

Taxonomic Studies on Three Marine Ciliates from China, Including a New Species (Ciliophora, Cyrtophorida)

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Abstract. The present work investigates the living morphology and infraciliature of three marine cyrtophorid ciliates, which were isolated from Qingdao, China. Compared with its congeners, *Orthotrochilia sinica* spec. nov. can be distinguished by a combination of features: body slender and elliptical in outline, size about $50\text{--}60 \times 20\text{--}25 \mu\text{m}$ *in vivo*, 18–21 somatic kineties, the length of the left perioral kinety treble the length of the right one, two ventrally located contractile vacuoles on the right side, and 25–32 nematodesmal rods. Based on current observations and the previous description, the diagnosis of *Trochiloides tenuis* (Deroux, 1976) Chen *et al.* 2011 is improved: cell size $30\text{--}40 \times 20\text{--}35 \mu\text{m}$ *in vivo*, oval shaped in outline; consistently three right kineties, four left kineties and seven postoral kineties; a single contractile vacuole; marine habitat. A second species of *Trochiloides*, *T. recta* (Kahl, 1923) Chen *et al.* 2011 is re-described based on a Chinese population. Furthermore, a key to the identification of species of the genus *Trochiloides* whose infraciliature data are available is supplied, and *Chlamydonyx trivialis* (Fenchel, 1965) comb. nov. [basonym: *Trochiloides trivialis* Fenchel, 1965] is suggested.

Key words: Cyrtophorida, infraciliature, marine ciliates, new species, taxonomy.

INTRODUCTION

Ciliates belonging to the order Cyrtophorida are an important component of periphytons in aquatic environments. Since the development of the protargol-impregnation method in the 1960s and, thereafter, the

application of molecular techniques, there has been great progress in studying the taxonomy and phylogeny of Cyrtophorida (Deroux 1976a, b, c; Dragesco 1966; Fauré-Fremiet 1965; Foissner 1979; Gao *et al.* 2012; Gong *et al.* 2005, 2007, 2008; Ma *et al.* 2006; Shao *et al.* 2008; Snoeyenbos-West *et al.* 2004). However, there remain many taxonomic problems, especially in respect to some poorly studied genera, such as *Orthotrochilia* Song, 2003 and *Trochiloides* Chen *et al.* 2011, which were treated as *nomen nudum* until recently (Chen *et al.* 2011, Song 2003). Both genera are similar in their

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general morphology (size, shape, contractile vacuoles, etc.) and both also have rather short postoral and left kineties, meaning that they are often confused with each other. *Orthotrochilia*, however, has two oral kineties, while *Trochilioides*, like most hartmannulids, has two circumoral kineties and one preoral kinety (Deroux 1976c). There are only a few nominal species in these two genera at present: only two in *Orthotrochilia* and ten in *Trochilioides*. Furthermore, most of these lack details concerning live features and/or infraciliature, and only a few have rather rudimentary descriptions, which result in many misidentifications or confusions (Deroux 1976c, Foissner 1984, Foissner *et al.* 1991).

During the last decade, extensive investigation of ciliate fauna has been carried out in China. Many taxa of cyrtophorids, including these two genera, have been re-defined and several new taxa and records have been reported (Chen *et al.* 2011, 2012; Gong *et al.* 2005; Gong and Song 2006; Pan *et al.* 2011, 2012; Song 2003; Song *et al.* 2009). As a new contribution, this present work describes a new species of *Orthotrochilia* and re-describes two species of *Trochilioides*.

MATERIALS AND METHODS

Orthotrochilia sinica spec. nov. was collected from coastal waters off Xiaogang Port in Qingdao (36°18'N, 120°43'E), China, on 30 November 2008. The water temperature was ca. 11°C and the salinity ca. 31‰.

Trochilioides tenuis was collected in the same place off Qingdao, China, on 11 March 2009. The water temperature was ca. 12°C and the salinity ca. 30‰.

Trochilioides recta was isolated from coastal waters off Qingdao, China, on 2 July 2009. The water temperature was ca. 24.5°C and the salinity ca. 31‰.

Living cells were observed at 100–1,000 × magnifications using bright-field and differential interference contrast microscopy. Protargol silver impregnation (Wilbert 1975) was applied to reveal the infraciliature. Counts, measurements and drawings of stained specimens were performed at 1,250 × magnification with the aid of a camera lucida. Terminology and systematics follow Corliss (1979).

RESULTS AND DISCUSSION

Order Cyrtophorida Fauré-Fremiet in Corliss, 1956 Family Dysteriidae Claparède and Lachmann, 1858 Genus *Orthotrochilia* Song, 2003

Orthotrochilia sinica spec. nov. (Fig. 1; Table 1)

Diagnosis: Marine *Orthotrochilia* with slender elliptical body shape, about 50–60 × 20–25 µm *in vivo*; 18–21 somatic kineties, two frontoventral kineties; the length of left perioral kinety treble the length of right one; two contractile vacuoles ventrally located on right side; 25–32 nematodesmal rods.

Slides deposition: A protargol slide (registration number: PHB08110301-1) with the holotype specimen and one protargol slide (registration number: PHB08110301-2) with paratype specimens are deposited in the Laboratory of Protozoology, OUC, China. Relevant specimens are marked by black ink circles on the cover-slip.

Type location: Coastal waters off Xiaogang Port in Qingdao (36°18'N, 120°43'E), China.

Description: Cell size about 50–60 × 20–25 µm *in vivo*. Body elliptical in outline with anterior end bluntly rounded and posterior tapered (Fig. 1A, F, I, J). Dorsal side vaulted with a longitudinal furrow in middle, while ventral side flattened (Fig. 1B, C, H, L). Cytostome sub-apically located, about 9 µm in width, and surrounded by 25–32 nematodesmal rods (Fig. 1F, D, M). Cytoplasm colourless, containing numerous small, greasily shining granules. Two contractile vacuoles, 4–5 µm in diameter, positioned in anterior and posterior 1/4 of body length, near right margin; contracting interval about 16 seconds (Fig. 1C, G). Podite, about 10 µm long, subcaudally positioned (Fig. 1I). Single macronucleus ellipsoid, about 25 × 11 µm *in vivo*, centrally located (Fig. 1G). Cilia about 10 µm long. Movement by slowly gliding on the substrate.

Infraciliature as shown in Fig. 1D, E, K, M. In total, 18–21 somatic kineties, the two right-most of which are almost equal in length, with both extending anteriorly and bending to the left side; the five right-most kineties terminating posteriorly at the same level, while others are progressively shorten from right to left (Fig. 1D, K, M). Equatorial fragment composed of 3–12 basal bodies, and terminal fragment consisting of 6–11 ones (Fig. 1D, E). About 4–6 kinetosome-like dots present near the base of the podite (Fig. 1D). Oral ciliature typical of the genus *Orthotrochilia*: two perioral kineties composed of dikinetids and distinctly separated; the left kinety triple the length of the right one (Fig. 1D, M).

Remarks and comparison: The genus *Orthotrochilia* was first established by Deroux (1976c). Because no type species was fixed, however, it was a *nomen nudum* according to Article 13.3.3 of ICZN (1999). Song (2003) re-studied *Orthotrochilia pilula* and re-established the genus by fixing *O. pilula* as the type species.

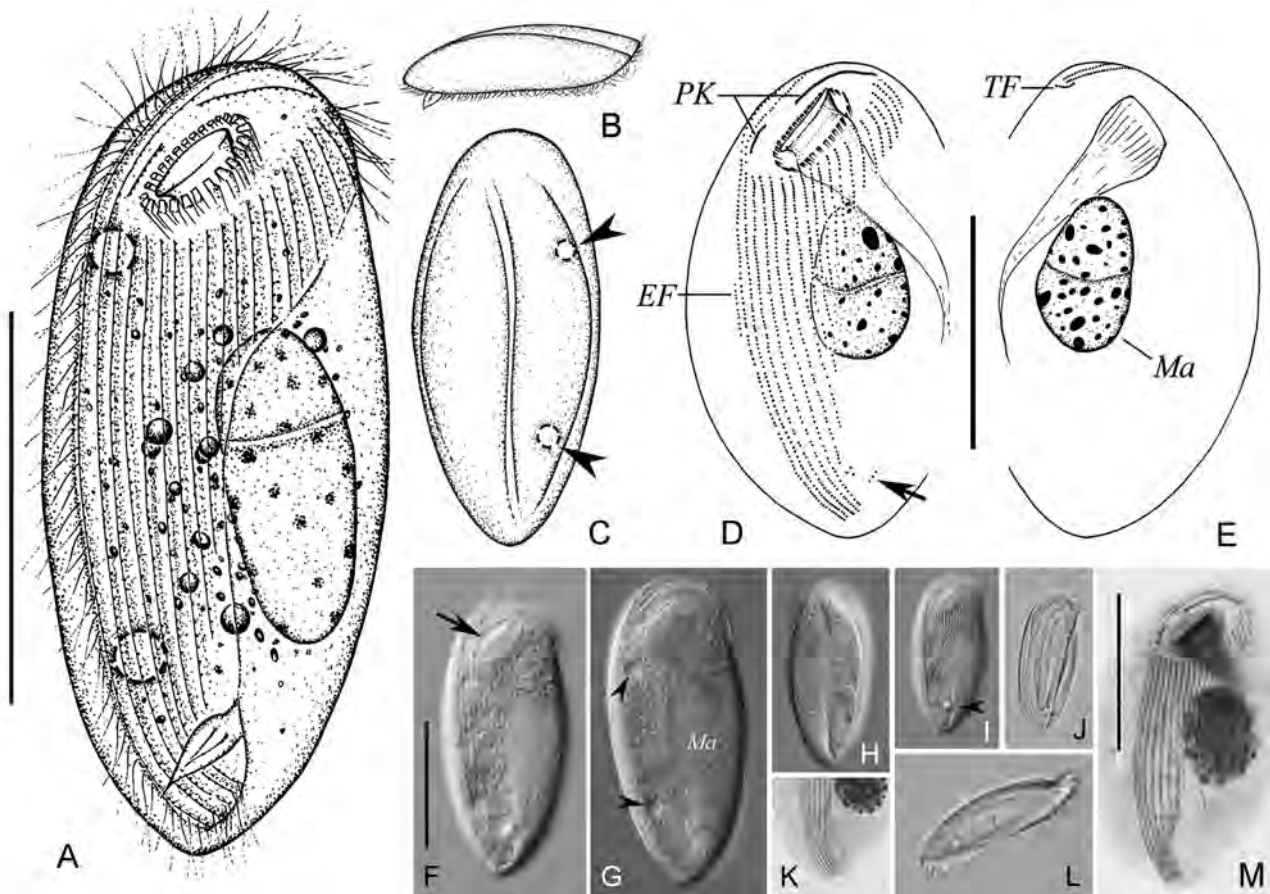


Fig. 1A–M. *Orthotrochilia sinica* spec. nov. from life (A–C, F–J, L) and after protargol impregnation (D, E, K, M). **A** – ventral view of a representative individual; **B, L** – lateral view; **C** – to show contractile vacuoles (arrowheads) and the furrow in dorsal side; **D, E** – infraciliature of ventral (**D**) and dorsal (**E**) side; **F, G** – ventral view, arrow (**F**) indicates cytostome and arrowheads (**G**) mark contractile vacuoles; **H** – dorsal view; **I, J** – ventral view of different individuals, arrowhead (**I**) indicates the podite; **K** – posterior portion; **M** – infraciliature. EF – equatorial fragment, Ma – macronucleus, PK – perioral kineties, TF – terminal fragment. Scale bars: 30 µm.

To date, only two species are included in this genus, i.e. *Orthotrochilia pilula* (Deroux, 1976) Song, 2003 and *O. agamaleivi* (Deroux, 1976) Song, 2003.

In terms of size and cell shape, the new species resembles *Orthotrochilia agamaleivi*. It can be distinguished, however, in that it has more somatic kineties (18–21 vs. 12–18), fewer nematodesmal rods (25–32 vs. ca. 40) and a longer left perioral kinety (triple the length of the right perioral kinety vs. nearly equal in length; Deroux 1976c). Meanwhile, compared to *O. pilula*, *O. sinica* is larger in size (50–60 × 20–25 µm vs. 20–30 × 15–20 µm), and possesses more somatic kineties (18–21 vs. 9) and nematodesmal rods (25–32 vs. ca. 6; Song 2003).

Genus *Trochiloides* Chen *et al.* 2011

Trochiloides tenuis (Deroux, 1976) Chen *et al.* 2011 (Fig. 2; Table 1)

According to Article 13.3.3 of ICZN (1999), *Trochiloides* Kahl, 1931 is a *nomen nudum*, so Chen *et al.* (2011) re-established the genus by fixing *T. recta* as type species. *Trochiloides tenuis* was originally reported by Deroux (1976c), based only on its infraciliature. So far no re-description has been attempted, we here redescribe it based on a Qingdao population.

Improved diagnosis: Small *Trochiloides*, 30–40 × 20–35 µm *in vivo*, body elliptical in outline; consistently three right kineties, four left kineties and seven

Table 1. Morphometric characteristics of *Orthotrochilia sinica* spec. nov. (the first line), *Trochilioides tenuis* (the second line), and *Trochilioides recta* (the third line) from protargol-impregnated specimens.

Characters	Min	Max	Mean	SD	CV	<i>n</i>
Body length (µm)	45	62	55.0	4.12	7.5	24
	24	33	28.8	2.71	9.4	18
	25	38	33.5	3.17	9.5	18
Body width (µm)	24	39	30.8	4.70	15.3	24
	14	21	17.8	1.96	11.0	18
	16	29	21.7	2.93	13.5	18
Number of somatic kineties	18	21	19.4	0.65	3.4	24
	14	14	14.0	0	0	18
	17	20	18.3	0.59	3.2	18
Number of right kineties	–	–	–	–	–	–
	3	3	3.0	0	0	18
	6	6	6.0	0	0	18
Number of left kineties	–	–	–	–	–	–
	4	4	4.0	0	0	18
	6	9	7.3	0.59	8.0	18
Number of postoral kineties	–	–	–	–	–	–
	7	7	7.0	0	0	18
	5	5	5.0	0	0	18
Number of basal bodies in EF	3	12	7.9	2.54	32.2	24
	0	8	3.4	2.48	72.9	16
	0	14	6.7	3.33	49.7	18
Number of basal bodies in TF	6	11	8.5	1.18	13.9	24
	2	4	2.6	0.51	19.6	16
	4	6	5.2	0.70	13.7	11
Number of nematodesmal rods	25	32	28.4	2.02	7.1	24
	–	–	–	–	–	–
	–	–	–	–	–	–
Length of macronucleus (µm)	17	22	19.7	1.46	7.4	24
	10	14	11.4	1.20	10.5	18
	11	18	13.9	0.43	3.1	18
Width of macronucleus (µm)	8	15	11.9	1.84	15.5	24
	5	8	6.3	0.84	13.3	18
	7	12	9.5	1.38	14.5	18

Abbreviations: CV – coefficient of variation in %; EF – equatorial fragment; Max – maximum; Mean – arithmetic mean; Min – minimum; *n* – number of individuals examined; SD – standard deviation; TF – terminal fragment.

– data not available.

postoral kineties; single contractile vacuole located at anterior of right side; marine habitat.

Description of Qingdao population: Cell size about 30–40 × 20–35 µm *in vivo*; body usually elliptical in outline with anterior end bluntly rounded and

posterior end somewhat tapered (Fig. 2A, H, I). Ventral side flat and dorsal side humped with several longitudinal stripes after protargol impregnation (Fig. 2D, J, P). Usually, rice-like pellicular granules irregularly distributed on dorsal side (Fig. 2K). Cytostome prominent,

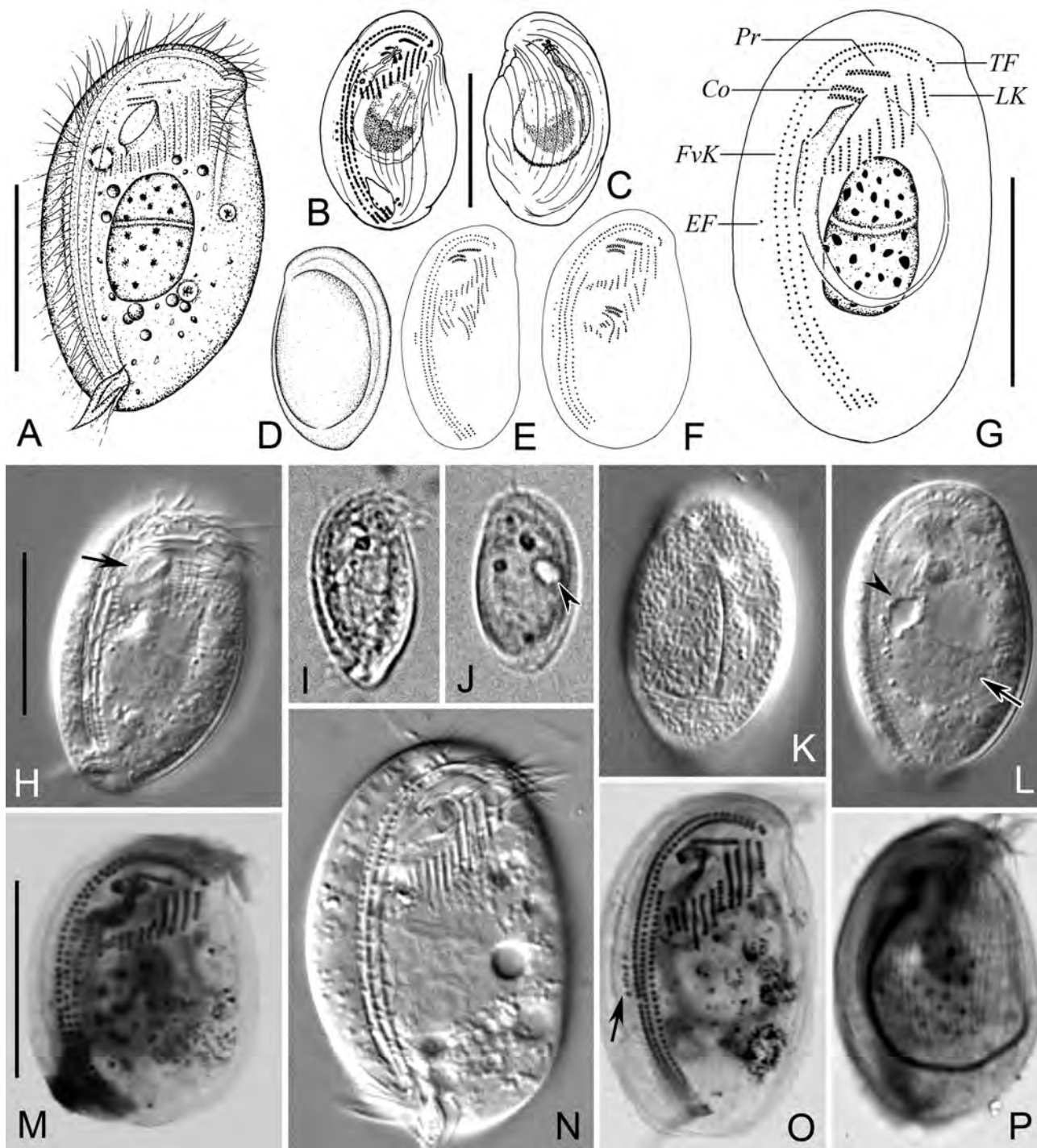


Fig. 2A–P. *Trochiloides tenuis* from life (A, D, H–L, N) and after protargol impregnation (B, C, E–G, M, O, P). **A** – ventral side of a representative individual; **B, C** – from Deroux (1976c); **D** – dorsal view *in vivo*; **E, F** – individuals in division; **G** – to show infraciliature; **H, I** – ventral views, arrow (H) refers to cytostome; **J** – dorsal view, arrowhead points to contractile vacuole; **K** – to show pellicle granules in dorsal side; **L** – details of cytoplasm, arrowhead marks contractile vacuole and arrow indicates macronucleus; **M, O** – infraciliature of different individuals, arrow (O) refers to equatorial fragment; **N** – ventral view; **P** – dorsal view. Co – circumoral kineties; EF – equatorial fragment; FvK – frontoventral kineties; LK – left kineties; Pr – preoral kinety; TF – terminal fragment. Scale bars: 20 μ m.

oval shaped, ventrally positioned in anterior 1/5 of cell (Fig. 1H); but nematodesmal rods not detected. Cytoplasm colourless, containing numerous small, greasily shining globules (2–3 μm across), and food vacuoles (4–8 μm across), which render cells slightly greyish (Fig. 2L, N). Single contractile vacuole, about 5 μm in diameter, located at anterior 1/3 of right side. Single oval macronucleus centrally positioned, about $11 \times 6 \mu\text{m}$ *in vivo* (Fig. 2A, L). Podite leaf shaped and 5 μm long, subcaudally located (Fig. 2A, H). Cilia 7 μm long. Movement by gliding on the substrate.

Infraciliature as shown in Fig. 2E, F, G, M, O, P. Consistently, three right kineties, seven postoral kineties and four left kineties surrounding the cytostome. Two right-most kineties almost equal in length, extending anteriorly to anterior margin (Fig. 2G, O). Posterior ends of right kineties terminating at the same level and a fragment, comprising three or four basal bodies, located on the left of them. Left and postoral kineties very short, the posterior ends of which are progressively shortened from right to left (Fig. 2M). Equatorial fragment composed of 0–8 basal bodies, and terminal fragment consisting of 2–4 ones (Fig. 2G, O). The oral ciliature composed of three dikinetid fragments: two circumoral kineties (Co) arranged in parallel, equal in length; and one preoral kinety, relatively long, obliquely arranged in front of Co (Fig. 2G, M, O). During cell division, the oral primordium of opisthe generated from three sections of postoral kineties (Fig. 2E, F).

Remarks: The infraciliature of the Qingdao population corresponds with the original description very well (Fig. 2B, C; Deroux 1976c), although our isolate is a little larger in size (23–33 μm long vs. 15–25 μm long after protargol impregnation). Considering that the size usually changes in different populations, we believe the two to be conspecific.

***Trochiloides recta* (Kahl, 1928) Chen *et al.* 2011 (Figs 3, 4; Table 1)**

Syn. *Trochilia recta* Kahl, 1928

Trochiloides recta is a relatively well-studied species. It was originally described by Kahl (1928) as a member of *Trochilia*. Subsequently, Kahl (1931) assigned it to the new genus *Trochiloides* according to its ciliary arrangement though he could not be the author of this genus (as mentioned above). Later it was re-described twice in more or less details (Deroux 1976c, Foissner *et al.* 1991). Therefore we just supply a brief description of the Qingdao population.

Description of Qingdao population: Cells about $35 \times 20 \mu\text{m}$ *in vivo*. Body broad oval in outline with both ends bluntly rounded (Fig. 3A, E, G). Dorsoventrally flattened about 2:1, ventral side flat, in right region slightly concave where the ventral ciliary rows are located; dorsal side somewhat humped (Fig. 3B, F). Cytostome ventrally positioned at anterior 1/5 of body length; about six conspicuous nematodesmal rods around it (Fig. 3A, E). Cytoplasm colourless, containing some greasily shining globules (2–3 μm across). Two contractile vacuoles, 5 μm in diameter, located at anterior and posterior 1/4 of cell (Fig. 3K). Podite about 6 μm long, caudally positioned (Fig. 3A, G). Single ellipsoid macronucleus in central region, about $15 \times 10 \mu\text{m}$ *in vivo* (Fig. 3K).

Infraciliature as shown in Fig. 3C, D, H–J, L, M. 17–20 somatic kineties in total, comprising six right kineties, six to nine left kineties and five postoral kineties. Two right-most kineties, almost equal in length, and extending anteriorly to anterior margin. Postoral and left kineties very short and distributed in anterior body half, the posterior ends of which are progressively shortened from right to left (Fig. 3J). Equatorial fragment (Fig. 3L) composed of 0–14 basal bodies, while terminal fragment consisting of 4–6 ones (Fig. 3H). Usually, five or six kinetosome-like granules present near the base of podite and arranged in a letter “C” pattern (Fig. 3C, D, I). Two circumoral kineties and one preoral kinety composed of dikinetids and obliquely arranged (Fig. 3C, D, H).

Remarks: According to Kahl (1931), *Trochiloides recta* is a euryhaline species and it occurs in waters with salinity from 3 to 25‰. Before the detailed infraciliature was first revealed by Deroux (1976c), several forms (Fig. 4D–F) had been reported under this name (Agamaliev 1974, Borror 1972, Dragesco 1966). Among these, *Trochiloides recta* sensu Agamaliev, 1974 (Fig. 4D) was assigned to *Orthotrochilia* as *O. agamalievi* by Deroux (1976c), while *Trochiloides recta* sensu Dragesco, 1966 (Fig. 4F) was considered as another species by Foissner *et al.* (1991). In terms of body size, shape, the number of contractile vacuoles and infraciliature, our isolate resembles the original population (Fig. 4A, B) and the population of Deroux (Fig. 4H) very well, and they must, therefore, be conspecific.

Foissner (1984) collected a freshwater isolate, namely *Trochiloides fimbriatus* Foissner, 1984 (Fig. 4N, O), whose infraciliature nearly has no difference from *T. recta*. However, the former has peculiar basket-

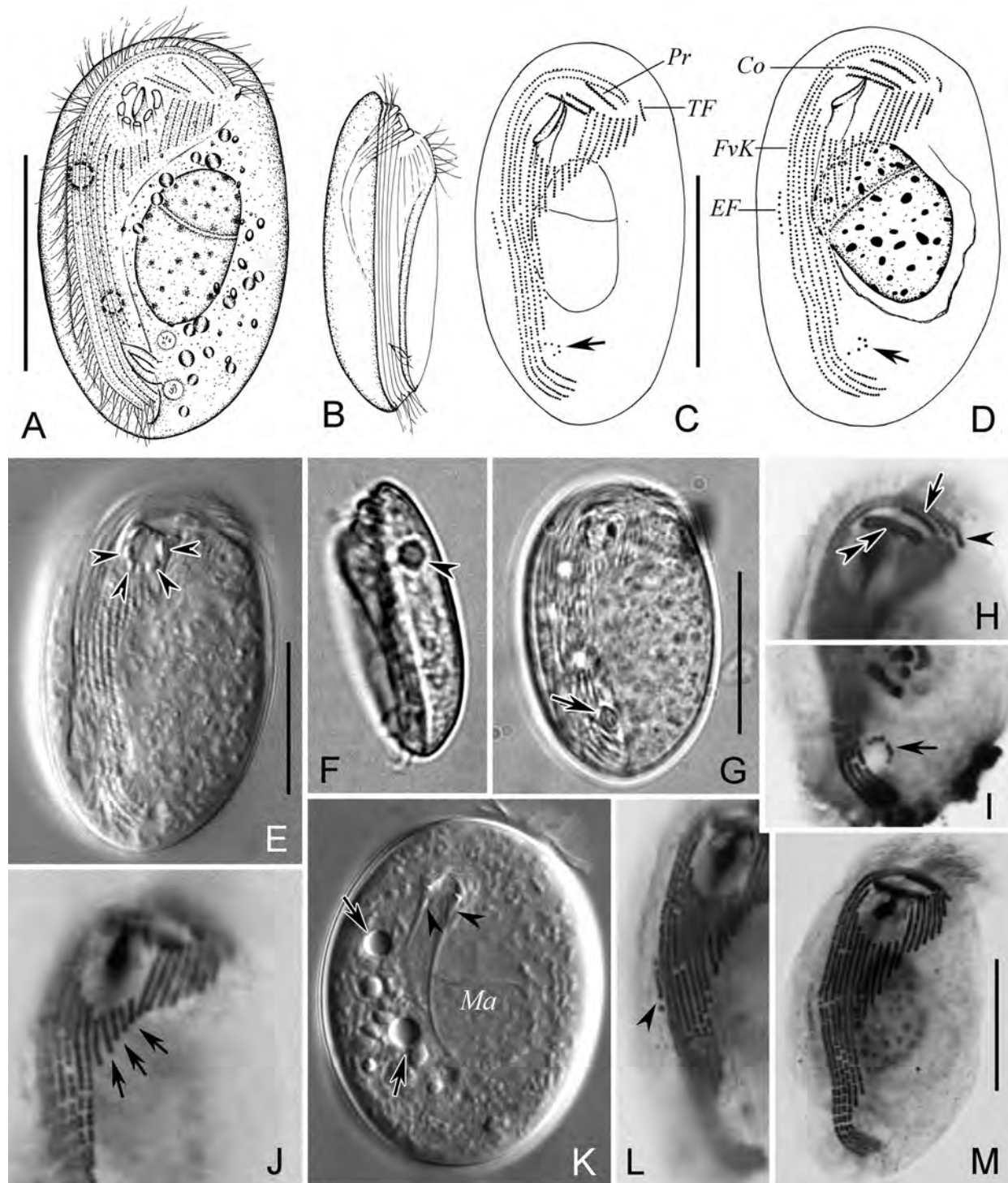


Fig. 3A–M. *Trochiloides recta* from life (A, B, E–G, K) and after protargol impregnation (C, D, H–J, L, M). **A** – ventral view of a typical individual; **B, F** – right view, arrowhead (F) indicates contractile vacuole; **C, D** – to show infraciliature, arrows mark kinetosome-like granules at base of podite; **E, G** – ventral view, arrowheads (E) point to tooth and arrow (G) shows podite; **H** – anterior portion, double arrowheads point to circumoral kineties, arrow refers to preoral kinety, arrowhead indicates terminal fragment; **I** – posterior portion, arrow marks kinetosome-like granules at base of podite; **J** – anterior portion, arrows point to postoral kineties; **K** – details of cytoplasm, arrows show contractile vacuoles, arrowheads refer to nematodesmal rods; **L** – right portion of ventral side, arrowhead points to equatorial fragment; **M** – infraciliature. Co – circumoral kineties, EF – equatorial fragment, FvK – frontoventral kineties, Ma – macronucleus, Pr – preoral kinety, TF – terminal fragment. Scale bars: 20 μm (A, C), 15 μm (E, G, M).

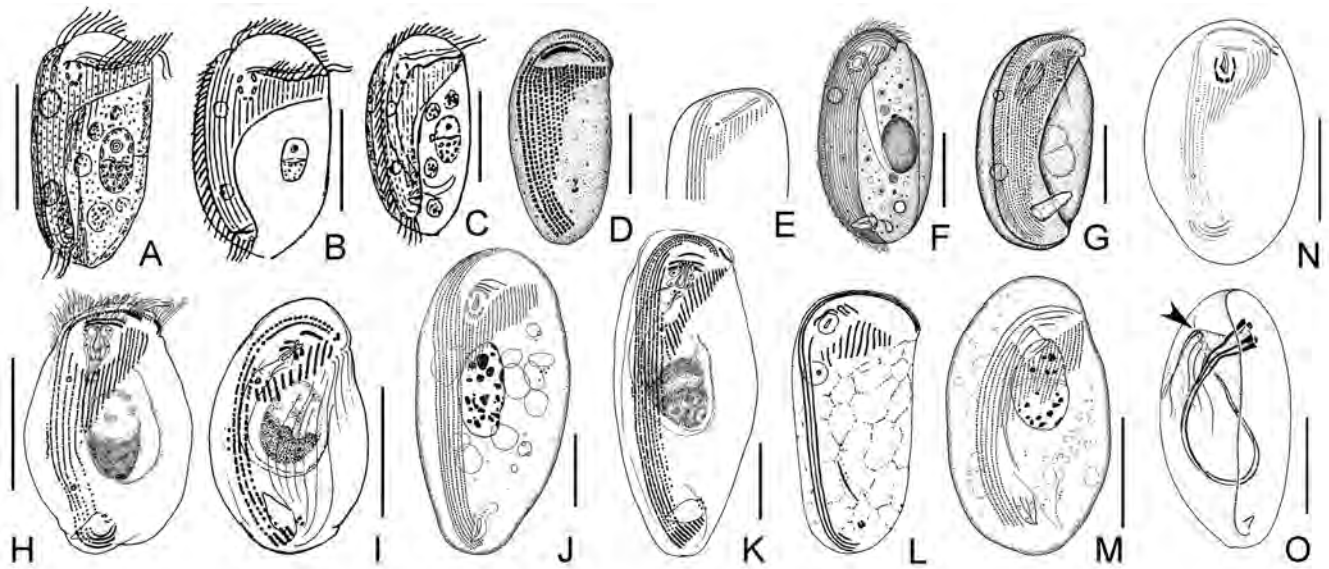


Fig. 4A–O. *Trochiloides recta* and its congeners whose infraciliature are known. **A–C, E, H** – *Trochiloides recta*, A and B from Kahl (1928), C from Kahl (1931), E from Borror (1972), H from Deroux (1976); **D** – *Orthotrochilia agamalievi* (syn. *Trochiloides recta* sensu Agamaliyev, 1974), from Agamaliyev (1974); **F** – *Trochiloides recta* sensu Dragesco, 1966, from Dragesco (1966); **G** – *Trochiloides trivialis*, from Fenchel (1965); **I** – *T. tenuis*, from Deroux (1976); **J, K** – *T. bathybius*, J from Jankowski (1967), K from Deroux (1976); **L** – *T. dispar*, from Fauré-Fremiet (1965); **M** – *T. littoralis*, from Jankowski (1967); **N, O** – *T. fimbriatus*, from Foissner (1984), arrowhead (O) points to the basket-curtain. Scale bars: 20 µm.

curtain which is not found in our and previous populations of *T. recta* and other species of *Trochiloides*. Additionally, it can be separated from *T. recta* by the location of the terminal fragment (left of frontoventral kineties vs. right of end of frontoventral kineties) and its orientation (vertical vs. horizontal). So both very likely are distinct species.

To date, besides *Trochiloides tenuis* (Fig. 4I), *T. recta* and *T. fimbriatus*, there are four species of *Trochiloides* whose infraciliature are known, namely, *Trochiloides dispar* Faure-Fremiet, 1965 (Fig. 4L), *Trochiloides bathybius* Jankowski, 1967 (Fig. 4J, K), *Trochiloides littoralis* Jankowski, 1967 (Fig. 4M), and

Trochiloides trivialis Fenchel, 1965 (Fig. 4G). Among these, the pattern of infraciliature of *T. trivialis* is quite different due to the fact that some of the postoral kineties are subterminally ended (Fenchel 1965). According to Chen *et al.* (2011), a critical feature through which it is possible to distinguish *Chlamydonyx* from *Trochiloides* is that part of the postoral and all of the left kineties extend to the posterior. Based on this criterion, *T. trivialis* should be transferred to the genus *Chlamydonyx* as *Chlamydonyx trivialis* (Fenchel, 1965) comb. nov.

A simple key to the identification of *Trochiloides* spp. with available infraciliature data can be proposed as below:

1	Five frontoventral kineties	<i>T. bathybius</i>
1'	Two frontoventral kineties	2
2	Three right kineties	3
2'	Five or six right kineties	4
3	Four left kineties	<i>T. tenuis</i>
3'	More than four left kineties	<i>T. dispar</i>
4	17 postoral kineties and left kineties in total	<i>T. littoralis</i>
4'	11–14 postoral kineties and left kineties in total	5
5	Absence of basket-curtain	<i>T. recta</i>
5'	Presence of basket-curtain	<i>T. fimbriatus</i>

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