



Zoological Journal of the Linnean Society, 2012, 164, 1–17. With 8 figures

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

HONGBO PAN^{1,2,3}, XIAOFENG LIN^{1*}, JUN GONG⁴, KHALED A. S. AL-RASHIED⁵ and WEIBO SONG²

¹Laboratory of Protozoology, Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, South China Normal University, Guangzhou, 510631, China

²Laboratory of Protozoology, Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, 266003, China

³College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201306, China

⁴Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, 264003, China

⁵Zoology Department, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Received 14 January 2011; revised 11 March 2011; accepted for publication 14 March 2011

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 flavidus* Deroux, 1976, and *Hypocoma acinetarum* Collin, 1907, are described. *Heterohartmannula* gen. nov. is mainly characterized by a combination of features: two circumoral kinetics obliquely arranged, podite not surrounded by somatic kinetics, 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 kinetics 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 flavidus* are supplied.

© 2012 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2012, 164, 1–17.
doi: 10.1111/j.1096-3642.2011.00751.x

ADDITIONAL KEYWORDS: *Aporthotrochilia* gen. nov. – *Aporthotrochilia pulex* comb. nov. – *Heterohartmannula fangi* sp. nov. – *Heterohartmannula* gen. nov. – *Hypocoma acinetarum* – marine ciliates – SSU rDNA – *Trochilia alveolata* sp. nov. – *Trochochilodon flavidus*.

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 kinetics usually restricted to ventral side and macronuclei heteromerous (Deroux, 1994). Both oral ciliature and somatic kinetics 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;

*Corresponding author. E-mail: xlin@scnu.edu.cn

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‰).

Aporthotrichilia 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°11'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 *Aporthotrichilia 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 *Aporthotrichilia* 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*, *Orthotrichilia*, *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

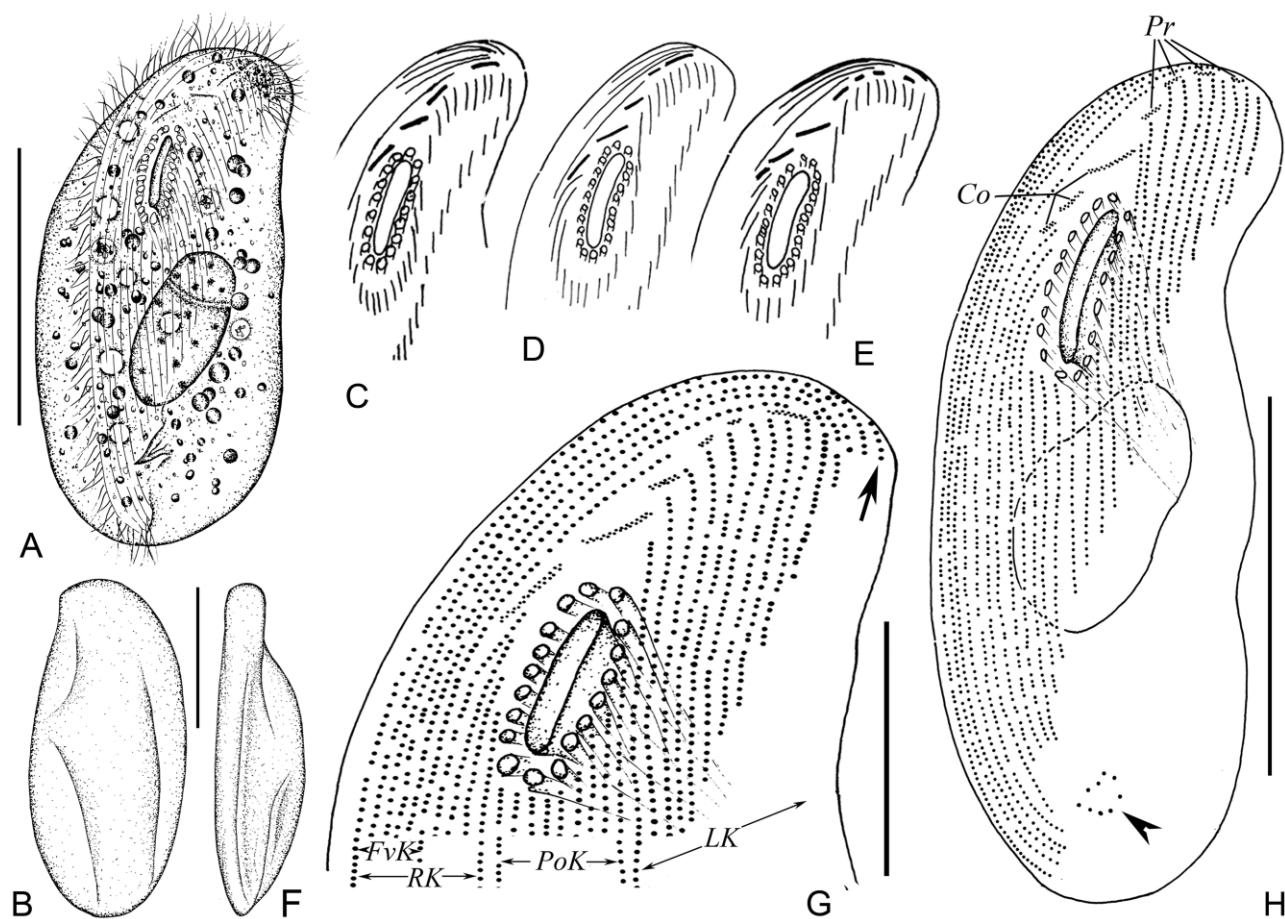


Figure 1. Morphology and infraciliature of *Heterohartmannula fungi* gen. et sp. nov. from life (A, B, F) and after protargol impregnation (C–E, G, H). A, ventral view of a typical individual. B, dorsal view. C–E, anterior of different individuals, to show variants of oral kinetics. F, lateral view. 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 kinetics; FvK, frontoventral kinetics; LK, left kinetics; PoK, postoral kinetics; Pr, preoral kinetics; RK, right kinetics. 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.

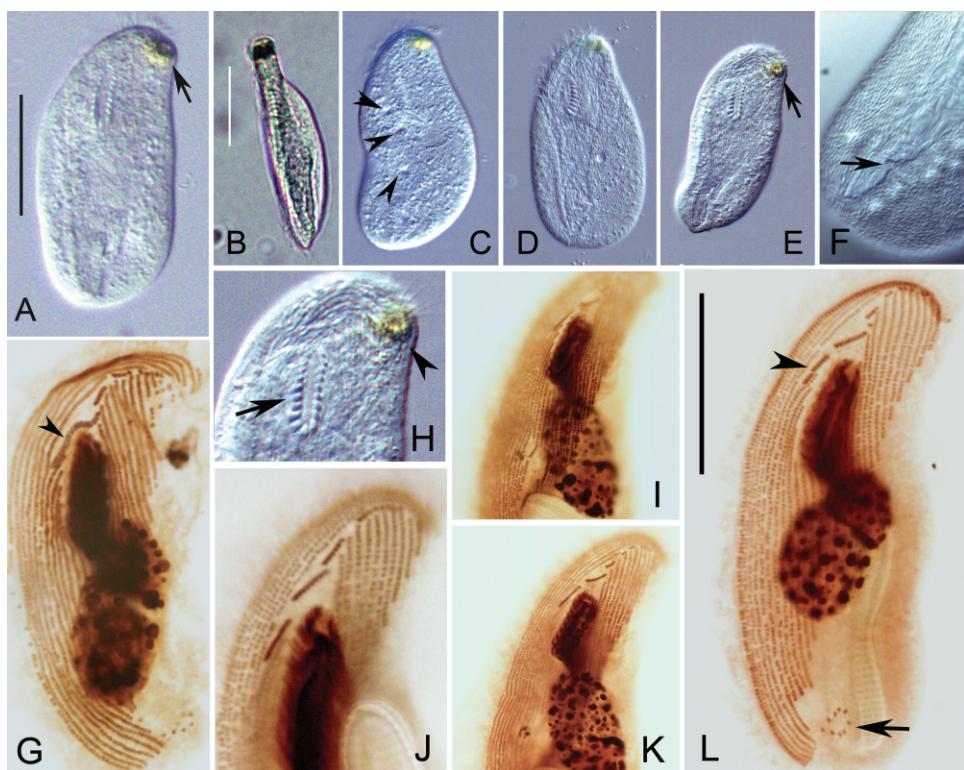


Figure 2. Photographs of *Heterohartmannula fangi* gen. et sp. nov. from life (A–F, H) and after protargol impregnation (G, I–L). A, ventral view of a typical individual, arrow points to the yellowish pigment spot. B, lateral view. C–E, to show shape variants, arrowheads mark contractile vacuoles, arrow 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 al.* 2004, differs from

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

Characters	<i>Heterohartmannula fangi</i>	<i>Hartmannula acrobates</i>	<i>Hartmannula sinica</i>	<i>Hartmannula angustipilosa</i>	<i>Hartmannula derouxi</i>
Body size <i>in vivo</i> (μm)	60–90 × 20–30	50–180 ×?	90–130 × 40–50	40–80 × 20–50	60–120 × 30–70
Pattern of circumoral kinetics	Obliquely arranged	Parallel arranged	Parallel arranged	Parallel arranged	Parallel arranged
Alveolar layer on the cell surface	Absent	Present	Present	Absent	Present
No. of somatic kinetics	28–32	32–37	24–31	24–28	42–53
No. of FvK	6	7	6–9	5–6	11–12
No. of right kinetics	10–12	9	10–13	5–6	12–19
No. of left kinetics	11–13	12–13	9–14	8–11	18–19
No. of postoral kinetics	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 <i>et al.</i> (2008)	Gong & Song (2004b)	Gong & Song (2004b)

FvK, frontoventral kinetics. ?, data not available.

Heterohartmannula fangi sp. nov. mainly by having more somatic kinetics (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 kinetics reduced to two fragments; several kinetal fragments positioned on the right posterior of frontoventral kinetics; postoral kinetics strongly shortened posteriad; terminal fragments consisting of several parallel arranged fragments.

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

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

Comparison: In general, *Aporthotrichilia* gen. nov. resembles *Orthotrichilia* Song, 2003 in oral ciliature and the pattern of postoral kinetics. 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 kinetics 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*), *Aporthotrichilia* can be clearly identified by having two perioral kinetics (vs. two circumoral and one preoral kinetics), several terminal fragments (vs. single), and several extra kinety fragments caudally positioned on right of frontoventral kinetics (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 kinetics 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 kinetics; Ma, macronucleus; Max., maximum; Mean, arithmetic mean; Min., minimum; N, number of specimens; PF, posterior fragments; SD, standard deviation; SK, somatic kinetics; TF, terminal fragments.

Redescription: Body size about $25\text{--}40 \times 15\text{--}20 \mu\text{m}$ *in vivo*; cells oval shaped with left margin sometimes slightly sigmoidal, both end bluntly rounded. Dors-oventrally 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 ($1\text{--}2 \mu\text{m}$ across) and few food vacuoles ($4\text{--}5 \mu\text{m}$ across). Two contractile vacuoles (*c.* $3 \mu\text{m}$ in diameter) positioned in anterior and posterior one-third near right margin (Fig. 3F, arrows). Podite inconspicuous, $4 \mu\text{m}$ long, subterminally positioned (Fig. 3H, arrow). Macronucleus ellipsoid, positioned in body centre, heteromerous. Micronucleus not detected. Cilia about $6 \mu\text{m}$ 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 kinetics, 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 kinetics postorally positioned, strongly shortened from right to left. Terminal fragments consisting of four to five fragments (Fig. 3G, Q). Equatorial fragment not detected. Perioral kinetics 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 kinetics of opisthe were developed from the

oral primordium in midbody, which is generated from sections of postoral kinetics; (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 kinetics and three or four posterior fragments; the postoral kinetics 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 kinetics (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 kinetics (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 $45\text{--}55 \times 15\text{--}20 \mu\text{m}$ *in vivo*; body outline elliptical, with five or six ridges on right side; cilium-free surface covered with

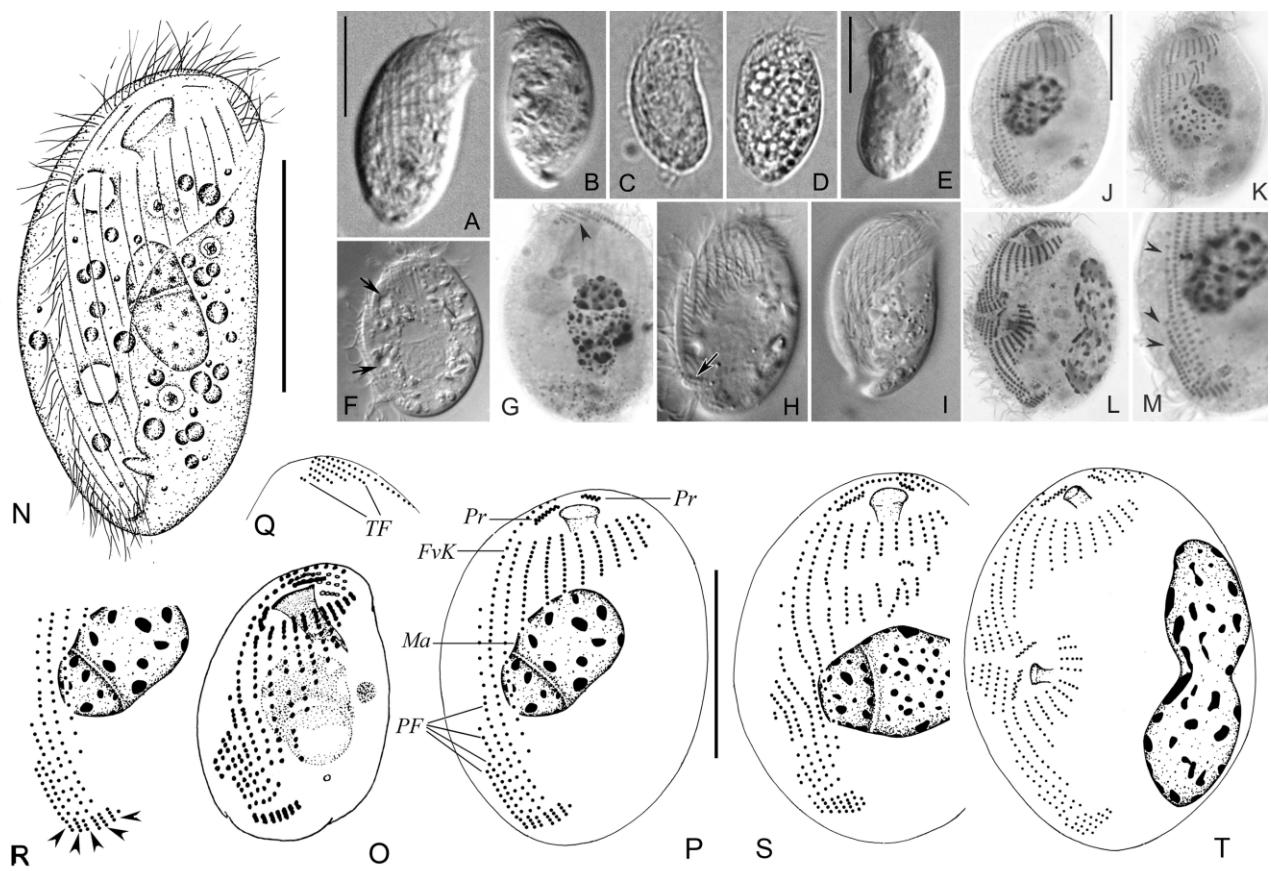


Figure 3. Morphology and infraciliature of *Aporthotrichilia pulex* gen. et comb. nov. from life (A–F, H, I, N) and after protargol impregnation (G, J–M, O–T). A, ventral view of a typical individual. B–E, H, I, showing shape variants of different individuals, arrow in (H) points to podite. F, ventral 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 kinetics; Ma, macronucleus; Pe, perioral kinetics; 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 $20 \mu\text{m}$ long, and extending to about posterior fifth of cell, supported by two nematodesmal rods (Fig. 4E). Contractile vacuole not detected. Macronucleus ellipsoidal, size about $23 \times 13 \mu\text{m}$ *in vivo*, centrally positioned, characteristically heteromerous. Micronucleus not detected. Cilia $8 \mu\text{m}$ 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).

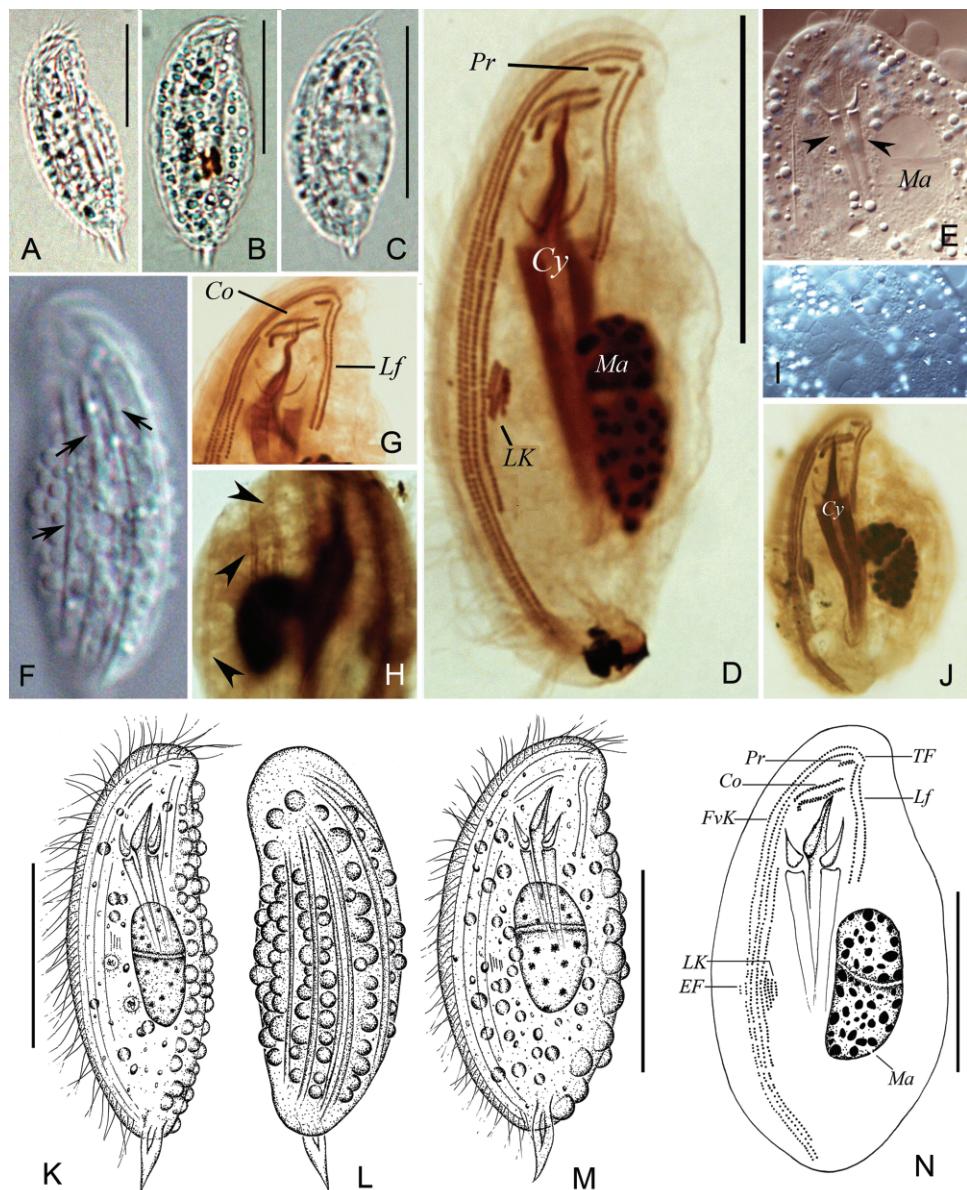


Figure 4. Morphology and infraciliature of *Trochilia alveolata* sp. nov. from life (A–C, E, F, I, K–M) and after protargol impregnation (D, G, H, J, N). A, left lateral view of a typical individual. B, C, left views of different individuals. D, left lateral view to show infraciliature. E, to show details 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 kinetics; Cy, cytopharynx; EF, equatorial fragment; FvK, frontoventral kinetics; Lf, left frontal kinetics; LK, left kinetics; 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 kinetics (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).

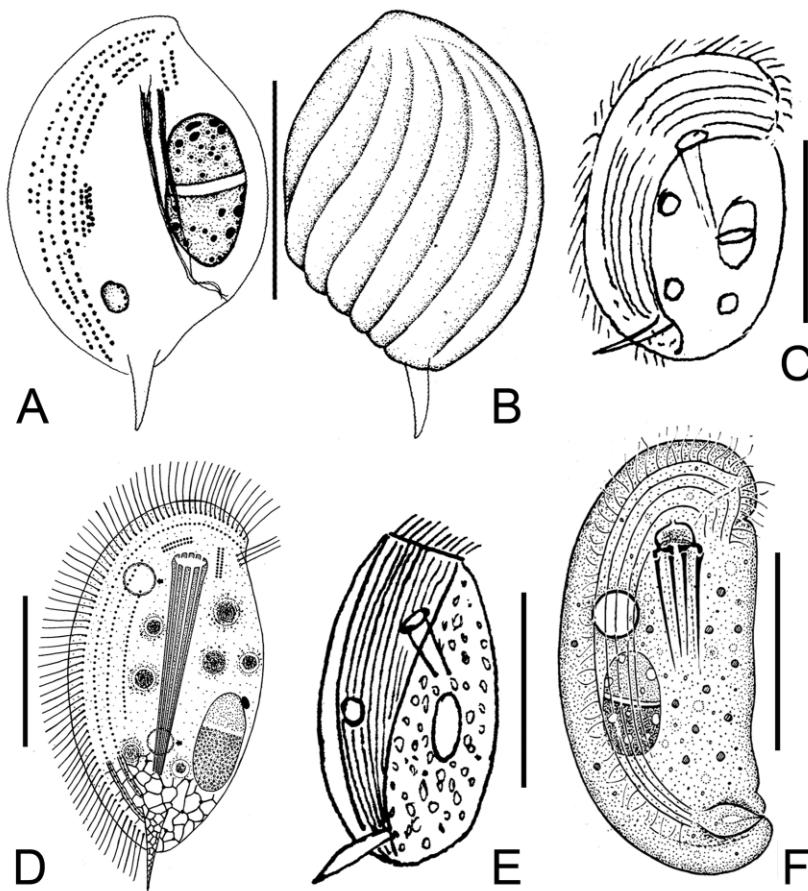


Figure 5. Congeners of *Trochilia alveolata* sp. nov. A, B, *Trochilia sigmoides*, from Gong *et al.* (2009b). C, *Trochilia salina*, from Kahl (1931). D, *Trochilia minuta*, from Foissner (1979). E, *Trochilia marina*, from Kahl (1931). F, *Trochilia petrani*, from Dragesco (1966). Scale bars = 10 µm (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: Dors-oventrally 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

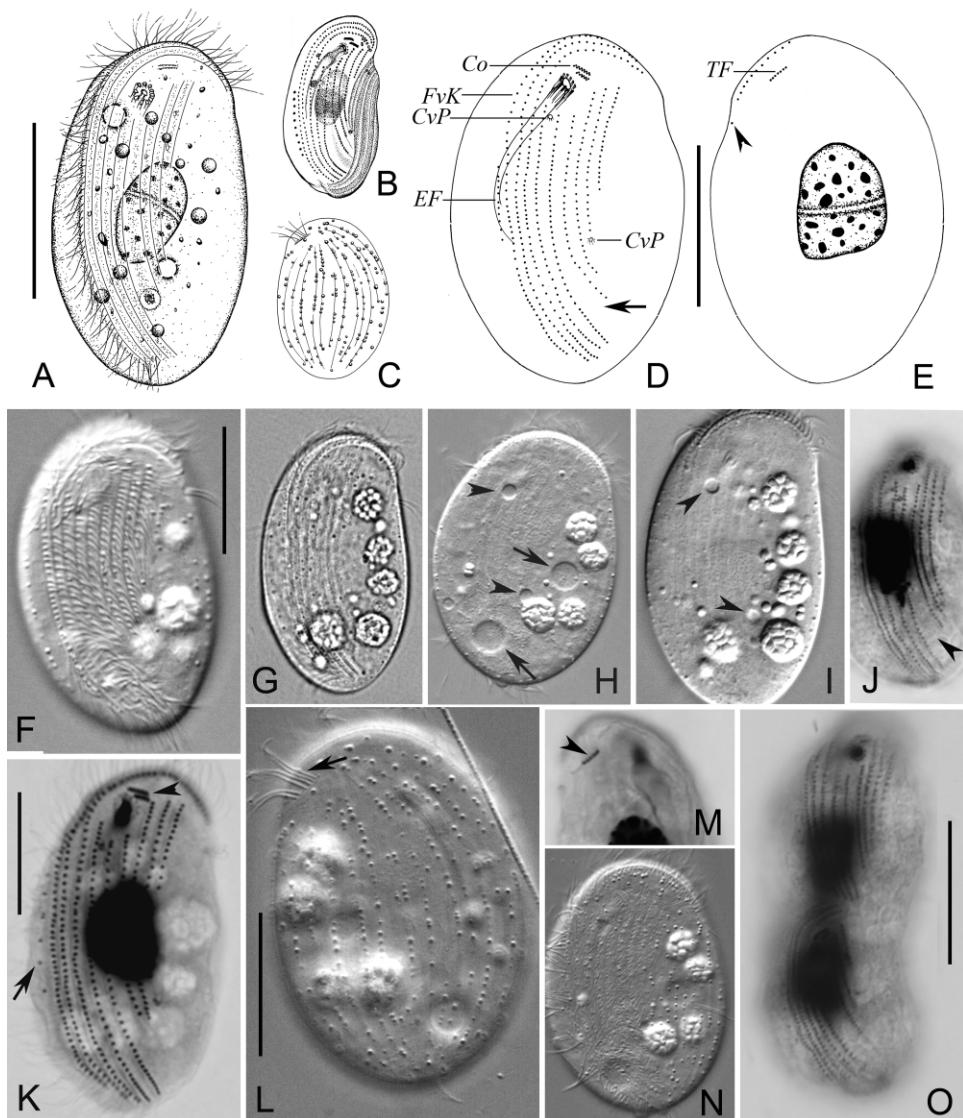


Figure 6. Morphology and infraciliature of *Trochochilodon flavus* from life (A, C, F–I, L, N) and after protargol impregnation (B, D, E, J, K, M, O). A, ventral view of a typical individual. B, ventral view, showing infraciliature, from Deroux (1976b). C, dorsal view, showing cortical granules. D, ventral 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.

sparingly 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,

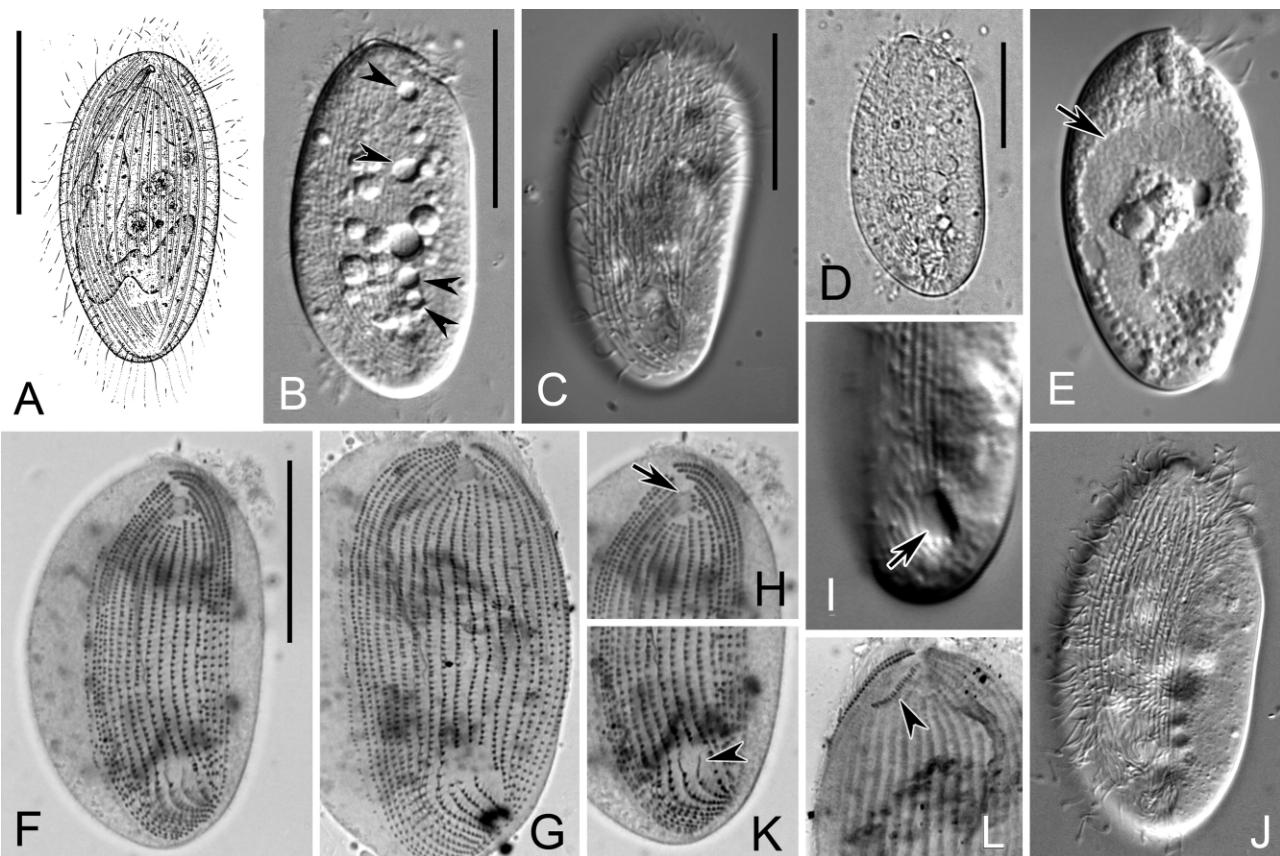


Figure 7. Morphology and infraciliature of *Hypocoma acinetarum* from life (A–E, I, J) and after protargol impregnation (F–H, K, L). A, ventral view, from Hu *et al.* (2003). B–E, ventral views of different individuals, arrowheads in (B) point to contractile vacuoles, arrow in (E) marks macronucleus. 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* $30\text{--}60 \times 15\text{--}30 \mu\text{m}$; 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. $35\text{--}60 \times 15\text{--}30 \mu\text{m}$. 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 ($3\text{--}5 \mu\text{m}$ across). Usually four contractile vacuoles, about $3\text{--}4 \mu\text{m}$ 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 $7 \mu\text{m}$ long, and on dorsal terminal fragment about $12 \mu\text{m}$ 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 ($35\text{--}60 \times 15\text{--}30$ vs. $30\text{--}50 \times 20\text{--}25 \mu\text{m}$) 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 (*Aporthotrichilia 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, *Aporthotrichilia 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 *Aporthotrichilia 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.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of China (Project no. 31071893), a Doctoral Research Fund from Shanghai Ocean University, a grant from FANEDD to J. G. (No. 2007B27) and the Center of Biodiversity Research, King Saud University, Saudi Arabia. Many thanks to Mr Weiwei

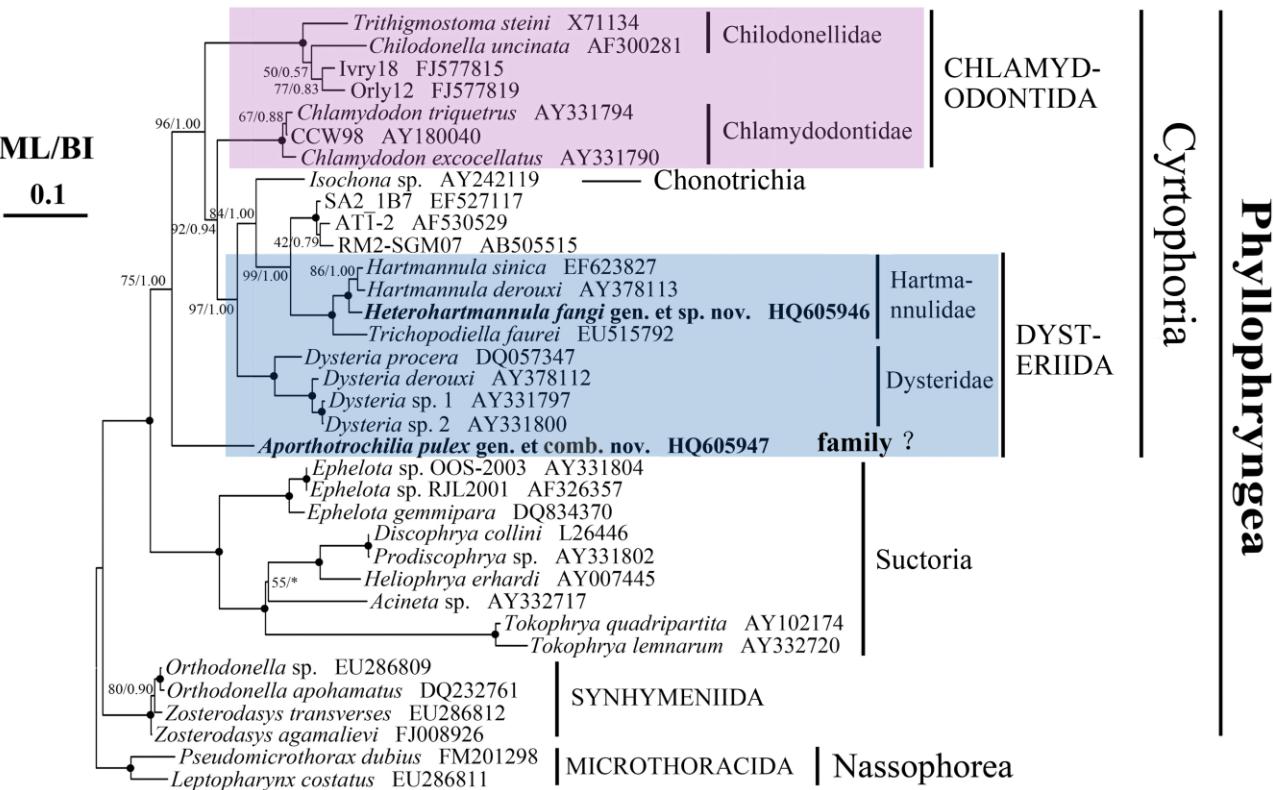


Figure 8. Phylogenetic trees based on the small subunit (SSU) rRNA sequences show the positions of two new genera, *Aporhotrochilia* gen. nov. and *Heterohartmannula* gen. nov., by maximum likelihood (ML) and Bayesian inference (BI). Numbers near branches are BI posterior probability value and ML bootstrap 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.

Liu, Mr Xinpeng Fan, Miss Jiamei Jiang, and Miss Jie Huang, for their help in sample collecting and suggestions. We are also grateful to three anonymous reviewers for critical comments.

REFERENCES

- Aesch E.** 2001. Catalogue of the generic names of ciliates (Protozoa, Ciliophora). *Denisia* **1:** 1–350.
- Carey PG.** 1991. *Marine interstitial ciliates. An illustrated key.* London: Chapman and Hall.
- Chen X, Gao S, Song W, Al-Rasheid KAS, Warren A, Gong J, Lin X.** 2010. *Parabirojmia multinucleata* spec. nov. and *Anteholosticha scutellum* (Cohn, 1866) Berger, 2003, marine ciliates (Ciliophora, Hypotrichida) from tropical waters in southern China, with notes on their small-subunit rRNA gene sequences. *International Journal of Systematic and Evolutionary Microbiology* **60:** 234–243.
- Chen X, Miao M, Song W, Warren A, Al-Rasheid KAS, Al-Farraj SA, Al-Quraishi SA.** 2008. Redescriptions of two poorly known marine suctorian ciliates, *Ephelota truncata* Fraipont, 1878 and *Ephelota mammillata* Dons, 1918 (Protozoa, Ciliophora, Suctoria), from Qingdao, China. *Acta Protozoologica* **47:** 247–256.
- Collin B.** 1907. Note préliminaire sur quelques Acinetiens: I. – *Ephelota gemmipara* (Hertwig); II. – *Hypocoma acinetarum* n. sp. Archives de zoologie expérimentale et générale. *Notes et Revues* **7:** 93–103.
- Corliss JO.** 1979. *The ciliated Protozoa. Characterization, classification and guide to the literature.* Oxford: Pergamon Press.
- Deroux G.** 1974. Les dispositifs adhésifs ciliaires chez les Cyrtophorida et la famille des Hypocomidae. *Protistologica* **10:** 379–396.
- Deroux G.** 1976a. Le plan cortical des Cyrtophorida unité d'expression et marges de variabilité. II. – Cyrtophorida a thigmotactisme ventral généralisé. *Protistologica* **12:** 483–500.
- Deroux G.** 1976b. Le plan cortical des Cyrtophorida unité d'expression et marges de variabilité. I. – Le cas des Pleiotrichopidae fam. nov., dans la nouvelle systématique. *Protistologica* **12:** 469–481.
- Deroux G.** 1976c. Plan cortical des Cyrtophorida. III. – Les structures différenciatrices chez les Dysterina. *Protistologica* **12:** 505–538.

- Deroux G.** 1994. Sous-Classe des Cyrtophorida Fauré-Fremiet in Corliss, 1956. *Traité de Zoologie* **2:** 401–431.
- Deroux G, Dragesco J.** 1968. Nouvelles données sur quelques ciliés holotriches cyrtophores à ciliature ventrale. *Protistologica* **4:** 365–403.
- Dragesco J.** 1966. Observations sur quelques ciliés libres. *Archiv für Protistenkunde* **109:** 155–206.
- Dragesco J, Dragesco-Kernéis A.** 1986. Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des Ciliés. *Faune Tropicale* **26:** 1–559.
- Fan X, Chen X, Song W, Al-Rasheid KAS, Warren A.** 2010. Two new marine scuticociliates, *Sathrophilus planus* n. sp. and *Pseudoplatynematum dengi* n. sp., with improved definition of *Pseudoplatynematum* (Ciliophora, Oligohymenophora). *European Journal of Protistology* **46:** 212–220.
- Fauré-Fremiet E.** 1957. *Trichopus lachmanni*, n. sp.; structure et morphogénèse. *Journal of Protozoology* **4:** 145–150.
- Fauré-Fremiet E.** 1965. Morphologie des Dysteriidæ (Ciliata Cyrtophorina). *Comptes Rendus de l'Academie des Sciences, Paris* **260:** 6679–6684.
- Foissner W.** 1979. Morphologie, Infraciliatur und Silberliniensystem von *Phascolodon vorticella* Stein, *Chlamydonella alpestris* nov. spec. und *Trochilia minuta* (Roux) (Ciliophora, Cyrtophorida). *Protistologica* **15:** 557–563.
- Foissner W, Blatterer H, Berger H, Kohmann F.** 1991. Taxonomische und Ökologische Revision der Ciliaten des Saprobiontsystems – Band I: Cyrtophorida, Oligotrichia, Hypotrichia, Colpodea. *Informationsberichte des Bayer. Landdesamtes für Wasserwirtschaft* **1/91:** 1–478.
- Gong J, Gao S, Roberts DM, Al-Rasheid KAS, Song W.** 2008. *Trichopodiella faurei* n. sp. (Ciliophora, Phyllopharyngea, Cyrtophoria): morphological description and phylogenetic analyses based on SSU rRNA and group I intron sequences. *Journal of Eukaryotic Microbiology* **55:** 492–500.
- Gong J, Lin X, Song W.** 2003. Redescription of a poorly-known marine cyrtophorid ciliate, *Dysteria pusilla* (Claparède et Lachmann, 1859) (Protozoa: Ciliophora: Cyrtophorida) from Qingdao, China. *Acta Protozoologica* **42:** 215–221.
- Gong J, Song W.** 2004a. Description of a new marine cyrtophorid ciliate, *Dysteria derouxi* nov. spec., with an updated key to 12 well-investigated *Dysteria* species (Ciliophora, Cyrtophorida). *European Journal of Protistology* **40:** 13–19.
- Gong J, Song W.** 2004b. Morphology and infraciliature of two marine species of *Hartmannula* (Protozoa, Ciliophora, Cyrtophorida), from scallop-farming waters off Qingdao (Tsingtao), China. *Journal of Natural History* **38:** 1327–1337.
- Gong J, Song W.** 2006. Description of a new marine cyrtophorid ciliate, *Brooklynella sinensis* n. sp. from China Sea with a new definition of the genus *Brooklynella* (Protozoa, Ciliophora, Cyrtophorida). *Zootaxa* **1113:** 41–49.
- Gong J, Song W, Warren A.** 2002. Redescriptions of two marine cyrtophorid ciliates, *Dysteria cristata* (Gourret and Roeser, 1888) and *Dysteria monostyla* (Ehrenberg, 1838) Kahl, 1931 (Protozoa, Ciliophora, Cyrtophorida), from China. *European Journal of Protistology* **38:** 213–222.
- Gong J, Song W, Warren A, Lin X, Roberts DM.** 2007. Microscopical observations on four marine *Dysteria* species (Ciliophora, Cyrtophorida). *European Journal of Protistology* **43:** 147–161.
- Gong J, Stoeck T, Yi Z, Miao M, Zhang Q, Roberts DM, Warren A, Song W.** 2009a. Small subunit rRNA phylogenies show that the class Nassophorea is not monophyletic (Phylum Ciliophora). *Journal of Eukaryotic Microbiology* **56:** 339–347.
- Gong J, Warren A, Song W.** 2009b. Cyrtophorids. In: Song W, Warren A, Hu X, eds. *Free-living ciliates in the Bohai and Yellow Sea, China*. Beijing: Science Press, 49–92.
- Guindon S, Gascuel O.** 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.
- Hall TA.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.
- Heuss K, Wilbert N.** 1973. Zur Morphologie und Ökologie von *Trochilia minuta* Roux, 1901 (Ciliata, Cyrtophorida). *Gewässer und Abwässer* **52:** 32–43.
- Hofmann AH.** 1987. Stomatogenesis in cyrtophorid ciliates. II. *Chilodonella cyprinid* (Moroff, 1902): the kinetofragment as an anlagen-complex. *European Journal of Protistology* **23:** 2–17.
- Hu X, Gong J, Song W.** 2003. Pathogenic ciliates in scallop-farming waters. In: Song W, Zhao Y, Xu K, Hu X, Gong J, eds. *Pathogenic protozoa in mariculture*. Beijing: Science Press, 145–178.
- Jiang J, Song W.** 2010. Two new Diophrys-like genera and their type species, *Apodiophrys ovalis* n. g., n. sp. and *Heterodiophrys zhui* n. g., n. sp. (Ciliophora: Euplotida), with notes on their molecular phylogeny. *Journal of Eukaryotic Microbiology* **57:** 354–361.
- Jiang J, Zhang Q, Hu X, Shao C, Al-Rasheid KAS, Song W.** 2010a. Two new marine ciliates, *Euplates sinicus* sp. nov. and *Euplates parabalteatus* sp. nov., and a new small subunit rRNA gene sequence of *Euplates rariseta* (Ciliophora, Spirotrichaea, Euplotida). *International Journal of Systematic and Evolutionary Microbiology* **60:** 1241–1251.
- Jiang J, Zhang Q, Warren A, Al-Rasheid KAS, Song W.** 2010b. Morphology and SSU rRNA gene-based phylogeny of two marine *Euplates* species, *E. orientalis* spec. nov. and *E. raikovi* Agamaliev, 1966 (Ciliophora, Euplotida). *European Journal of Protistology* **46:** 121–132.
- Kahl A.** 1931. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotrichia außer den im 1. Teil behandelten Prostomata. *Die Tierwelt Deutschlands* **21:** 181–389.
- Kahl A.** 1933. Ciliata libera et ectocommensalia. In: Grimpe G, Wagler E, eds. *Die Tierwelt der Nord- und Ostsee* 23 (Teil II, c3). Leipzig: Akademische Verlagsgesellschaft Becker & Erler, 147–183.
- Li L, Huang J, Song W, Shin MK, Al-Rasheid KAS, Berger H.** 2010b. *Apogastrostyla rigescens* (Kahl, 1932) gen. nov., comb. nov. (Ciliophora, Hypotrichia): morphology, notes on cell division, SSU rRNA gene sequence data, and neotypification. *Acta Protozoologica* **49:** 195–212.

- Li J, Lin X, Yi Z, Clamp JC, Liu W, Al-Rasheid KAS.** 2010a. Molecules or morphogenesis: how to determine the phylogenetic assignment of *Paratetrahymena* (Protista, Ciliophora, Oligohymenophorea)? *Zoologica Scripta* **39**: 499–510.
- Li L, Song W.** 2006. Phylogenetic positions of two cyrtophorid ciliates, *Dysteria procera* and *Hartmannula derouxi* (Ciliophora: Phyllopharyngea: Dysteriida) inferred from the complete small subunit ribosomal RNA gene sequences. *Acta Protozoologica* **45**: 265–270.
- Li L, Zhang Q, Al-Rasheid KAS, Kwon CB, Shin MK.** 2010c. Morphological redescriptions of *Aspidisca magna* Kahl, 1932 and *A. leptaspis* Fresenius, 1865 (Ciliophora, Euplotida), with notes on morphogenetic process in *A. magna*. *Acta Protozoologica* **49**: 327–337.
- Liu W, Li J, Gao S, Gong J, Lin X, Liu H, Song W.** 2009. Morphological studies and molecular data on a new marine ciliate, *Apokeronopsis sinica* n. sp. (Ciliophora: Urostylida), from the South China Sea. *Zootaxa* **2005**: 57–66.
- Liu W, Shao C, Gong J, Li J, Lin X, Song W.** 2010. Morphology, morphogenesis and molecular phylogeny of a new marine urystyliid ciliate (Ciliophora, Stichotrichia) from the South China Sea, and an overview of the convergent evolution of midventral complex within the Spirotrichea. *Zoological Journal of the Linnean Society* **158**: 697–710.
- Miao M, Wang Y, Song W, Clamp JC, Al-Rasheid KAS.** 2010. Description of *Eurystomatella sinica* n. gen., n. sp. with establishment of a new family Eurystomatellidae n. fam. (Protista, Ciliophora, Scuticociliatia) and analyses of its phylogeny inferred from sequences of the small-subunit rRNA gene. *International Journal of Systematic and Evolutionary Microbiology* **60**: 460–468.
- Nylander JA.** 2004. *MrModeltest v2*. Distributed by the author. Evolutionary Biology Center, Uppsala University.
- Pan H, Gao F, Li J, Lin X, Al-Farraj SA, Al-Rasheid KAS.** 2010a. Morpholgy and phylogeny of two new pleurostomatid ciliates, *Epiphyllum shenzhenense* n. sp. and *Loxophyllum spirellum* n. sp. (Protozoa, Ciliophora) from a mangrove wetland, South China. *Journal of Eukaryotic Microbiology* **57**: 421–428.
- Pan H, Huang J, Hu X, Fan X, Al-Rasheid KAS, Song W.** 2010b. Morphology and SSU rRNA gene sequence of three marine ciliates from Yellow Sea, China, including one new species, *Uronema heteromarinum* nov. spec. (Ciliophora, Scuticociliatida). *Acta Protozoologica* **49**: 45–59.
- Pan H, Hu X, Gong J, Lin X, Al-Rasheid KAS, Al-Farraj SA, Warren A.** 2011. Morphological redescriptions of four marine ciliates (Ciliophora: Cyrtophorida: Dysteriidae) from Qingdao, China. *European Journal of Protistology* **47**: 197–207.
- Petz W, Song W, Wilbert N.** 1995. Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia* **40**: 1–223.
- Ronquist F, Huelsenbeck JP.** 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Shao C, Song W, Gong J.** 2008. Morphology and morphogenesis of a new marine cyrtophorid ciliate, *Hartmannula sinica* nov. spec. (Protozoa, Ciliophora, Cyrtophorida) from China. *European Journal of Protistology* **44**: 1–12.
- Shen Z, Jie H, Lin X, Yi Z, Li J, Song W.** 2010. Morphological and molecular characterization of *Aspidisca hongkongensis* spec. nov. (Ciliophora, Euplotida) from the South China Sea. *European Journal of Protistology* **46**: 204–211.
- Snoeyenbos-West OLO, Cole J, Campbell A, Coats DW, Katz LA.** 2004. Molecular phylogeny of phyllopharyngean ciliates and their group I introns. *Journal of Eukaryotic Microbiology* **51**: 441–450.
- Song W.** 2003. On two marine cyrtophorid ciliates from China, with description of *Chlamydonella derouxi* nov. spec. and *Orthotrichilia pilula* (Deroux, 1976) nov. comb., and reestablishment of the genus *Orthotrichilia* nov. gen. (Protozoa, Ciliophora, Cyrtophorida). *Hydrobiologia* **499**: 169–177.
- Song W, Wilbert N.** 2002. Faunistic studies on marine ciliates from the Antarctic benthic area, including descriptions of one epizoic form, 6 new species and 2 new genera (Protozoa: Ciliophora). *Acta Protozoologica* **41**: 23–61.
- Sun P, Song W, Xu D.** 2007. Two new marine species of *Pseudovorticella* (Ciliophora, Peritrichia) from Qingdao, north China. *Acta Protozoologica* **46**: 55–64.
- Wang Y, Hu X, Long H, Al-Rasheid KAS, Al-Farraj SA, Song W.** 2008. Morphological studies indicate that *Pleuronema grolierei* nov. spec. and *P. coronatum* Kent, 1881 represent different sections of the genus *Pleuronema* (Ciliophora: Scuticociliatida). *European Journal of Protistology* **44**: 131–140.
- Xu D, Song W.** 2006. Hapantotypification and morphological redescription of the marine planktonic ciliate, *Spirostrombidium cinctum* (Kahl, 1932) Petz, Song et Wilbert, 1995 (Ciliophora: Oligotrichida). *Acta Protozoologica* **45**: 17–25.
- Yi Z, Song W, Stoeck T, Al-Rasheid K, Al-Khedhairy K, Gong J, Ma H, Chen Z.** 2009. Phylogenetic analyses suggest that *Psammomitra* (Ciliophora, Urostylida) should represent an urystyliid family, based on SSrRNA and alpha-tubulin gene sequence information. *Zoological Journal of the Linnean Society* **157**: 227–236.