Taxonomy of five species of cyrtophorids (Protozoa: Ciliophora) including consideration of the phylogeny of two new genera

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Cyrtophorids are a specialized group of ciliated protozoa with multitudinous morphotypes. In the present work, the morphology and infraciliature of two new and three rarely known species, including two new genera of cyrtophorid ciliates, ***Heterohartmannula fangi* gen. et sp. nov.**, ***Aporthotrochilia pulex* (Deroux, 1976) gen. et comb. nov.**, ***Trochilia alveolata* sp. nov.**, *Trochochilodon flavus* Deroux, 1976, and *Hypocoma acinetarum* Collin, 1907, are described. *Heterohartmannula* gen. nov. is mainly characterized by a combination of features: two circumoral kineties obliquely arranged, podite not surrounded by somatic kineties, and no distinct gap between left and right ciliary field. *Aporthotrochilia* gen. nov. is diagnosed mainly by: podite present, oral ciliature reduced to two fragments, several kinety fragments positioned on the right posterior of frontoventral kineties and several terminal fragments. Phylogenetic analyses based on the small subunit rRNA (SSU rRNA) gene sequences support the establishment of two new genera and indicate that *Heterohartmannula* is most closely related to *Hartmannula*, and *Aporthotrochilia* is basal to the Cyrtophoria-Chonotrichia clade. *Trochilia alveolata* sp. nov. differs from its congeners mainly by having a conspicuous alveolar layer. In addition, detailed live and infraciliature data of *Hypocoma acinetarum* and *Trochochilodon flavus* are supplied.

INTRODUCTION

The cyrtophorid ciliates usually occur in aquatic habitats, and some of them are ectocommensal or parasitic of aquatic animals (Corliss, 1979; Hofmann, 1987). Cyrtophorids represent one of the most specialized groups in the Ciliophora, which are morphologically characterized by having toothed buccal basket, somatic kineties usually restricted to ventral side and macronuclei heteromerous (Deroux, 1994). Both oral ciliature and somatic kineties are of great morphological diversity, which gives rise to the high species diversity of the cyrtophorids. To date, there are over 40 genera in this group, inclusive of Hypocomatina (Kahl, 1931, 1933; Fauré-Fremiet, 1965;

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Deroux, 1976a, b, c; Corliss, 1979; Foissner, 1979; Carey, 1991; Song & Wilbert, 2002; Song, 2003; Gong & Song, 2006). Many investigations have been carried out in the last three decades (Dragesco & Dragesco-Kernéis, 1986; Foissner *et al.*, 1991; Petz, Song & Wilbert, 1995; Gong *et al.*, 2002, 2003, 2007, 2008; Gong & Song, 2004a, b; Shao, Song & Gong, 2008; Pan *et al.*, 2011), however, some known cyrtophorids still need to be redefined and new taxa have yet to be discovered.

In the past two decades, studies on ciliate biodiversity in China have mainly focused on the coastal waters off the northern seas (Xu & Song, 2006; Sun, Song & Xu, 2007; Chen *et al.*, 2008; Wang *et al.*, 2008; Fan *et al.*, 2010; Jiang *et al.*, 2010a, b; Li *et al.*, 2010c; Miao *et al.*, 2010; Pan *et al.*, 2010a, b). Recently, ciliate faunistic investigations in the coastal habitats off the South China Sea were carried out. These works demonstrated the high diversity in this subtropical area (Liu *et al.*, 2009, 2010; Chen *et al.*, 2010; Jiang & Song, 2010; Li *et al.*, 2010a; Shen *et al.*, 2010). Here we describe two new and three rarely known species, which were collected from the coast off both the northern and southern China seas. Two new genera are suggested.

MATERIAL AND METHODS

Heterohartmannula fangi gen. et sp. nov. was collected on 7 April 2010 from coastal waters off Donghai Island, Zhanjiang (21°14′N, 110°23′E), China (temperature 24 °C, and salinity 27‰).

Aporthotrochilia pulex (Deroux, 1976) gen. et comb. nov. was sampled on 15 December 2009 from coastal waters off Zhanjiang (21°14′N, 110°23′E), China (temperature 24 °C, salinity 25‰, pH 7.4).

Trochilia alveolata sp. nov. was isolated on 7 January 2010 from Clear Water Bay, Hong Kong (22°12′N, 114°117′E), with water temperature 23 °C and salinity 16‰.

Trochochilodon flavus Deroux 1976 was collected on 3 September 2009 from a sandy beach at Qingdao (36°04′ N, 120°23′ E), China. The water temperature was *c.* 25 °C and salinity was 31‰.

Hypocoma acinetarum Collin, 1907 was sampled on 10 May 2010 from coastal waters off Qingdao, China, using the slide method (Li *et al.*, 2010b). Water temperature was *c.* 17 °C and salinity was around 30‰.

Living cells were observed at 100–1000 × magnifications using bright-field and differential interference contrast microscopy. The counts, measurements, and drawings of stained specimens were performed at 1250 × with the aid of a camera lucida. The terminology and systematic scheme are mainly according to Corliss (1979) and Gong *et al.* (2009a).

Genomic DNA extraction, PCR amplification, and small subunit (SSU) rDNA gene cloning and sequencing were performed according to the method described by Yi *et al.* (2009).

Other than the SSU rRNA gene sequences of *Heterohartmannula fangi* and *Aporthotrochilia pulex*, the sequences used in this study were obtained from the National Center for Biotechnology Information GenBank database and all available SSU rRNA gene sequences of the class Phyllophrygea were included. *Pseudomicrothorax dubius* and *Leptopharynx costatus* were selected as the outgroups. Sequences were first aligned with CLUSTAL W implemented in BIOEDIT 7.0 (Hall, 1999) and further modified manually using BIOEDIT. The final alignment of 1462 characters and 35 taxa was used to construct phylogenetic trees.

The program MrModeltest v. 2.0 (Nylander, 2004) selected the general time reversible + Proportion Invariant (= 0.2613) + Gamma (= 0.4983) as the best model for both maximum likelihood (ML) and Bayesian inference (BI). A ML tree was constructed with the PhyML v. 2.4.4 program (Guindon & Gascuel, 2003). The reliability of internal branches was assessed using a nonparametric bootstrap method with 1000 replicates. A BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) using the Markov chain Monte Carlo algorithm. The program was run for 1 000 000 generations with a sample frequency of 100 generations and the first 2500 trees were discarded as burn-in. The remaining trees were used to calculate posterior probabilities (PP) using a majority rule consensus.

The following uncommon term is used in the present work:

Posterior fragment: In *Aporthotrochilia* gen. nov., several short kineties caudally positioned on the right of frontoventral kineties.

RESULTS AND DISCUSSION

Order: Cyrtophorida Fauré-Fremiet
in Corliss, 1956

Suborder: Dysteriina Deroux, 1976

Family: Hartmannulidae Poche, 1913

Genus: *Heterohartmannula* gen. nov.

Diagnosis: Dorsoventrally flattened Hartmannulidae with a tail-shaped podite; two circumoral kineties distinctly detached, obliquely arranged in a line with fragmented preoral kinety.

Type species: Heterohartmannula fangi sp. nov.

Table 1. Morphometric characteristics of ***Heterohartmannula fangi*** gen. et sp. nov. (1st line) and *Trochochilodon flavus* (2nd line) from protargol-impregnated specimens

Characters	Min	Max	Mean	SD	CV	N
Body length	49	89	61.0	10.04	16.5	22
	32	47	40.2	4.26	10.6	24
Body Width	20	32	24.4	3.09	12.7	22
	17	29	22.3	2.62	11.7	24
No. of somatic kineties	28	32	29.7	1.13	3.8	22
	9	9	9.0	0	0	24
No. of FvK	6	7	6.4	0.49	7.7	22
	3	3	3.0	0	0	24
No. of right kineties	10	12	10.7	0.65	6.1	22
	5	5	5.0	0	0	24
No. of left kineties	11	13	11.9	0.68	5.7	22
	4	4	4.0	0	0	24
No. of postoral kineties	5	9	7.0	1.05	15.0	22
	3	3	3.0	0	0	24
No. of basal bodies in TF	3	8	6.0	1.14	19.0	21
	5	7	5.3	0.56	10.6	24
No. of basal bodies in EF	–	–	–	–	–	–
	5	22	13.1	6.28	47.9	24
No. of nematodesmal rods	17	22	19.3	1.28	6.6	19
	–	–	–	–	–	–
No. of preoral fragments	2	4	3.0	0.53	17.7	22
	–	–	–	–	–	–
Length of macronucleus	13	28	18.0	3.02	16.8	22
	10	16	12.6	1.79	14.2	24
Width of macronucleus	8	15	10.9	1.25	11.5	22
	6	12	7.7	1.16	15.1	24

All measurements in μm. Abbreviations: CV, coefficient of variation (%); EF, equatorial fragment; FvK, frontoventral kineties; Max, maximum; Mean, arithmetic mean; Min, minimum; *N*, number of specimens; SD, standard deviation; TF, terminal fragment.

Etymology: The prefix 'hetero' indicates that the new genus is different from the well-known genus *Hartmannula*; feminine gender.

Remarks: The family Hartmannulidae is characterized by: presence of podite, left kineties as a continuous field and macronucleus heteromerous (Deroux, 1976c; Corliss, 1979). The genus *Heterohartmannula* corresponds well to these features. Thus it belongs to Hartmannulidae.

Compared with other related genera in Hartmannulidae (e.g. *Aegyriana, Brooklynella, Chlamydonyx, Hartmannula, Orthotrochilia, Trochilioides*), the oral ciliature of *Heterohartmannula* gen. nov. is unique: the circumoral kineties are detached and obliquely arranged in a line (vs. closely arranged as equal mark-like), and the preoral kinety is composed of several (e.g. two to four) distinctly detached fragments (vs. single

and continuous) (Deroux, 1976c; Song & Wilbert, 2002; Song, 2003; Gong & Song, 2004b, 2006). These differences support the establishment of a new genus.

HETEROHARTMANNULA FANGI SP. NOV.
(FIGS 1, 2; TABLE 1)

Diagnosis: Marine *Heterohartmannula* c. 60–90 × 20–30 μm *in vivo*, body oval-shaped in outline; pellicle of cilium-free field covered by densely arranged bacteria; 28–32 ventral kineties, the right-most six of which extend apically; about 19 nematodesmal rods; preoral kinety usually composed of three fragments; about six contractile vacuoles ventrally located; a yellowish pigment spot near the anterior tip of cell; podite subterminally positioned.

Type deposition: The holotype slide of protargol-impregnated specimens is deposited in the Laboratory
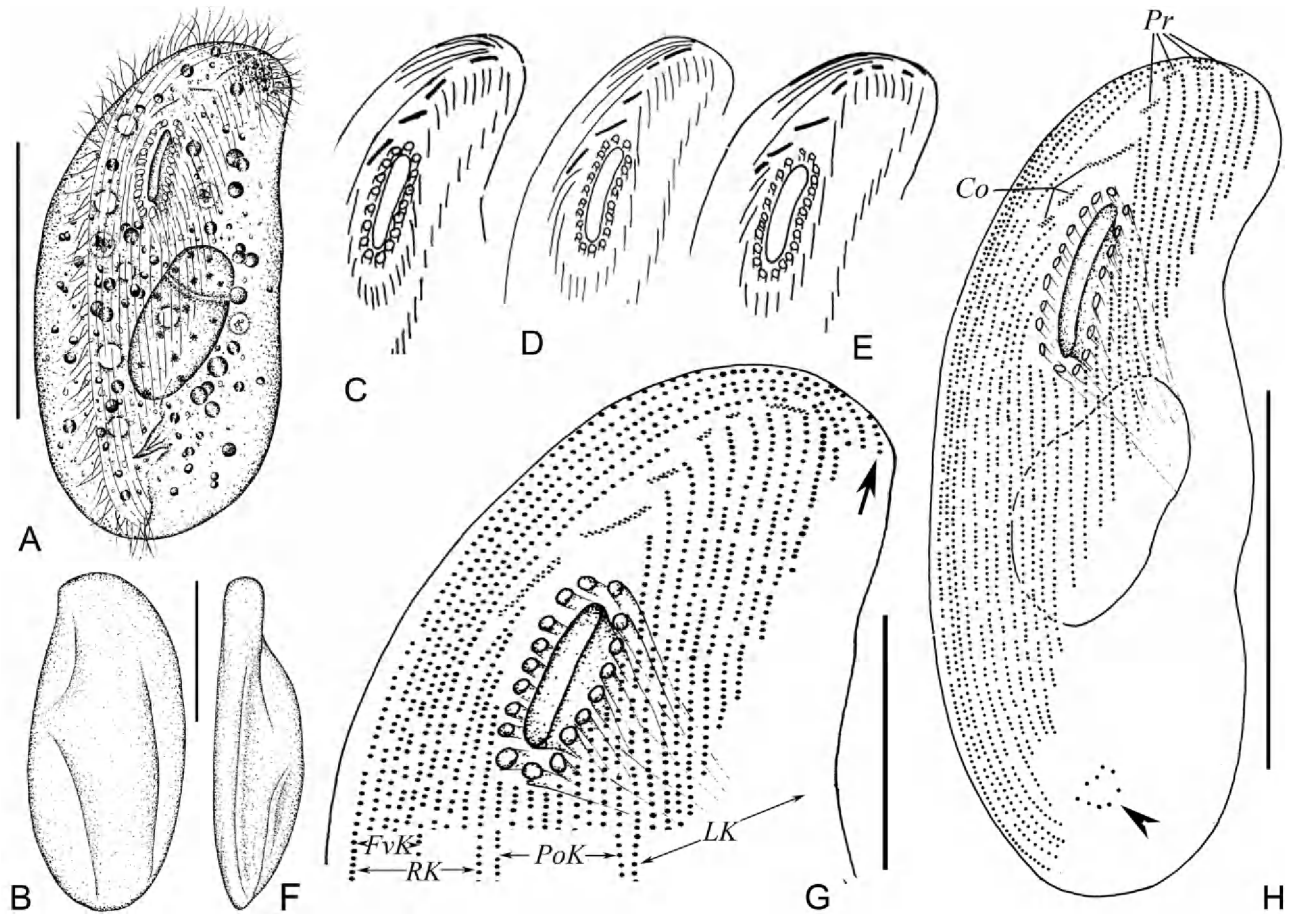
Figure 1. Morphology and infraciliature of ***Heterohartmannula fangi*** **gen. et sp. nov.** from life (A, B, F) and after protargol impregnation (C–E, G, H). A, ventral view of a typical individual. B, dorsal view. C–E, anterior of different individuals, to show variants of oral kineties. F, lateral view. G, anterior portion to show infraciliature, arrow marks terminal fragment. H, ventral view, arrowhead points to kinetosome-like granules near base of podite. Abbreviations: Co, circumoral kineties; FvK, frontoventral kineties; LK, left kineties; PoK, postoral kineties; Pr, preoral kineties; RK, right kineties. Scale bars = 40 μm (A, G, H), 25 μm (F).

of Protozoology, Ocean University of China, China (No. PHB10040701). A paratype slide is in the National History Museum, London, UK (registration number 2010:11:7:2).

Type locality: Coastal waters off Donghai Island, Zhanjiang (21°14′N, 110°23′E), China (temperature 24 °C, salinity 27‰).

Dedication: We dedicate this new species to our respected colleague, Prof. Dr Jingyun Fang, Peking University, China, in recognition of his tremendous contributions to the fields of biodiversity and plant ecology.

Description: Cell size about 60–90 × 20–30 μm *in vivo*, body highly flexible and slightly contractile, usually long oval in outline. Right margin more convex than left, with anterior 'beak' projecting to left; usually ventral surface flat and dorsal surface vaulted in midbody (Figs 1A, B, F, 2A–E). Pellicle of cilium-free field conspicuously covered with densely arranged bacteria (Fig. 2F). Podite about 7 μm in length, subterminally positioned. Cytostome slit-like, surrounded by 17–22 straight tooth-tipped nematodesmal rods, extending posteriorly slightly leftwards (Figs 1G, 2H). Cytoplasm colourless or greyish, usually with numerous granules (1–2 μm across), which render the cell more or less opaque. One small patch-like pigment spot positioned at anterior left of cell, usually yellowish to dark yellow in colour (Fig. 2H, arrowhead). About six contractile vacuoles (4 μm in diameter), most of which are longitudinally arranged on ventral side (Figs 1A, 2C). Cilia about 7 μm long. Movement slow, usually crawling on substrate.
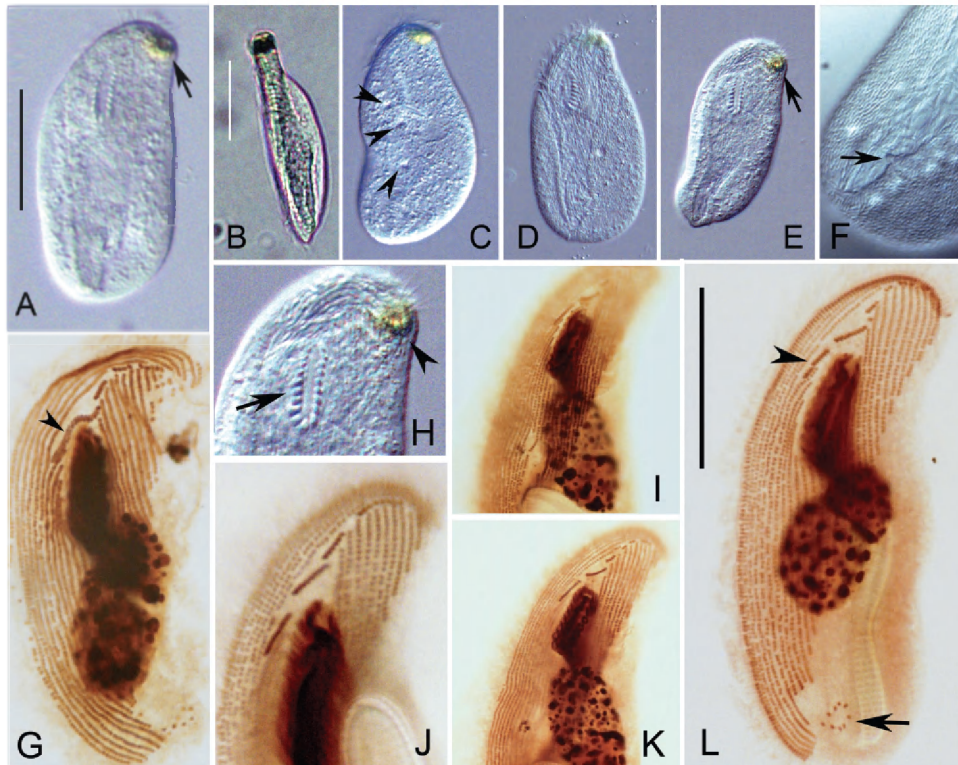
Figure 2. Photographs of ***Heterohartmannula fangi* gen.** et **sp. nov.** from life (A–F, H) and after protargol impregnation (G, I–L). A, ventral view of a typical individual, arrow points to the yellowish pigment spot. B, lateral view. C–E, to show shape variants, arrowheads mark contractile vacuoles, arrow indicates the yellowish pigment spot. F, posterior of cell, arrow refers to podite. G, ventral view, arrowhead shows that two circumoral kineties converge as one. H, anterior portion, arrow points to cytostome and arrowhead indicates the yellowish pigment granule. I–K, anterior portion of different individuals. L, ventral view, arrowhead marks the right circumoral kinety, arrow points to kinetosome-like granules. Scale bars = 40 μm.

Infraciliature as shown in Figures 1C–E, G, H, 2G, I–L. 28–32 somatic kineties, the right-most six to seven of which extend apically. Ten to 12 right, five to nine postoral, and 11–13 left kineties surrounding the oral area; left and postoral kineties posteriorly shortened progressively from right to left (Figs 1G, H, 2G, L). Terminal fragment (Fig. 1G) consisting of three to eight basal bodies; equatorial fragment not detected. About nine kinetosome-like dots present near the base of podite (arrowhead in Fig. 1H and arrow in Fig. 2L). Macronucleus ellipsoidal, heteromerous, about 18×11 μm in size. Micronucleus invisible.

Two circumoral kineties obliquely arranged, the right of which is sometimes divided into two fragments (three of 22 individuals) (Figs 1C–E, 2J–L), and they seldom connect with each other (one of 22 individuals) (Fig. 2G). Preoral kineties usually comprising two to four fragments (Figs 1C–E, 2J–L).

Comparison: Considering general morphology (e.g. cell size and shape, numbers of somatic kineties and contractile vacuoles), *Heterohartmannula fangi* sp. nov. resembles *Hartmannula angustipilosa*. However, it can be separated from the latter by obliquely (vs. in parallel) arranged circumoral kineties, the number of nematodesmal rods (17–22 vs. ten–11) and the presence of bacteria on the pellicle (vs. absence) (Gong & Song, 2004b; Table 2).

Hartmannula sinica Shao *et al.* 2008 is similar to *Heterohartmannula fangi* sp. nov. in terms of body size, numbers of somatic kineties, and nematodesmal rods (Shao *et al.*, 2008). Nevertheless, *Hartmannula sinica* can be clearly identified by having an alveolar layer on its cell surface (vs. absence in latter) and more contractile vacuoles (*c.* 15 vs. *c.* six) (Table 2).

Heterohartmannula fangi sp. nov. has the same number of nematodesmal rods as *Hartmannula acrobates* Poche, 1913. However, it differs from the latter by the character of cell surface (partly covered by bacteria vs. by an alveolar layer), and number of somatic kineties (28–32 vs. 32–37) (Deroux & Dragesco, 1968; Table 2).

Except for differences in oral kineties, *Hartmannula derouxi* Gong et Song, 2004, differs from

Table 2. Comparison of *Heterohartmannula fangi* gen. et sp. nov. and its congeners

Characters	Heterohartmannula fangi	Hartmannula acrobates	Hartmannula sinica	Hartmannula angustipilosa	Hartmannula derouxi
Body size *in vivo* (μm)	60–90 × 20–30	50–180 ×?	90–130 × 40–50	40–80 × 20–50	60–120 × 30–70
Pattern of circumoral kineties	Obliquely arranged	Parallel arranged	Parallel arranged	Parallel arranged	Parallel arranged
Alveolar layer on the cell surface	Absent	Present	Present	Absent	Present
No. of somatic kineties	28–32	32–37	24–31	24–28	42–53
No. of FvK	6	7	6–9	5–6	11–12
No. of right kineties	10–12	9	10–13	5–6	12–19
No. of left kineties	11–13	12–13	9–14	8–11	18–19
No. of postoral kineties	5–9	12–16	3–6	10–12	10–15
No. of nematodesmal rods	17–22	18–22	20–24	10–11	27–32
No. of contractile vacuoles	6 or 7	?	c. 15	4–7	20–28
Data sources	Present work	Deroux & Dragesco (1968)	Shao *et al.* (2008)	Gong & Song (2004b)	Gong & Song (2004b)

FvK, frontoventral kineties. ?, data not available.

Heterohartmannula fangi sp. nov. mainly by having more somatic kineties (42–53 vs. 28–32), more contractile vacuoles (20–28 vs. *c.* six), and more nematodesmal rods (27–32 vs. 17–22) (Gong & Song, 2004b; Table 2).

GENUS: *APORTHOTROCHILIA* GEN. NOV.

Diagnosis: Dorsoventrally compressed Hartmannulidae with a podite; oral kineties reduced to two fragments; several kinetal fragments positioned on the right posterior of frontoventral kineties; postoral kineties strongly shortened posteriad; terminal fragments consisting of several parallel arranged fragments.

Type species: Aporthotrochilia pulex (Deroux, 1976) comb. nov. (basionym: *Trichopodiella pulex* Deroux, 1976).

Etymology: Composite of Greek prefix apo- (derived from) and the generic name *Orthotrochilia*; feminine gender.

Comparison: In general, *Aporthotrochilia* gen. nov. resembles *Orthotrochilia* Song, 2003 in oral ciliature and the pattern of postoral kineties. However, the new genus can be distinguished by the presence of several extra kinetal fragments, i.e. the posterior fragments are on right posterior of frontoventral kineties and the higher number of terminal fragments (more than one vs. single) (Song, 2003).

Compared with other closely related genera (e.g. *Microxysma, Trochilioides, Chlamydonyx*), *Aporthotrochilia* can be clearly identified by having two perioral kineties (vs. two circumoral and one preoral kineties), several terminal fragments (vs. single), and several extra kinety fragments caudally positioned on right of frontoventral kineties (vs. none) (Deroux, 1976c).

APORTHOTROCHILIA PULEX (DEROUX, 1976) COMB. NOV. (FIG. 3; TABLE 3)

Basionym: Trichopodiella pulex Deroux, 1976.

New diagnosis: Body oval in outline, size *in vivo* 25–40 × 15–20 μm; a small podite subterminally positioned; two contractile vacuoles situated on right side; one frontoventral kinety and three to five posterior fragments; nine to ten ventral kineties in postoral field; four to five terminal fragments; marine habitat.

Slide deposition: One voucher slide with protargol specimens is deposited in the Natural History Museum, London, UK with registration number 2010:11:7:3. Another two slides are deposited in the Laboratory of Protozoology, Ocean University of China (no. PHB09121509).

Table 3. Morphometric characteristics of *Aporthotrochilia pulex* **gen.** et **comb. nov.** from protargol-impregnated specimens

Character	Min.	Max.	Mean	SD	CV	N
Body length	23	31	27.7	2.35	8.5	25
Body width	11	20	16.3	2.49	15.3	25
No. of SK	14	16	14.7	0.56	3.8	25
No. of FvK	1	1	1.0	0	0	25
No. of PF	3	5	4.0	0.29	7.3	25
No. of TF	4	5	4.7	0.48	10.2	21
Length of Ma	9	15	11.6	1.58	13.6	25
Width of Ma	5	8	6.0	1.10	18.3	25

All measurements in μm. CV, coefficient of variation (%); FvK, frontoventral kineties; Ma, macronucleus; Max., maximum; Mean, arithmetic mean; Min., minimum; *N*, number of specimens; PF, posterior fragments; SD, standard deviation; SK, somatic kineties; TF, terminal fragments.

Redescription: Body size about 25–40 × 15–20 μm *in vivo*; cells oval shaped with left margin sometimes slightly sigmoidal, both end bluntly rounded. Dorsoventrally flattened, ventral side flat and dorsal side slightly vaulted (Fig. 3A–E, I, N). Cytostome relatively small, subanteriorly positioned, nematodesmal rods hard to detect. Cytoplasm colourless or greyish, with several tiny, greasily shining granules (1–2 μm across) and few food vacuoles (4–5 μm across). Two contractile vacuoles (*c.* 3 μm in diameter) positioned in anterior and posterior one-third near right margin (Fig. 3F, arrows). Podite inconspicuous, 4 μm long, subterminally positioned (Fig. 3H, arrow). Macronucleus ellipsoid, positioned in body centre, heteromerous. Micronucleus not detected. Cilia about 6 μm long. Movement by slowly gliding on substrate.

Infraciliature as shown in Figure 3G, J, K, L, M, P–T. The short three to five right-most of the ventral kineties, which are anteriorly shortened progressively from left to right and posteriorly positioned, forming posterior fragments (PFs); the longest PF only extending forward to anterior one-third of body length (Fig. 3M, P, R). One frontoventral kinety (FvK) positioned next to PF extending apically. Both FvK and PF terminating posteriorly at the same level, and basal bodies densely arranged at their posterior ends (Fig. 3R, arrowheads). Nine to ten ventral kineties postorally positioned, strongly shortened from right to left. Terminal fragments consisting of four to five fragments (Fig. 3G, Q). Equatorial fragment not detected. Perioral kineties positioned anterior to cytostome, consisting of two fragments, both distinctly separated and composed of dikinetids.

Morphogenesis: Only two specimens in middle and late stages of the morphogenetic process have been observed (Fig. 3K, L, S, T). These showed that (1) perioral kineties of opisthe were developed from the oral primordium in midbody, which is generated from sections of postoral kineties; (2) the posterior fragments of both proter and opisthe stem from the parental posterior fragments.

Remarks: Deroux (1976c) described a species under the name of *Trichopodiella pulex* with its infraciliature (Fig. 3O) in detail: one frontoventral kinety, 11 postoral kineties and three or four posterior fragments; the postoral kineties strongly shortened. However, he did not note whether the podite is present. Our isolate corresponds very well to the original report in terms of infraciliature, except for the only difference in the number of postoral kineties (ten vs. 11), which is, however, considered minor. Therefore, our isolate should represent a population of *Trichopodiella pulex*.

However, during our study, we found this organism was quite different from other *Trichopodiella* spp. by: the presence of podite (vs. absence), four to five terminal fragments (vs. single), three to five distinct posterior fragments (vs. none) and two perioral kineties (vs. single) (Fauré-Fremiet, 1957; Deroux & Dragesco, 1968; Deroux, 1976c; Gong *et al.*, 2008). Therefore, we concluded that this small species should represent a distinct genus, and assigned it to the new genus *Aporthotrochilia* as *Aporthotrochilia pulex* (Deroux, 1976) gen. et comb. nov.
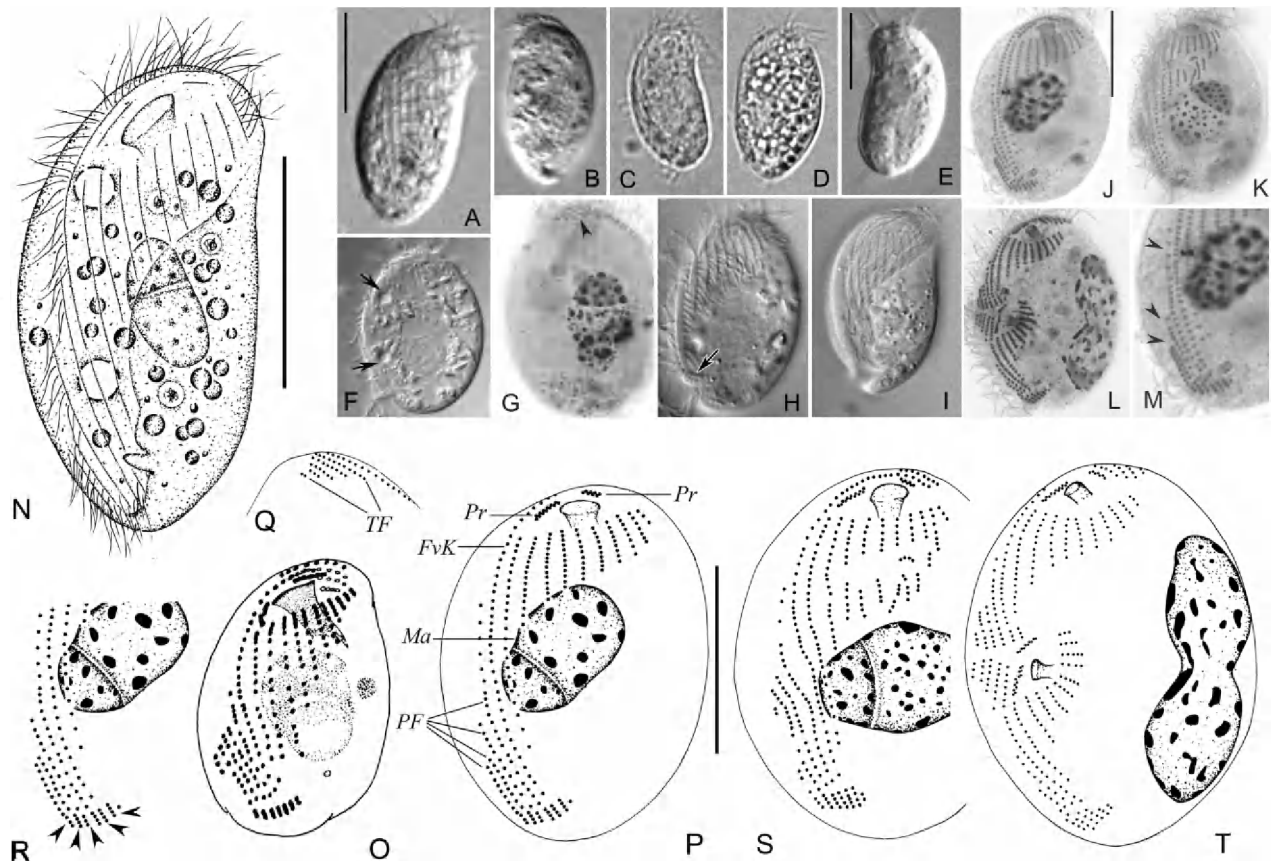
SUBORDER: DYSTERIINA DEROUX, 1976

FAMILY: DYSTERIIDAE CLAPARÈDE & LACHMANN, 1858

GENUS: *TROCHILIA* DUJARDIN, 1841

TROCHILIA ALVEOLATA SP. NOV.

(FIG. 4, TABLE 4)

Diagnosis: Small-sized *Trochilia* about 45–55 × 15–20 μm *in vivo*; body outline elliptical, with five or six ridges on right side; cilium-free surface covered with

Figure 3. Morphology and infraciliature of *Aporthotrochilia pulex* **gen.** et **comb. nov.** from life (A–F, H, I, N) and after protargol impregnation (G, J–M, O–T). A, ventral view of a typical individual. B–E, H, I, showing shape variants of different individuals, arrow in (H) points to podite. F, ventral view, arrows mark contractile vacuoles. G, dorsal view, arrowhead points to terminal fragments. J, ventral view. K. ventral view, to show the infraciliature of an individual in middle stage of morphogenesis. L, ventral view of an individual in late stage of morphogenesis. M, posterior portion, arrowheads indicate posterior fragments. N, ventral view of a typical individual. O, ventral view, from Deroux (1976c). P, ventral view. Q, anterior portion of dorsal side. R, posterior of ventral side, arrowheads point to densely arranged basal bodies in caudal. S, ventral view, to show the infraciliature of an individual in middle stage of morphogenesis. T, ventral view of an individual in late stage of morphogenesis. Abbreviations: FvK, frontoventral kineties; Ma, macronucleus; Pe, perioral kineties; PF, posterior fragments; TF, terminal fragments. Scale bars = 20 μm.

a conspicuous alveolar layer; four right somatic kineties, the right-most two of which extend dorsoapically; two left frontal kineties which extend to about one-third of body length; podite caudally positioned.

Type deposition: The holotype, as a protargol-impregnated slide, has been deposited in the Laboratory of Protozoology, Ocean University of China (No. PHB10010703). A paratype slide is in the National History Museum, London, UK, with the registration number 2010:11:7:1.

Type locality: Coastal waters off Clear Water Bay, Hong Kong (22°12′N, 114°117′E), water temperature 23 °C and salinity 16‰.

Etymology: The name refers to the feature of having distinct alveoli.

Description: Cell size about 55–45 × 15–20 μm *in vivo*, bilaterally flattened about 1:2. From side view, body elongated or oval in outline; left side flattened and right side vaulted with about five or six ridges. Anterior margin rounded and posterior slightly tapering (Fig. 4A–C, F, K–M). Pellicle of cilium-free field conspicuously covered with a thick alveolar layer that is composed of multiple vacuoles and detectable under 200 × magnification (Fig. 4F, L). Podite slender, about 8 μm long, caudally positioned. Cytoplasm colourless or greyish, usually with many tiny shining granules (1–2 μm across) and one or two ingested diatoms,

Table 4. Morphometric characteristics of *Trochilia alveolata* **sp. nov.** (upper line) and *Hypocoma acinetarum* (lower line) from protargol-impregnated specimens

Character	Min	Max	Mean	SD	CV	N
Body length	38	56	47.3	4.96	10.5	16
	35	57	46.5	5.63	12.1	19
Body width	17	29	24.8	3.47	14.0	16
	18	28	21.0	2.26	10.8	19
No. of right kineties	4	4	4.0	0	0	16
	9	13	11.1	1.2	10.8	19
No. of left kineties	4	5	4.7	0.48	10.2	16
	6	8	6.3	0.58	9.2	19
No. of FvK	2	2	2.0	0	0	16
	–	–	–	–	–	–
No. of basal bodies in FvK	124	164	147.7	11.78	8.0	11
	–	–	–	–	–	–
No. of basal bodies in TF	2	4	3.1	0.74	23.9	15
	9	16	13.1	2.15	16.4	17
No. of basal bodies in EF	1	9	3.1	2.13	68.7	16
	17	40	27.8	6.47	23.3	16
No. of basal bodies in Lf	30	43	36.3	3.38	9.3	16
	–	–	–	–	–	–
Length of macronucleus	12	20	16.4	2.28	13.9	16
	–	–	–	–	–	–
Width of macronucleus	6	10	7.7	1.20	15.6	16
	–	–	–	–	–	–

All measurements in μm. Abbreviations: CV, coefficient of variation (%); EF, equatorial fragment; FvK, frontoventral kineties; Lf, left frontal kineties; Max, maximum; Mean, arithmetic mean; Min, minimum; N, number of specimens; SD, standard deviation; TF, terminal fragment.

which render the cell more or less opaque. Cytostome in anterior quarter of cell. Cytopharynx diagonally orientated, about 20 μm long, and extending to about posterior fifth of cell, supported by two nematodesmal rods (Fig. 4E). Contractile vacuole not detected. Macronucleus ellipsoidal, size about 23 × 13 μm *in vivo*, centrally positioned, characteristically heteromerous. Micronucleus not detected. Cilia 8 μm long *in vivo*. Movement always slow, by crawling on substrate.

Infraciliature as shown in Figure 4D, G, H, J, N. Four right kineties, the two right-most of which are almost equal in length (*c.* 147 kinetosomes) and both extend anteriorly and bend to dorsal side; the other two kineties shortened at both ends from right to left (Fig. 4D, J, N). At midbody about five closely spaced, very short left kineties. Two relatively long left frontal kineties, composed of 30–43 kinetosomes, positioned at the left side of cytostome, extending to about one-third of body length. One straight terminal fragment comprising three kinetosomes. Equatorial fragment consisting of about three kinetosomes.

Oral ciliature typical of the genus *Trochilia* (Fig. 4D, G, N): two parallel circumoral kineties and one short preoral kinety, all of which are composed of dikinetids.

Comparison and remarks: The genus *Trochilia* Dujardin, 1841, is characterized by a combination of the following features: (1) lateral compressed but no ventral grooves; (2) two nematodesmal rods; (3) left field kineties divided into left kineties and left frontal kineties, the latter group always composed of two rows (Heuss & Wilbert, 1973; Deroux, 1976c; Gong, Warren & Song, 2009b).

Amongst *Trochilia* species that have been described using modern methods, three species are similar to *Trochilia alveolata* sp. nov., *Trochilia minuta* (Roux, 1899) Kahl 1931; *Trochilia petrani* Dragesco, 1966, and *Trochilia sigmoides* Dujardin, 1841.

Trochilia petrani (Fig. 5F) resembles *T. alveolata* sp. nov. in terms of cell size, but it can be distinguished by the absence of both the alveolar layer and the ridges on right side (vs. present in latter) (Dragesco, 1966).
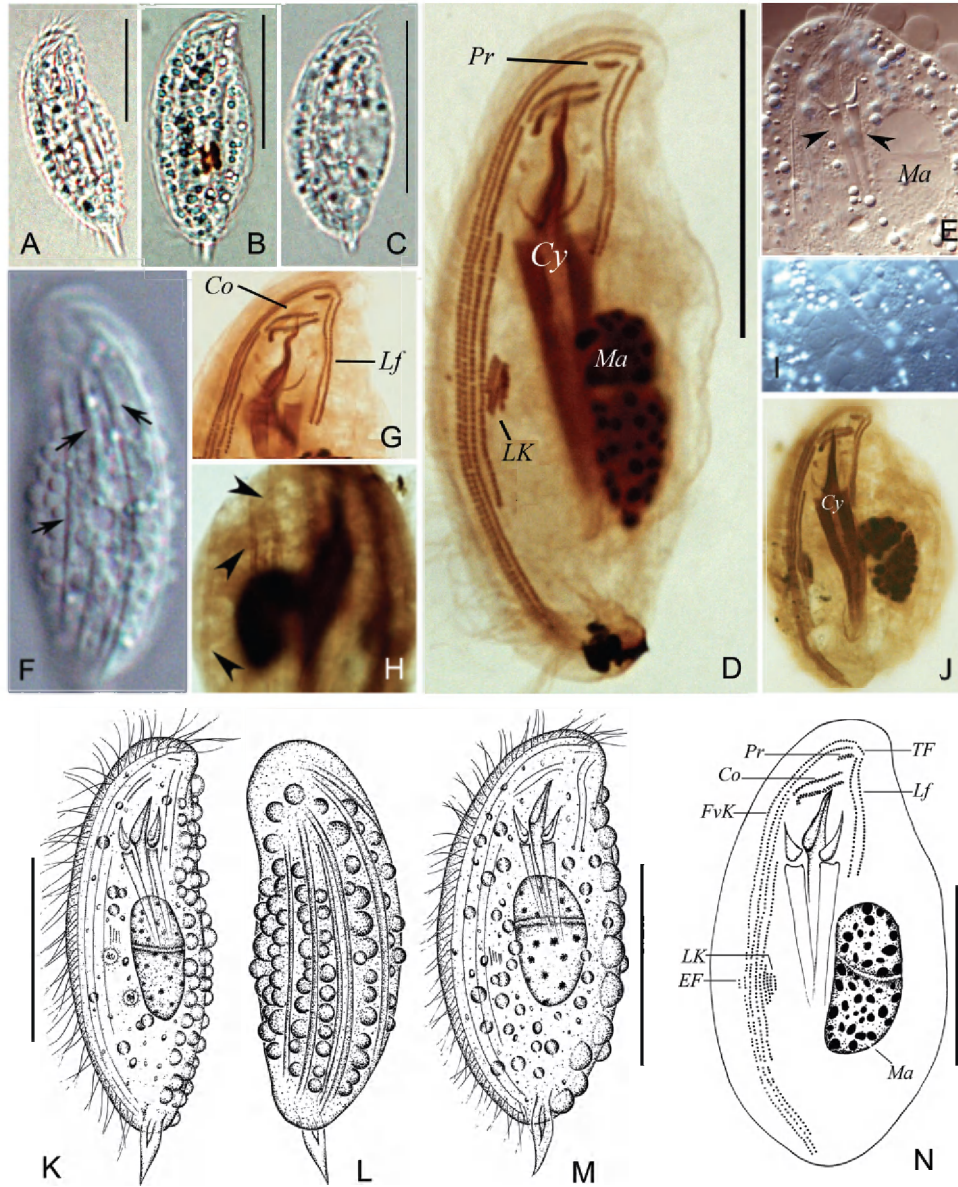
Figure 4. Morphology and infraciliature of ***Trochilia alveolata* sp. nov.** from life (A–C, E, F, I, K–M) and after protargol impregnation (D, G, H, J, N). A, left lateral view of a typical individual. B, C, left views of different individuals. D, left lateral view to show infraciliature. E, to show details of oral apparatus structure, arrowheads mark nematodesmal rods. F, right lateral view to show the alveolar layer on the cell surface, arrows point to ridges. G, anterior part of cell. H, right lateral view, arrowheads refer to ridges on the surface. I, details of cell surface. J, right view of a small individual. K, left lateral view of a typical individual. L, right lateral view to show ridges and well-developed alveoli. M, left lateral view of a fat individual. N, left lateral view to show the infraciliature. Abbreviations: Co, circumoral kineties; Cy, cytopharynx; EF, equatorial fragment; FvK, frontoventral kineties; Lf, left frontal kineties; LK, left kineties; Ma, macronucleus; Pr, preoral kinety; TF, terminal fragment. Scale bars = 30 μm (A–J), 25 μm (K–N).

Trochilia minuta and *T. sigmoides* are similar to the new species in terms of the presence of ridges on the right side. However, *T. minuta* (Fig. 5D) can be separated from *T. alveolata* sp. nov. by having relatively shorter left frontal kineties (containing *c.* six kinetosomes vs. 30–43 kinetosomes) and the absence of alveolar layer (vs. present in latter) (Foissner, 1979). Compared with the new species, *T. sigmoides* (Fig. 5A, B) is smaller (20–28 vs. 45–55 μm in length), oval in body outline (vs. elliptical) and has no alveolar layer (vs. present), and hence can be identified (Gong *et al.*, 2009b).
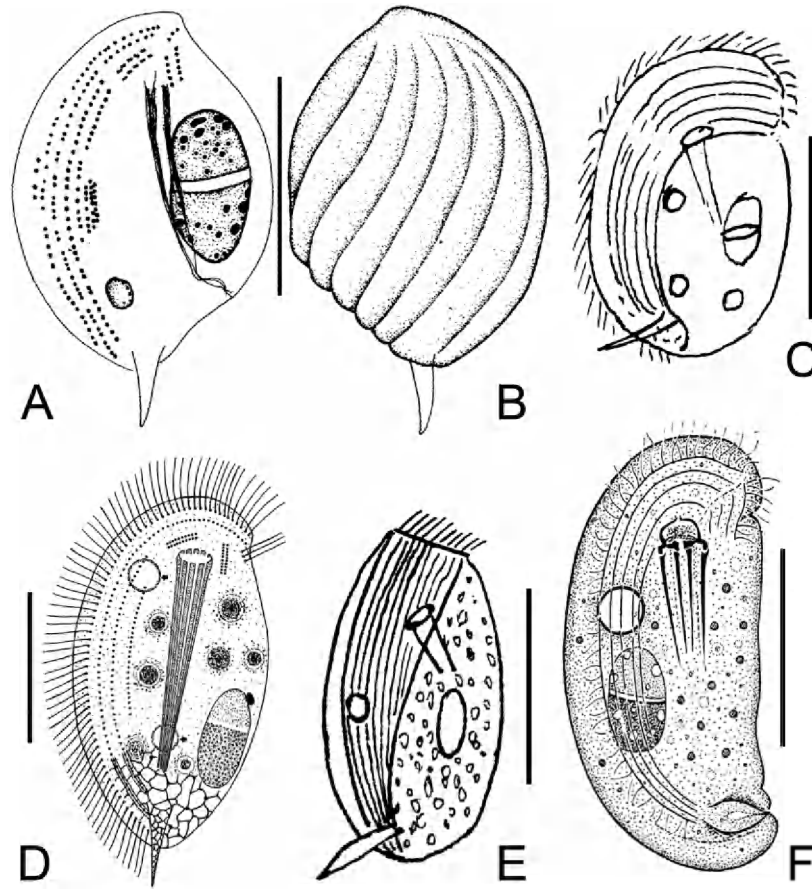
Figure 5. Congeners of ***Trochilia alveolata* sp. nov.** A, B, *Trochilia sigmoides*, from Gong *et al*. (2009b). C, *Trochilia salina*, from Kahl (1931). D, *Trochilia minuta*, from Foissner (1979). E, *Trochilia marina*, from Kahl (1931). F, *Trochilia petrani*, from Dragesco (1966). Scale bars = 10 μm (D); 15 μm (A, B); 20 μm (C, E, F).

Trochilia marina Mereschkowski, 1881, and *T. salina* Entz, 1879, whose infraciliatures are not known, are also similar to *T. alveolata* sp. nov. in cell shape and marine habitat. The former (Fig. 5E) is characterized by smaller size (*c*. 33 vs. 45–55 μm in length) and the absence of alveolar layer (vs. present in latter). The latter (Fig. 5C) can be identified from *T. alveolata* sp. nov. by smaller size (*c*. 20 vs. 45–55 μm in length) and the absence of ridges on the right side (vs. present) (Kahl, 1931).

SUBORDER: DYSTERIINA DEROUX, 1976

FAMILY: PLESIOTRICHOPIDAE DEROUX, 1976

GENUS: *TROCHOCHILODON* DEROUX, 1976

Deroux (1976b) reported *Trochochilodon* with detailed infraciliature of the type species *Trochochilodon flavus* but no live information. The genus *Trochochilodon*, in our opinion, has not been clearly defined yet. Based on the data available, an improved diagnosis of the genus is supplied here.

Improved diagnosis for genus Trochochilodon: Dorsoventrally compressed plesiotrichopids; right kineties clearly separated from left kineties in posterior region of cell; left kineties posteriorly shortened from right to left; oral ciliature consisting of two circumoral kineties only; no podite.

TROCHOCHILODON FLAVUS DEROUX, 1976 [NOM. CORRECT. AESCHT, 2001 (pro *T. FLAVUM*)]
(FIG. 6, TABLE 1)

Based on current careful observations of both morphology and infraciliature, we supply here an improved diagnosis and a detailed description.

Improved diagnosis: Marine *Trochochilodon*, size 40–60 × 20–30 μm *in vivo*, cell oval in outline; nine ventral kineties, including five right and four left kineties; three right-most kineties extending apically; about 12 nematodesmal rods; cortical granules
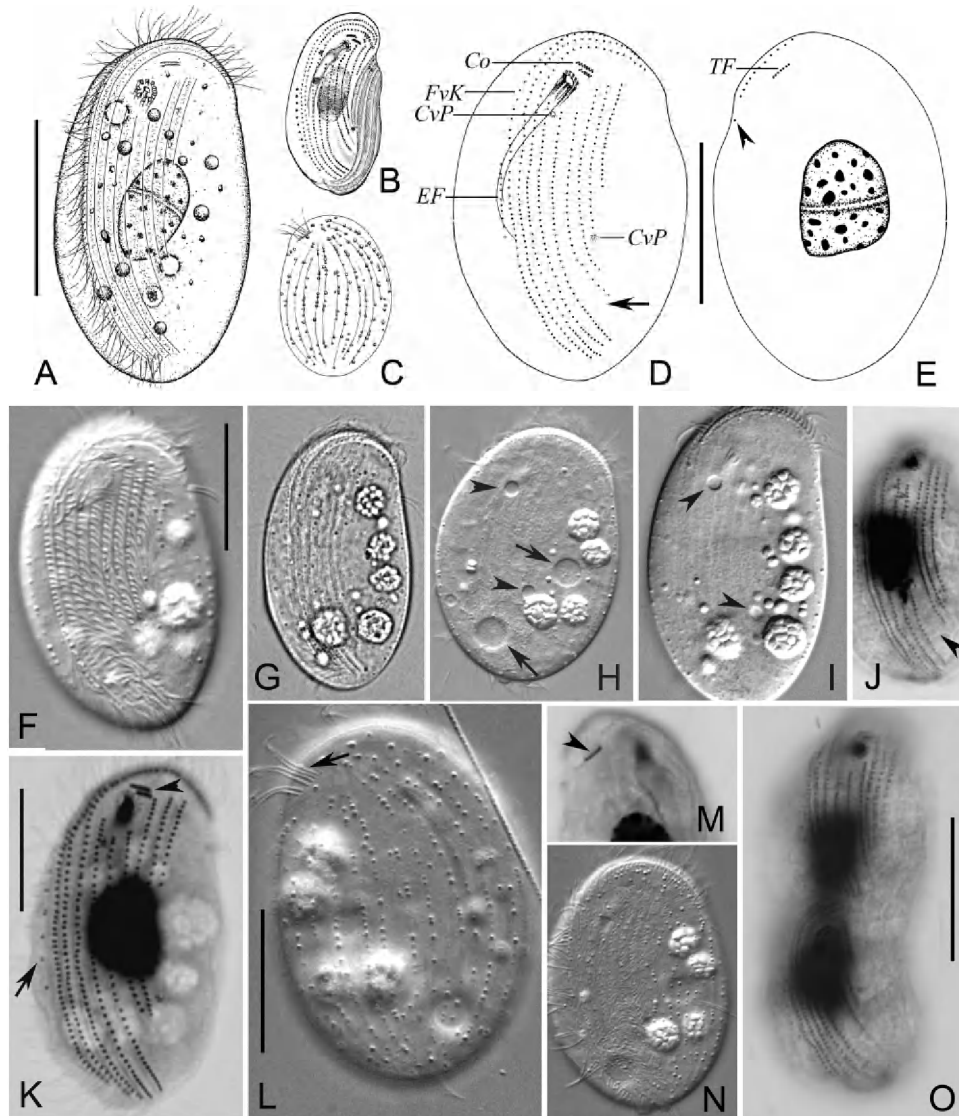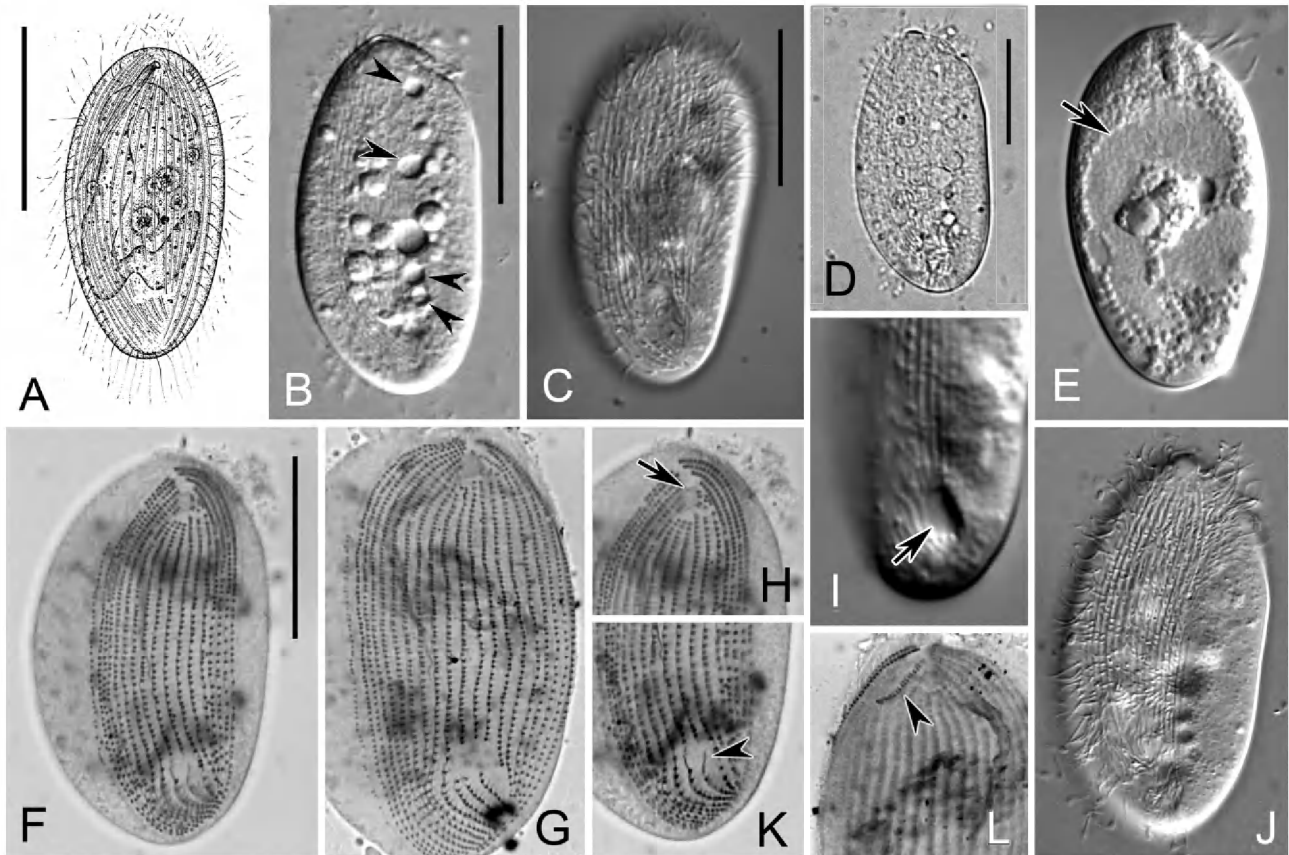
Figure 6. Morphology and infraciliature of *Trochochilodon flavus* from life (A, C, F–I, L, N) and after protargol impregnation (B, D, E, J, K, M, O). A, ventral view of a typical individual. B, ventral view, showing infraciliature, from Deroux (1976b). C, dorsal view, showing cortical granules. D, ventral view, arrow points to the gap between right and left kineties. E, dorsal view, arrowhead indicates the extra basal body in left margin. F, G, ventral views of different individuals. H, I, to show contractile vacuoles (arrowheads) and food vacuoles (arrows in H). J, ventral view, arrowhead marks the gap between right and left kineties. K, ventral view, arrow refers to equatorial fragment, arrowhead shows circumoral kineties. L, dorsal view, arrow shows terminal cilia. M, anterior of dorsal side, arrowhead points to terminal fragment. N, ventral view. O, ventral view of an individual in a late stage of morphogenesis. Abbreviations: Co, circumoral kineties; CvP, contractile vacuole pore; EF, equatorial fragment; FvK, frontoventral kineties; TF, terminal fragment. Scale bars = 25 μm.

sparsely arranged in lines in the dorsal pellicle; two contractile vacuoles, diagonally positioned.

Description based on Qingdao population: Cell size *c.* 45–55 × 25–30 μm *in vivo,* body shape usually oval or slightly kidney-like with both ends broadly rounded (Fig. 6A, F–I). Cell dorsoventrally flattened, ventral surface flat, and dorsal side slightly vaulted. Pellicle

somewhat rough, some cortical granules (*c.* 1 μm across) arranged in curved lines on cilia-free area (Fig. 6C, L, N). Cytoplasm colourless and hyaline, with different sized granules (1–6 μm in diameter) and food vacuoles (4–5 μm across). Cytostome inconspicuous, positioned in anterior one-sixth of body length; cytopharynx diagonally orientated, supported by 12 nematodesmal rods. Two contractile vacuoles,

Figure 7. Morphology and infraciliature of *Hypocoma acinetarum* from life (A–E, I, J) and after protargol impregnation (F–H, K, L). A, ventral view, from Hu *et al.* (2003). B–E, ventral views of different individuals, arrowheads in (B) point to contractile vacuoles, arrow in (E) marks macronucleus. F, G, ventral view, showing infraciliature. H, K, anterior (H) and posterior (K) parts of ventral view, arrow indicates the cytostome, arrowhead marks the gap in posterior of middle kineties. I, posterior part of ventral side, arrow points to depressed area. J, ventral view of a typical individual. L, dorsal view, arrowhead shows the terminal fragment. Scale bars = 25 μm.

about 3 μm in diameter, diagonally positioned (Fig. 6A, H, I). Macronucleus heteromerous and ellipsoidal, about 15 × 10 μm *in vivo*, obliquely positioned in midbody. Micronuclei not detected. Cilia about 12 μm long *in vivo*. Movement usually by gliding on substrate.

Infraciliature as shown in Figure 6D, E, J, K, M, O. A total of nine somatic kineties, which are divided into five right and four left kineties by a conspicuous gap in posterior region (arrow in Fig. 6D and arrowhead in Fig. 6J); the right-most three kineties extending apically and bending to left margin; the middle three kineties arranged in postoral region; the posterior ends of left kineties shortened progressively from right to left. Terminal fragment comprising five basal bodies (Fig. 6M, arrowhead); equatorial fragment consisted of five to 22 basal bodies (Fig. 6D, K). Usually one or two basal bodies (arrowhead in Fig. 6E) recognized in left margin, positioned in anterior one-sixth of body

length. After protargol impregnation, two contractile vacuole pores visible (Fig. 6D).

Oral kineties composed of two short, parallel arranged circumoral kineties. Outer circumoral kinety slightly longer than the inner one (Fig. 6D, K).

Remarks: Trochochilodon flavus was originally described by Deroux (1976b) with detailed infraciliature (Fig. 6B) but no live characters. Considering that the infraciliature of our population is the same as the original, we confirm that our form is *T. flavus*.

SUBORDER: HYPOCOMATINA DEROUX, 1976

FAMILY: HYPOCOMIDAE BÜTSCHLI, 1889

GENUS: *HYPOCOMA* GRUBER, 1884

HYPOCOMA ACINETARUM COLLIN, 1907
(FIG. 7, TABLE 4)

Deroux (1974) revealed the infraciliature and morphogenesis of *Hypocoma acinetarum*, and recently

Hu, Gong & Song (2003) briefly reported a Chinese population. With the current population, we had the opportunity to carry out further investigations and so an improved definition can be given here.

Improved diagnosis: Marine *Hypocoma*, cell size *in vivo* 30–60 × 15–30 µm; oval-shaped in outline; 17–20 ventral kineties consisting of nine to 13 right and six to eight left kineties; about four contractile vacuoles arranged in a longitudinal row on left side of cell; one macronucleus, C-shaped.

Redescription: Body *in vivo* c. 35–60 × 15–30 µm. Cell usually oval-shaped in outline, with right margin convex and left margin straight or slightly concave (Fig. 7A–E, J). A depressed region positioned in posterior of ventral side (Fig. 7I, arrow). Cytoplasm colourless or greyish, containing several granules (3–5 µm across). Usually four contractile vacuoles, about 3–4 µm in diameter, longitudinally arranged on left (Fig. 7B, arrowheads). Cytostome simply and inconspicuous, positioned near the anterior end of cell, no distinct nematodesmal rods detected. Macronucleus C-shaped, centrally positioned (Fig. 7E, arrow). Cilia on ventral side about 7 µm long, and on dorsal terminal fragment about 12 µm long. Movement by swimming moderately fast.

Infraciliature as shown in Figure 7F–H, K, L. Sixteen to 19 somatic kineties consisting of nine to 13 right and six to eight left kineties, forming sutures at both anterior and posterior ends. Left kineties straight, and right kineties slightly curved to left at both ends. Anterior ends of all kineties surrounding the cytostome. The right-most and left-most kineties converge in caudal region. About three or four middle kineties interrupted in posterior part, their posterior ends positioned in the depressed area and forming a naked region (Fig. 7K, arrowhead). Equatorial fragment composed of 17–40 basal bodies. Terminal fragment consisting of nine to 16 basal bodies (Fig. 7L, arrowhead). Oral kineties not observed.

Remarks: Collin (1907) first reported *Hypocoma acinetarum* with a general morphological description. The morphometric data of our form correspond well with the original report in terms of cell shape and size, number of contractile vacuoles, and shape of macronucleus. Thus it is confirmed that our isolate is *Hypocoma acinetarum*.

Deroux (1974) first revealed the infraciliature of *Hypocoma acinetarum*. Differently from the present population, his form has slightly more kineties (20 vs. 16–19).

Hu *et al.* (2003) also reported a Chinese population without information on the contractile vacuoles. Compared with their population, our isolate is a little larger in size (35–60 × 15–30 vs. 30–50 × 20–25 µm) and has fewer kineties (16–19 vs. 17–20).

PHYLOGENY OF TWO NEW GENERA (FIG. 8)

Deposition of sequences
The new SSU rDNA sequences have been deposited in the GenBank database with the following accession numbers: HQ605947 (*Aporthotrochilia pulex* gen. et comb. nov.) and HQ605946 (*Heterohartmannula fangi* gen. et. sp. nov.).

Phylogenetic analyses
Phylogenetic trees by ML and BI strongly support the monophyly of Subkinetalia, Phyllophryngea, Suctorida, Synhymeniida and the genus *Dysteria*. The topology shown in Figure 8 indicates *Heterohartmannula fangi* gen. et sp. nov. clustering with *Hartmannula* spp., then forming a sister clade to *Trichopodiella faurei*. Furthermore, *Aporthotrochilia pulex* gen. et comb. nov. occupies the basal position of the chonotrichid–cyrtophorid clade with moderate values (ML/BI, 75/1.00).

Discussion based on phylogenetic trees
In previous studies (Snoeyenbos-West *et al.*, 2004; Li & Song, 2006; Gong *et al.*, 2008, 2009a), Phyllophryngea and Suctoria have been considered as monophyletic, which is also strongly supported by our research. *Heterohartmannula fangi* gen. et sp. nov. is placed closer to *Hartmannula* than to *Trichopodiella*, which corresponds well to morphological comparisons that both *Heterohartmannula* and *Hartmannula* have distinct podites but *Trichopodiella* does not.

It is striking that *Aporthotrochilia pulex* gen. et comb. nov. locates at the base of the Cyrtophoria-Chonotrichia group in the molecular trees. This result disagrees with the morphological taxonomy in which *A. pulex* belongs to Hartmannulidae and hence would have been expected to cluster with *Trichopodiella, Heterohartmannula,* and *Hartmannula*. We have double-checked the macronucleus of *A. pulex*, and confirm that it is juxtaposed heteromerous (vs. centrally heteromerous in Chilodonellina). What morphological character makes the phylogenetic position of *A. pulex* so special, and in which family (possibly new?) it should be classified, remain open questions for further discussion.

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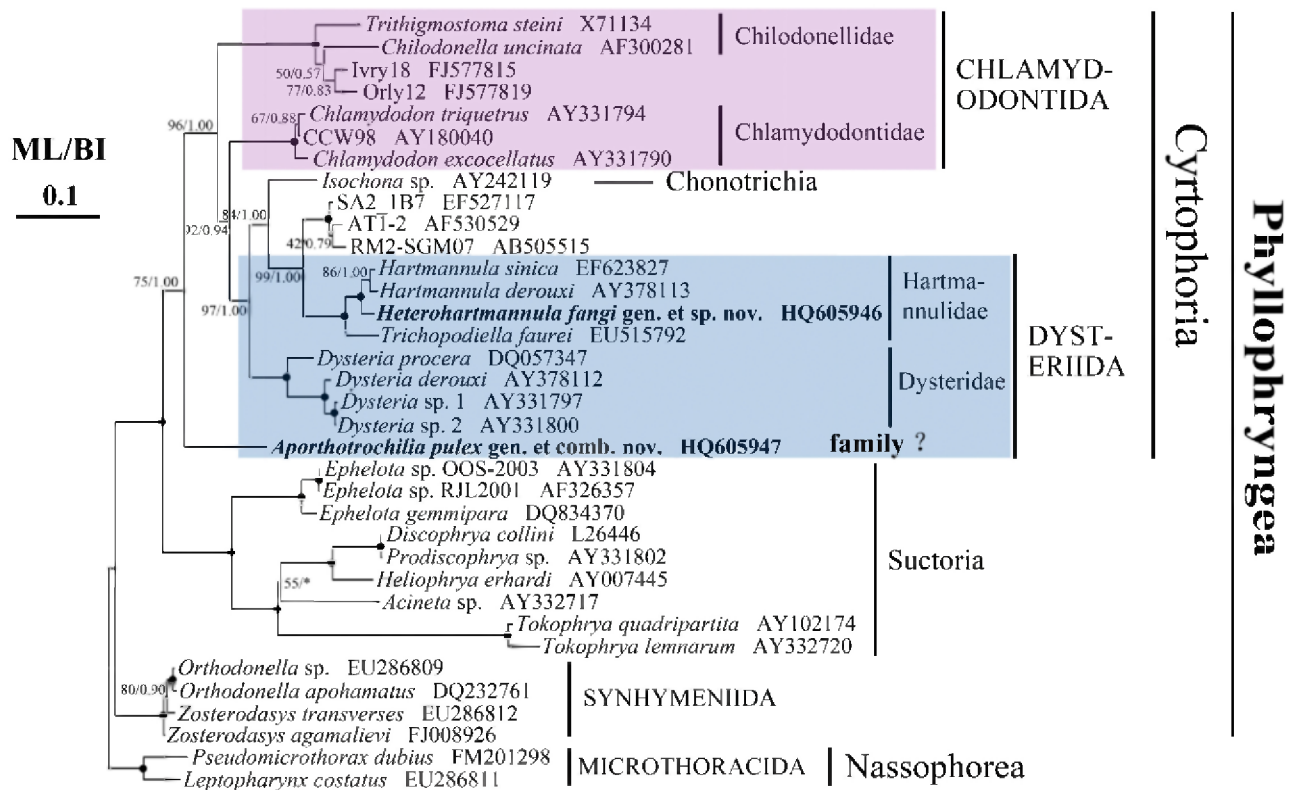
Figure 8. Phylogenetic trees based on the small subunit (SSU) rRNA sequences show the positions of two new genera, *Aporthotrochilia* **gen. nov.** and *Heterohartmannula* **gen. nov.**, by maximum likelihood (ML) and Bayesian inference (BI). Numbers near branches are BI posterior probability value and ML bootstrap values. The asterisk (*) reflects disagreement between phylogenies. Black dots on nodes indicate maximal (100% ML, 1.00 BI) support in both analyses. Cyrtophoria is highlighted. The scale bar corresponds to ten substitutions per 100 nucleotide positions.

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