine structure of anterior end to show rod-shaped extrusomes, arrowhead indicating cilia of the dorsal brush; **G** – dividing cell, showing cortical furrows along somatic kineties; **H, I** – typical individual, indicating contractile vacuole; **J** – cortical granules between somatic kineties (arrowheads); **K** – fine structure of the mid-body to show cytoplasmic granules and rod-shaped extrusomes (arrowheads); **L–N** – ciliary pattern of anterior body end, showing circumoral kinety narrowly spaces oralized somatic monokinetids (arrowheads), and dorsal brush rows 1–4; **O** – ciliary pattern in mid-body and many scattered macronuclei; **P** – overview showing circumoral kinety and somatic kineties. B1–4 – dorsal brush rows 1–4, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, Ma – macronuclei, OB – oral bulge, SK – somatic kinety. Scale bars: 90 µm (**A–C, H, I**), 70 µm (**P**).

comprising three, five, seven and two dikinetids respectively (number of specimens = 15) (Figs 1C, F, 2M, N). Cilia of dorsal brush about 3–4 µm long.

Oral bulge inconspicuous after protargol staining (Fig. 2L, M, P). Circumoral kinety inconspicuous and composed of dikinetids which are at the anterior end of each somatic kinety (Figs 1C, E, F, 2L, M, P).

Comparison: Considering the general morphology in terms of body length and the number of macronuclei, five species should be compared with *Chaenea paucis-*

Table 1. Morphometric data of *Chaenea paucistriata* spec. nov. (upper rows) and *C. sinica* spec. nov. (lower rows). All data based on protargol-impregnated specimens. CV – coefficient of variation in %, Max – maximum, Min – minimum, n – number of specimens investigated, SD – standard deviation of the mean.

Characters	Min	Max	Mean	SD	CV	n
Body, length in μm	103	165	141.1	19.9	14.1	15
	77	195	139.6	29.6	21.2	24
Body, width in μm	17	30	24.0	4.4	18.4	15
	27	47	34.5	4.0	11.6	24
Body, length:width ratio	4.7	7.5	5.8	0.9	15.5	15
	2.2	4.2	3.1	0.5	16.2	16
Anterior body end to the 1st	9	22	15.2	3.8	25.3	15
macronucleus, distance	10	17	13.6	2.5	18.1	10
Somatic kineties, number	8	8	8.0	0	0	15
	17	21	18.7	1.1	5.7	25
Macronuclei, number	63	94	81.9	11.3	13.7	15
	71	164	114.1	29.8	26.2	17
Macronucleus, length in μm	2	5	3.5	1.1	30.6	15
	2	4	3.5	0.7	21.0	15
Macronucleus, width in μm	1	2	1.5	0.5	35.2	15
	1	2	1.5	0.5	33.7	15
Oralized somatic monokinetids in	6	7	6.5	0.5	8.0	15
a somatic kinety, number	6	9	7.2	0.9	13.1	15
Kinetids in a somatic kinety, number	60	89	77.0	8.5	11.0	15
	89	188	139.7	30.7	22.0	15
Anterior body end to the beginning	5	8	6.5	0.7	11.4	15
of DB, distance	4	6	5.6	0.7	12.5	10
Anterior body end to the end of	8	10	9.7	0.6	6.4	15
DB1, distance	6	11	9.2	2.2	23.9	10
Anterior body end to the end of	10	12	11.7	0.6	5.1	15
DB2, distance	14	15	14.5	0.5	3.6	10
Anterior body end to the end of	12	14	13.8	0.6	4.1	15
DB3, distance	14	15	14.1	0.3	2.2	10
Anterior body end to the end of	8	9	8.9	0.4	4.0	15
DB4, distance	6	11	8.9	1.9	21.5	10
DB1, number of dikinetids	3	3	3.0	0	0	15
	3	7	5.7	1.3	23.5	10
DB2, number of dikinetids	5	5	5.0	0	0	15
	10	11	10.5	0.5	5.0	10
DB3, number of dikinetids	7	7	7.0	0	0	15
	11	13	11.9	0.9	7.4	10
DB4, number of dikinetids	2	2	2	0	0	15
	3	6	4.0	0.9	23.6	10

triata spec. nov., namely *C. teres*, *C. vorax*, *C. simulans*, *C. stricta* and an unidentified *Chaenea* species from Petz *et al.* (1995) (Figs 3A–E, G–M; Table 2).

Chaenea teres is similar to the new species in terms of body size, length of the extrusome and the presence of fine cortical granules; it can be distinguished, however, in having more somatic kineties (12–14 vs. 8), and more dikinetids in dorsal brush row 3 (14–17 vs. 7) and 4 (5–7 vs. 2) (Figs 3A–D; Table 2; Petz *et al.* 1995).

Chaenea vorax differs from *C. paucistriata* in having a smaller body length (100–180 μm vs. 180–250 μm), more somatic kineties (11 or 12 vs. constantly 8) and shorter extrusomes (5–6 μm vs. 8 μm) (Figs 3L, M; Table 2; Song and Packroff 1997).

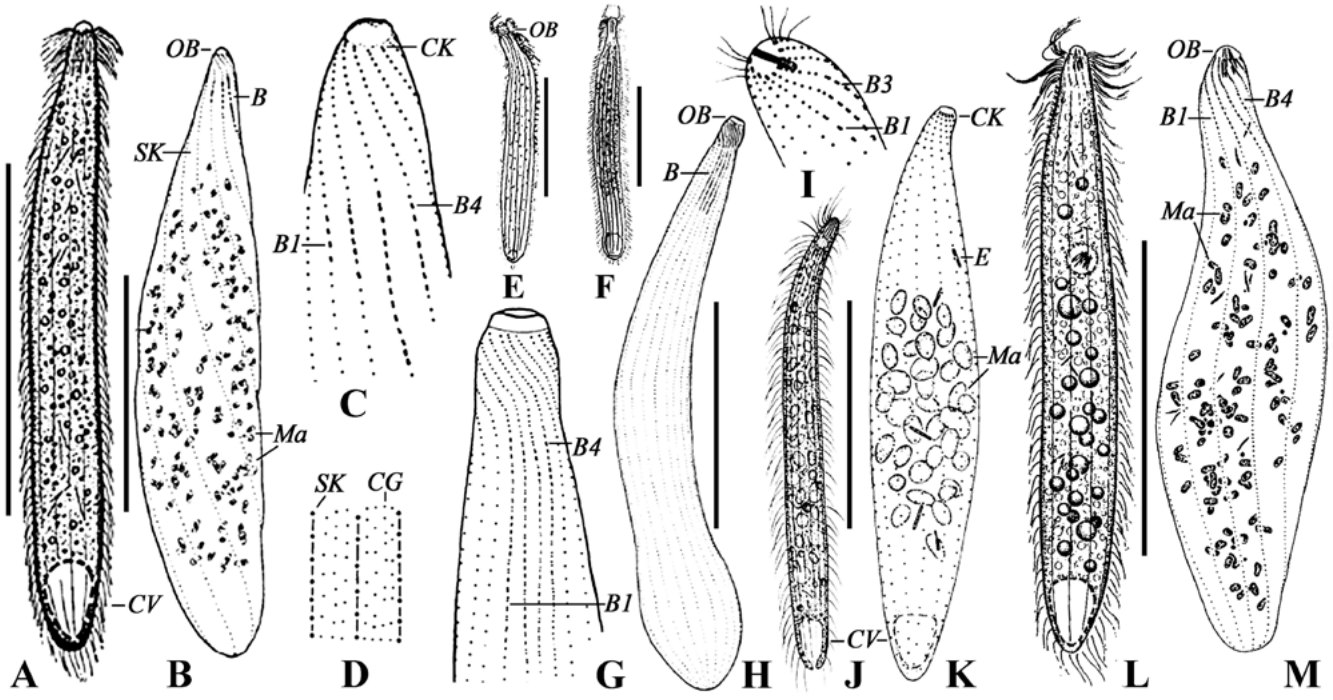
Chaenea simulans can be separated from the new species by having a longer body length (250–350 μm vs. 180–250 μm), more somatic kineties (12–14 vs. constantly 8) and a different habitat (brackish water with salinity 1‰ vs. marine water with salinity about 30‰) (Fig. 3E; Table 2; Kahl 1930).

Chaenea stricta (Dujardin, 1841) Foissner *et al.*, 1995 can be distinguished from the new organism through its smaller body length (90–130 μm vs. 180–250 μm), greater number of somatic kineties (11 or 12 vs. constantly 8), and different habitat (freshwater vs. marine water) (Figs 3I–K; Table 2; Foissner *et al.* 1995).

Although *in vivo* characteristics of *Chaenea* sp. sensu Petz *et al.*, 1995 are not available, it differs from the new species in having more somatic kineties (16–20 vs. constantly 8), and longer extrusomes (12–15 μm vs. ca. 8 μm) (Figs 3G, H; Table 2; Petz *et al.* 1995).

Chaenea sinica spec. nov. (Figs 4, 5; Table 1)

Diagnosis: Extended cell size *in vivo* usually about 200 × 20 μm. On average 19 somatic kineties.



Figs 3A–M. Morphology of some closely-related congeners of *Chaenea paucistriata* spec. nov. and *Chaenea sinica* spec. nov. **A–D** – *C. teres* (from Petz *et al*., 1995), general view of living cell (A), overview of ciliary pattern (B), detail of ciliary pattern in anterior body end (C), surface view showing cortical granulation (D); **E** – *C. simulans* (from Kahl, 1930); **F** – *C. robusta* (from Kahl, 1930) **G, H** – *Chaenea* sp. (from Petz *et al*., 1995), detail of ciliary pattern in anterior body (G), overview of ciliary pattern (H); **I–K** – *C. stricta* (from Foissner, 1984), detail of ciliary pattern in anterior body (I), general view (J), overview of ciliary pattern (K); **L, M** – *C. vorax* (from Song and Packroff, 1997), general view (L), overview of ciliary pattern (M). B – dorsal brush, B1–4 – dorsal brush rows 1–4, CG – cortical granules, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, Ma – macronuclei, OB – oral bulge, SK – somatic kinety. Scale bars: 100 μm (**A, H, L**), 50 μm (**B, J, K, M**), 150 μm (**E, F**).

Table 2. Comparison of *Chaenea paucistriata* spec. nov. and *C. sinica* spec. nov. with their closely related congeners. SK – somatic kineties, Ma – macronuclei, – data not available.

Species	Body length, *in vivo* (μm)	SK, number	Ma, number	Extrusome length (μm)	habitat	Data source
C. paucistriata spec. nov.	180–250	8	63–94	8	marine	present work
C. sinica spec. nov.	140–240	17–21	71–164	6–8	marine	present work
C. teres	120–270	12–14	hundreds	9	marine	Petz *et al.* 1995
C. vorax	100–180	11 or 12	80–110	5–6	marine	Song and Packroff 1997
C. simulans	250–350	12–14	>100	–	brackish water (1‰)	Kahl 1930
C. stricta	90–130	11 or 12	20–30	–	freshwater	Foissner 1984
C. robusta	300–400	ca. 15	ca. 50	12–15	marine	Kahl 1930
Chaenea sp.	–	16–20	ca. 150	12–15	marine	Petz *et al.* 1995

Dorsal brush rows 1–4 consisting of 3–7, 10 or 11, 11–13, and 3–6 dikinetids, respectively. About 71–164 macronuclei.

Type locality: Coastal waters of Bohai Bay (37°37'N, 121°22'E), China.

Type material: A protargol slide containing the holotype specimen marked with an ink circle has been deposited in the Laboratory of Protozoology, Ocean University of China (Registry no. WYG2006032701).

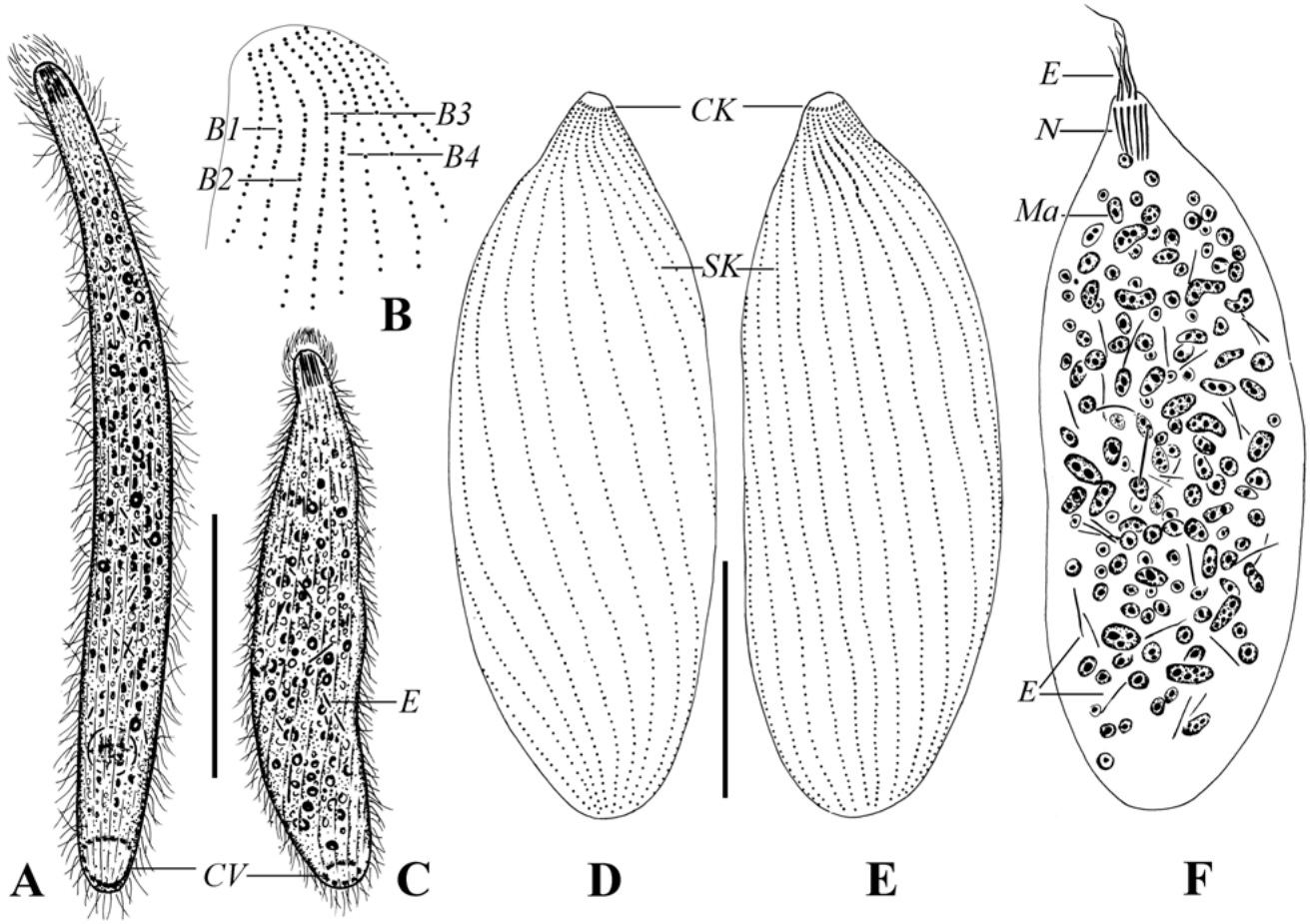
Etymology: The species-group name *sinicus, -a, -um* [m, f, n] reflects the fact that this organism was discovered in China.

Description: Extended cell size about 140–240 × 14–25 μm *in vivo*, with a length:width ratio of about 10–13:1; cell flexible and contractile; anterior body portion slightly narrowed and posterior part rounded (Figs 4A, C, 5A–E). Oral bulge inconspicuous (Fig. 5A–C). 71–164 ellipsoid macronuclei, with size about 2–4 × 1–2 μm, scattered in cytoplasm (Figs 4F, 5N). Single contractile vacuole located at the posterior body end (Figs 4A, C, 5B). Extrusomes rod-like, about 6–8 μm long, attached in batches to oral bulge, and scattered in cell (Figs 4A, C, 5F, G); extruded ones can be observed outside oral bulge, about 12–16 μm long (Figs 4F, 5L). Cortex flexible with distinct furrows present in some contracted specimens (Fig. 5E). Cell colour greyish due to packed food vacuoles, ca. 10 μm in diameter, and cytoplasmic granules, ellipsoid or round, ca. 1–4 μm in diameter (Figs 4A, C, 5C). Movement by slowly crawling on bottom of Petri dish, with anterior body portion continually contracting. 17–21 somatic kineties mainly consisting of monokinetids and extending the entire body length, each of which consists of 6–9 narrowly spaced oralized somatic monokinetids and 89–188 ordinarily spaced somatic monokinetids (Figs 4D, E, 5H–K). Somatic cilia about 5–6 μm long (Fig. 5D). Four dorsal brush rows consisting of 3–7, 10 or 11, 11–13, and 3–6 dikinetids respectively (Figs 4B, 5H–J). Cilia of dorsal brush undetectable in living cells, but observable in protargol stained specimens and about 2.5 μm long (Figs 4B, 5H–J, M). Circumoral kinety inconspicuous, composed of dikinetids which are at anterior end of each somatic kinety (Figs 4D, E, 5I). Nematodesmata, which can be observed after protargol staining, arising from circumoral kinety (Figs 4F, 5I).

Comparison: Considering the cell size, the number of macronuclei and somatic kineties, *Chaenea sinica* spec. nov. can be distinguished from most congeners. But, *Chaenea* sp. *sensu* Petz *et al.*, (1995) and *Chaenea robusta* Kahl, 1930 need to be compared with our new species.

Although no information regarding its living cell is available, *Chaenea* sp. *sensu* Petz *et al.* (1995) still differs from the new form in possessing longer extrusomes (12–15 μm vs. 6–8 μm) and more dikinetids, in particular in the dorsal brush rows (22–28, 25–30, 25–32, 24–29 vs. 3–7, 10 or 11, 11–13, 3–6). These two species can therefore be separated (Figs 3G, H; Table 2; Petz *et al.* 1995).

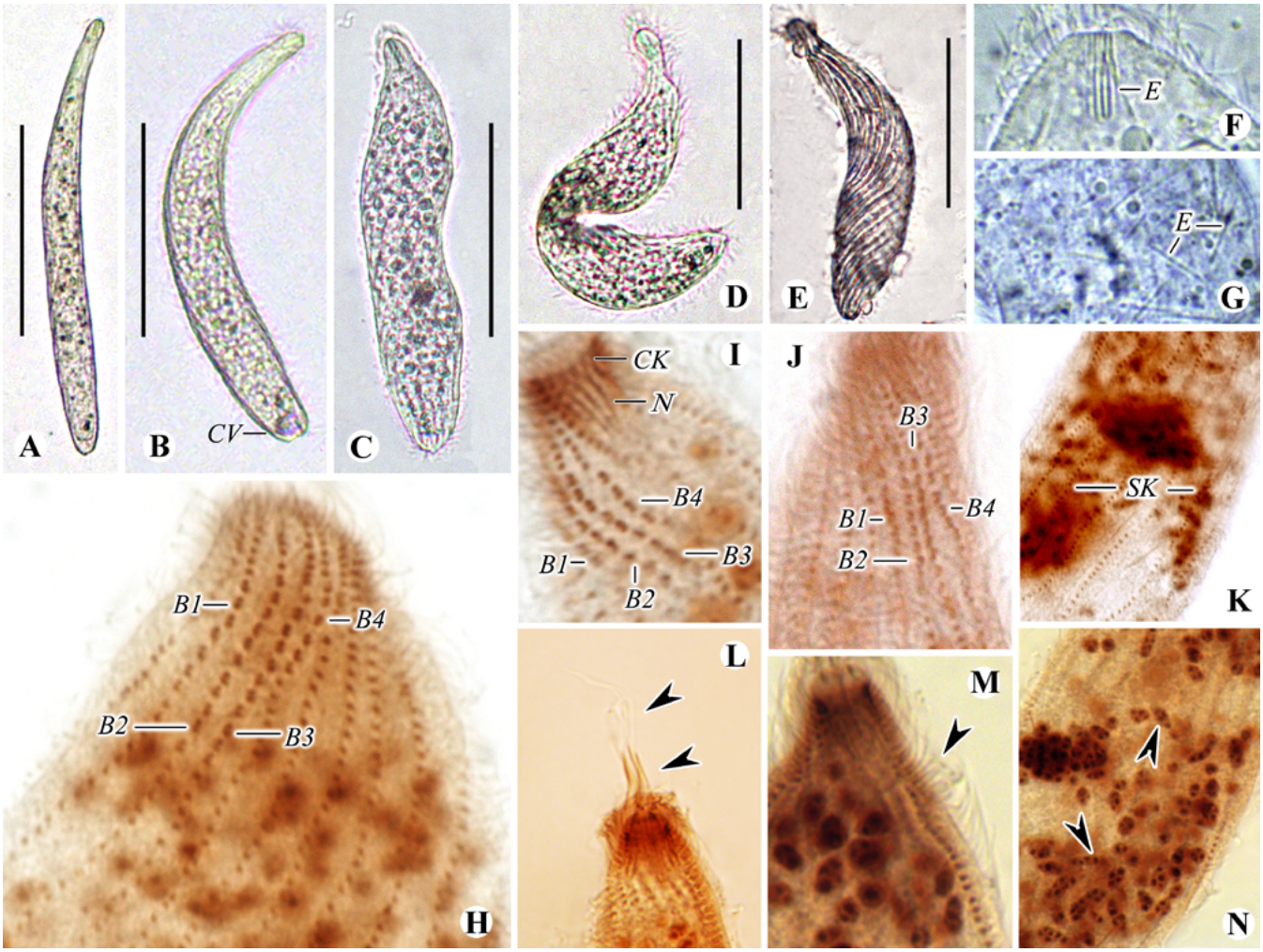
Chaenea robusta can be distinguished from the new species by having: (1) a longer body (300–400 μm vs. 140–240 μm), (2) longer dorsal brush cilia (8 μm vs. ca. 2.5 μm) and extrusomes (12–15 μm vs. 6–8 μm), and (3) fewer somatic kineties (about 15 vs. 19 on average) (Fig. 3F; Table 2; Kahl 1930).



Figs 4A–F. *Chaenea sinica* spec. nov. *in vivo* (A, C) and after protargol staining (B, D–F). **A** – typical extended individual; **B** – detail of anterior part of dorsal ciliary pattern; **C** – contracted individual; **D, E** – overview of ciliary pattern of ventral (**D**) and dorsal (**E**) side; **F** – distribution of macronuclei, extrusomes, and nematodesmata. B1–4 – dorsal brush rows 1–4, CK – circumoral kinety, CV – contractile vacuole, E – extrusome, Ma – macronuclei, N – nematodesmata, SK – somatic kinety. Scales bars: 50 µm.

Key to the identification of fifteen *Chaenea* species:

1	Posterior body end distinctly pointed	2
	Posterior body end rounded	3
2	Body length *in vivo* 625–833 µm	*C. crassa*
	Body length *in vivo* 130–150 µm	*C. limicola*
3	Body length *in vivo* ca. 1000 µm	*C. gigas*
	Body length *in vivo* smaller than 650 µm	4
4	Macronuclear nodules doughnut-shaped or horseshoe-shaped	5
	Macronuclear nodules ellipsoid	6
5	Five or six macronuclear nodules	*C. minor*
	11–21 macronuclear nodules	*C. mirabilis*
6	Two or three macronuclear nodules	7
	More than 20 macronuclear nodules	8
7	Anterior body part distinctly swollen	*C. tesselata*
	Anterior body part not distinctly swollen	*C. sapropelica*
8	Fewer than 11 or more than 21 somatic kineties	9
	11–21 somatic kineties	10



Figs 5A–N. *Chaenea sinica* spec. nov. *in vivo* (**A–G**) and after protargol impregnation (**H–N**). **A, B** – typical individuals; **C–E** – contracted and twisted cells; **F, G** – rod-shaped extrusomes attached to oral bulge (**F**) and scattered in cytoplasm (**G**); **H–J** – ciliary pattern of anterior body end, showing circumoral kinety, dorsal brush rows 1–4, and nematodesmata; **K** – middle part of body, showing somatic kineties; **L** – anterior body end, arrowheads denote the extruded extrusomes outside the oral bulge; **M** – anterior body end, arrowhead marks cilia of the dorsal brush; **N** – many scattered macronuclear nodules throughout cytoplasm (arrowheads). B1–4 – dorsal brush rows 1–4, CK – circumoral kinety, CV – contractile vacuole, E – extrusome, N – nematodesmata, SK – somatic kinety. Scales bars: 100 µm.

9	Eight somatic kineties	*C. paucistriata*
	34 somatic kineties	*C. psammophila*
10	Freshwater or brackish water (1‰) habitat	11
	Marine habitat	12
11	Body length *in vivo* 250–350 µm, more than 100 macronuclear nodules	*C. simulans*
	Body length *in vivo* 90–130 µm, 20–30 macronuclear nodules	*C. stricta*
12	Extrusome 12–15 µm long	*C. robusta*
	Extrusome 5–9 µm long	13
13	17–21 somatic kineties	*C. sinica*
	11–14 somatic kineties	14
14	Extrusome wedge-shaped, 5–6 µm long; cilia of dorsal brush 2 µm long	*C. vorax*
	Extrusome rod-shaped, 9 µm long; cilia of dorsal brush 10 µm long	*C. teres*

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