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European Journal of Protistology 51 (2015) 142–157

European Journal of PROTISTOLOGY

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Biodiversity of marine scuticociliates (Protozoa, Ciliophora) from China: Description of seven morphotypes including a new species, *Philaster sinensis* spec. nov.

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Received 21 August 2014; received in revised form 17 February 2015; accepted 18 February 2015
Available online 25 February 2015

Abstract

Seven marine scuticociliates, *Philaster sinensis* spec. nov., *Pseudocohnilembus hargisi* Evans and Thompson, 1964. J. Protozool. 11, 344, *Parauronema virginianum* Thompson, 1967. J. Protozool. 14, 731, *Uronemella filificum* (Kahl, 1931. Tierwelt. Dtl. 21, 181) Song and Wilbert, 2002. Zool. Anz. 241, 317, *Cohnilembus verminus* Kahl, 1931, *Parauronema longum* Song, 1995. J. Ocean Univ. China. 25, 461 and *Glauconema trihymene* Thompson, 1966. J. Protozool. 13, 393, collected from Chinese coastal waters, were investigated using live observations, silver impregnation methods, and, in the case of the new species, SSU rDNA sequencing. *Philaster sinensis* spec. nov. can be recognized by the combination of the following characters: body cylindrical, approximately 130–150 × 35–55 μm in vivo; apical end slightly to distinctly pointed, posterior generally rounded; 19–22 somatic kineties; M1 triangular, consisting of 13 or 14 transverse rows of kinetosomes; M2 comprising 10–12 longitudinal rows; CVP positioned at end of SK1; marine habitat. We also provide improved diagnoses for *Pseudocohnilembus hargisi*, *Parauronema virginianum*, *Uronemella filificum* and *Parauronema longum* based on their original descriptions as well as the present work. Phylogenetic analyses support the monophyly of the genus *Philaster*.

Keywords: Marine habitat; *Philaster sinensis* spec. nov.; Phylogeny; Scuticociliates

Introduction

Ciliates in the subclass Scuticociliatia Small, 1967 are commonly found in ecosystems worldwide and exhibit both a great biological and morphologic diversity (Foissner and Wilbert 1981; Fan et al. 2011a,b; Foissner et al., 1994, 2013; Pan et al. 2011; 2013c; Seo et al. 2013; Song and Wilbert 2002; Thompson and Kaneshiro, 1968; Whang et al. 2013).

Abbreviations: AIC, Akaike information criterion; BI, Bayesian inference; CV, contractile vacuole; CVP, contractile vacuole pore; MCMC, Markov chain Monte Carlo; ML, maximum-likelihood; M1, membranelle 1; M2, membranelle 2; M3, membranelle 3; PM, paroral membrane; SK1, first somatic kinety; SK2, second somatic kinety.

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http://dx.doi.org/10.1016/j.ejop.2015.02.005
0932-4739/

Fig. 1. Map (A) and photographs of biotopes (B–I) in which the samples were collected. (A) Map showing collecting sites. (B) Ganhaiyuan beach in Rizhao, Shandong province. (C) Yangkou beach, Qingdao. (D) Daya Bay, Guangdong province. (E) Mariculture pond in Jiaozhou, Shandong province. (F) Beach of Nanyao, Qingdao. (G) Mangrove wetland in Shenzhen. (H) Mariculture pond at Techengdao in Zhanjiang, Guangdong province. (I) Mangrove wetland in Gaoqiao.

About 20 genera and 40 species of scuticociliates have been reported in Chinese coastal waters (Fan et al., 2011a, 2011b; Pan et al. 2010, 2011, 2013c; Song et al. 2009). Because of their small size and the high degree of similarity in infraciliature, many scuticociliates are identified based upon a combination of characteristics observed in vivo and silver impregnation (Ma and Song 2003; Pan et al. 2010; Song and Wilbert 2000; Song et al. 2001; Thompson 1964). Recent investigations have revealed a high diversity of scuticociliates in Chinese coastal waters, and the discovery of new species highlights the need to conduct further studies on this group (Fan et al., 2011a,b; Hu et al. 2008; Pan et al. 2011; Song et al. 2009; Wang et al., 2008a,b, 2009). In recent years, molecular phylogenetic analyses based on SSU rDNA sequences have been increasingly used to investigate the evolutionary relationships among Scuticociliatia. The relationships among this species-rich assemblage remain unresolved as molecular and morphological interpretations often conflict (Fan et al. 2009; Gao and Katz 2014; Gao et al., 2010, 2012a,b, 2013, 2014; Miao et al. 2008; Yi et al. 2009).

As part of an on-going faunistic study of marine ciliates in China, the morphology and infraciliature of seven scuticociliates were studied, including the molecular phylogenetic assignment of *Philaster sinensis* spec. nov.

Material and methods

Geographic distribution and ecological features

Environmental samples were collected between July 2010 and December 2012 from mariculture ponds, mangrove wetlands or coastal waters along the coastal line of eastern China (Fig. 1A–I). Investigations were conducted mainly in six cities distributed in Shandong and Leizhou peninsulas (Qingdao, Rizhao, Jiaozhou, Zhanjiang, Hong Kong and

Shenzhen), as well as Zhujiang estuary. The environmental features of the collecting sites were: Nanyao beach (Qingdao, Fig. 1F): distance from water ca. 10 m; pH 8.0 in upper 5 cm of sediment; mean particle size about 0.2 mm; Yangkou beach (Qingdao, Fig. 1C): distance from water ca. 30 m; pH 7.9 in upper 5 cm of sediment; mean particle size about 0.25 mm; Ganhaiyuan beach (Rizhao, Fig. 1B): distance from water ca. 100 m; pH 7.4 in upper 5 cm of sediment; mean particle size about 0.18 mm; Jiaozhou mariculture pond (Fig. 1E): pH 7.8 in mariculture water; mariculture pond at Techengdao (Fig. 1H): pH 7.6 in mariculture water; mangrove wetland in Gaoqiao (Fig. 1I): distance from water ca. 500 m; pH 7.8 in upper 5 cm of sediment; mean particle size ca. 0.15 mm; a mangrove wetland near Shenzhen (Fig. 1G): distance from water ca. 1000 m, pH 7.8 in upper 5 cm of sediment, mean particle size about 0.15 mm; Daya Bay (Fig. 1D): pH 8.2 in mariculture water.

Ciliate collection and identification

Philaster sinensis spec. nov. nuclear DNAwas collected from sand at Nanyao beach (36°06′39″N; 120°35′26″E), Qingdao, northern China, on 27 October 2011, when the water temperature was 13 °C and pH 8.0. *Pseudocohnilembus hargisi* was collected from Yangkou beach (36°14′18″N; 120°40′23″E), Qingdao, on 13 October 2010, when the water temperature was 15 °C, and pH 7.9. *Cohnilembus verminus* was collected from Ganhaiyuan beach, (35°24′53″N; 119°34′13″E), Rizhao, northern China, on 25 October 2010, when the water temperature was 11 °C, and pH 7.4. In each case, sand and seawater were taken from the top 5 cm of the sand layer.

Parauronema virginianum was collected from water near the bottom of a mariculture pond in Jiaozhou (36°12′32″N; 120°7′28″E), northern China, on 3 July 2010, when the water temperature was 20 °C and pH 7.8.

Uronemella filificum was isolated from a mariculture pond at Techengdao (21°08′59″N; 110°26′28″E), Zhanjiang, southern China, on 25 November 2010 and 6 November 2010, the water temperature was 18 °C and the pH 7.6, while in the latter the water temperature was 21 °C, pH 7.7.

Parauronema longum was collected from a mangrove wetland at Gaoqiao, Zhanjiang (21°32′56″N; 109°37′22″E), on 6 November 2010, when the water temperature was 25 °C and pH 7.8. Sediment plus seawater were taken from the original sites.

Glauconema trihymene was isolated from Daya Bay (22°27′20″N; 114°41′30″E), southern China, on 21 April 2011, when the water temperature was 20 °C and the pH 8.2.

Individuals were observed in vivo using differential interference contrast microscopy. Protargol staining was used in order to reveal the infraciliature (Pan et al., 2013a). Counts and measurements of stained specimens were performed at magnifications of 100–1250×. Drawings were carried out with the help of a camera lucida (Pan et al. 2013b). Systematics and terminology are mainly according to Lynn (2008).

Protargol-impregnated slides containing voucher specimens of six known species (*Pseudocohnilembus hargisi*, *Parauronema virginianum*, *Uronemella filificum*, *Cohnilembus verminus*, *Parauronema longum*, *Glauconema trihymene*), are deposited in the Laboratory of Protozoology, Ocean University of China, with registration numbers PXM-20101013, PXM-2010070302, PXM-2010112501, PXM-20101025, PXM-2010110601 and PXM-2011042101, respectively.

DNA extraction, PCR amplification and sequencing

Philaster sinensis spec. nov. nuclear DNA was extracted from cells ($n > 5$) from the same clone which was used for morphological studies using the DNeasy Tissue kit (Qiagen, CA). The PCR amplifications of the SSU rDNA were performed as described by Gao et al. (2013). Purified PCR product of the appropriate size was directly sequenced or inserted into the pMD$^{\mathrm{TM}}$18-T vector (Takara Biotechnology, Dalian Co., Ltd.) and sequenced on an ABI-PRISM 3730 automatic sequencer (Applied Biosystems).

Sequence availability and phylogenetic analyses

The SSU rDNA sequence of *Philaster sinensis* spec. nov. was analysed along with 74 other sequences obtained from GenBank database (accession numbers see Fig. 7). Sequences were aligned using Clustal W implemented in BioEdit 7.0 (Hall 1999). Bayesian inference (BI) analyses were performed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) using the GTR + I + G model selected by MrModeltest v.2.2 (Nylander 2004) according to the Akaike information criterion (AIC) criterion. Markov chain Monte Carlo (MCMC) simulations were run with two sets of four chains for 2500,000 generations, with trees sampled every 100 generations. The first 25% of sampled trees were discarded as burn-in, and then all remaining trees were used to calculate posterior probabilities using a majority rule consensus. Modeltest 3.7 (Posada and Crandall 1998) using the AIC selected the GTR + I (=0.6986) + G (=0.4938) model, which was used for maximum-likelihood (ML) analyses constructed using RaxML-HPC2 (Stamatakis et al. 2008) at the CIPRES website (http://www.phylo.org/). The reliability of internal branches was assessed using nonparametric bootstrapping with 1000 replicates. Phylogenetic trees were visualized with MEGA v.4 (Tamura et al. 2007).

Results and discussion

Philaster sinensis spec. nov. (Fig. 2A–O; Table 1)

Diagnosis: Body cylindrical, approximately 130–150 × 35–55 μm in vivo; apical end slightly to distinctly pointed,

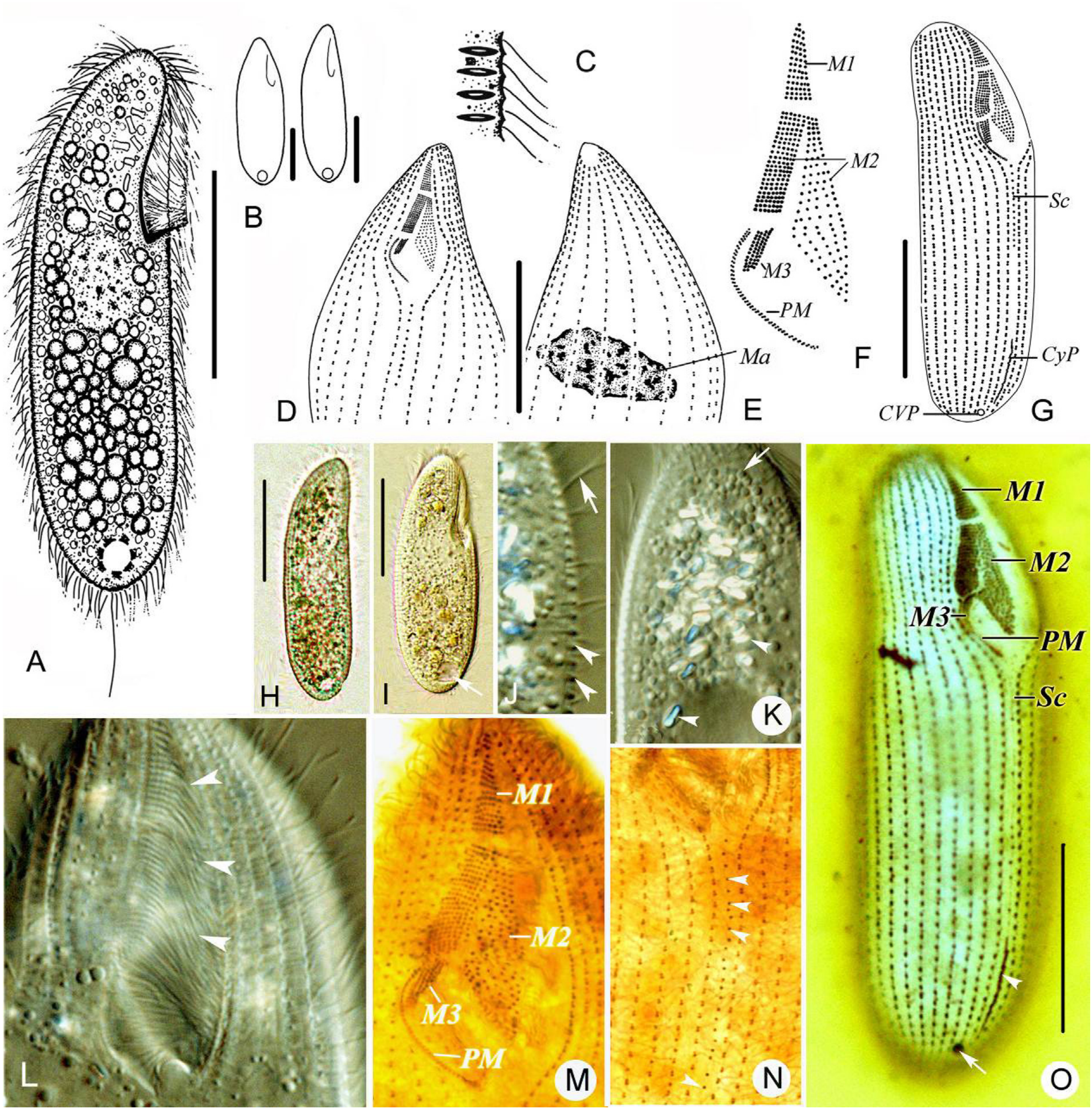

Fig. 2. *Philaster sinensis* spec. nov. in vivo (A–C, H–L), after protargol (D–F, M, N) and silver nitrate staining (G, O). (A, H) Right lateral view of a representative individual. (B) Different body shapes. (C) Detailed view of cortex to demonstrate arrangement of extrusomes. (D, E) Ventral (D) and dorsal (E) views of holotype specimen, showing infraciliature and nuclear apparatus. (F) Detailed structure of buccal area. (G, O) Ventral view, showing infraciliature, arrow in (O) refers to CVP and arrowhead marks cytoproct. (I) Ventral view, arrow marks CV. (J) Ventral view, arrowheads show extrusomes, arrow exhibits somatic cilia. (K) Ventral view, arrowheads show irregularly-shaped crystals, arrow marks extrusomes. (L) Ventral view, arrowheads mark PM. (M) Infraciliature of buccal area. (N) Posterior region, arrowheads show scutica whose basal bodies are serially arranged. CVP, contractile vacuole pore; CyP, cytoproct; M1, 2, 3, membranelles 1, 2, and 3; Ma, macronucleus; PM, paroral membrane; Sc, scutica. Bars, 50 μm (D–G, O), 55 μm (A), 60 μm (B, H, I).

posterior generally rounded; length of buccal field approximately 35% of body. Extrusomes bar-shaped. 19–22 somatic kineties; membranelle 1 triangular, consisting of 13 or 14 transverse rows of kinetosomes; membranelle 2 comprising 10–12 longitudinal rows. One spherical to oval macronucleus centrally located. CV caudally positioned; CVP positioned at end of SK1. Marine habitat.

Type locality: A sand beach at Nanyao, Qingdao, northern China (36°06′39″N; 120°35′26″E).

Habitat: Coastal water and sand, salinity 31‰, pH 8.0

Deposition of slides: A protargol slide containing the holotype specimen (Fig. 2D, E), and a silver nitrate slide containing paratype specimens are deposited in the Laboratory of Protozoology, Ocean University of China with registration

Table 1. Morphometric characterization of Philaster sinensis spec. nov. (Ps), Parauronema virginianum (Pv), Uronemella filificum (Uf), and Glauconema trihymene (Gt).

Character	Species	Min	Max	Mean	M	SD	CV	n
Body, length	Ps	130	151	140.1	142.0	5.7	4.1	18
	Pv	52	74	66.9	68.0	2.7	10.1	25
	Uf	28	39	33.6	34.0	4.4	13.1	23
	Gt	27	35	31.7	29.0	5.2	16.4	23
Body, width	Ps	38	56	47.7	46.0	4.7	9.9	18
	Pv	12	14	13.1	13.0	1.8	13.7	25
	Uf	16	23	18.9	19.0	1.9	10.1	23
	Gt	15	22	18.7	18.0	3.3	17.6	23
Somatic kineties, number	Ps	19	22	20.4	21.0	0.7	3.1	18
	Pv	11	11	11	11.0	0	0	22
	Uf	15	18	16.6	16.0	3.6	19.4	20
	Gt	17	17	17	17.0	3.5	20.6	21
Buccal field, length	Ps	43	54	48.4	48.0	6.7	13.8	18
	Pv	11	15	12.8	13.0	1.2	9.4	25
	Uf	14	24	19.6	19.0	1.5	7.7	23
	Gt	11	13	12.3	12.0	0.3	2.4	19
Somatic kinety 1, number of basal bodies	Ps	49	52	51.1	51.0	5.8	10.4	18
	Pv	23	26	24.7	24.0	1.2	4.9	25
	Uf	20	22	21.2	21.0	0.5	2.5	23
	Gt	22	25	23.7	23.0	2.5	10.4	19
Macronucleus, length	Ps	22	31	26.7	26.0	2.8	10.5	18
	Pv	4	6	5.8	5.0	0.5	8.6	24
	Uf	5	6	5.3	5.0	1.1	20.8	21
	Gt	7	10	8.5	8.0	1.8	21.2	22
Macronucleus, width	Ps	17	28	22	21	2.4	10.9	18
	Pv	8	10	9.1	9.0	1.8	19.8	24
	Uf	6	7	6.4	6.0	1.3	20.3	21
	Gt	7	9	7.6	8.0	0.2	2.6	22

Data based on protargol-impregnated specimens. Measurements in µm. CV – coefficient of variation in %; M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of specimens investigated; SD – standard deviation.

numbers PXM-2011102701-01 and PXM-2011102701-02, respectively.

Etymology: The species-group name '*sinensis*' refers to the fact that this species was first isolated from Chinese coastal waters.

Description: Body cylindrical, size in vivo approximately 130–150 × 35–55 µm. When viewed from ventral side, apical end slightly pointed (Fig. 2A, H, I). Body asymmetrical in outline when viewed from ventral side with anterior end slightly curved sideways (Fig. 2B). Buccal field large and depressed extending to about 1/3 of body length with buccal cilia short (7–10 µm long) and uniform (Fig. 2A, L). Somatic cilia densely arranged and about 6–8 µm long (Fig. 2C, H–J). Pellicle smooth, with rod-shaped, very fine, short (ca. 3.0 × 0.5 µm) extrusomes arranged in rows (Fig. 2C, J). Cytoplasm colourless to slightly greyish, with many spherical food vacuoles (1–6 µm across) and rod- or dumbbell-like crystals (Fig. 2A, K). Single caudal cilium approximately 10 µm in length (Fig. 2A). Single large spherical to ovoid macronucleus centrally located with many small nucleoli. One CV positioned caudally, 6–10 µm across, pulsating at intervals of about 10 s (Fig. 2I).

Moving rapidly and rotating around the longitudinal axis of body. Nineteen to 22 somatic kineties, extending entire length of body, composed of dikinetids (Fig. 2D, E). One long kinety forming scutica between first and last kinety, consisting of more than 40 pairs of basal bodies (Fig. 2N). Buccal apparatus as shown in Fig. 2F, M: M1 triangular, consisting of 13 or 14 transverse rows of kinetosomes; M2 divided into two parts with a gap in between: one part bar-shaped, comprising six longitudinal rows of basal bodies, the other triangle-shaped, comprising 10–12 longitudinal rows; M3 short and reduced in comparison to the other membranelles and consisting of four longitudinal rows of basal bodies. PM commencing right of anterior edge of M3. Silverline system typical of genus, CVP positioned at end of SK1 (Fig. 2G, O).

Remarks and comparison: Hitherto, *Philaster* Fabre-Domergue, 1885 comprised six nominal species: *Philaster digitiformis* Fabre-Domergue, 1885 (type species by monotypy); *P. apodigitiformis* Miao et al., 2009; *P. hiatti* Thompson, 1969; *P. bergeri* Grolière, 1980; *P. resedaceus* Tucolesco, 1962; and *P. salinus* Tucolesco, 1962. *Philaster sinensis* spec. nov. can be easily separated from *P. resedaceus*

Table 2. Morphometric characterization of Cohnilembus verminus (Cv), Parauronema longum (Pl), and Pseudocohnilembus hargisi (Ph).

Character	Species	Min	Max	Mean	M	SD	CV	n
Body, length	Cv	129	146	130.4	132.0	2.1	1.6	25
	Pl	52	68	62.3	61.0	5.7	9.1	25
	Ph	28	42	37.4	34.0	9.8	17.1	25
Body, width	Cv	20	26	24.4	23.0	2.2	13.9	25
	Pl	23	26	24.5	24.0	2.3	9.4	25
	Ph	16	20	18.3	17.0	2.4	13.1	25
Somatic kineties, number	Cv	10	11	10.4	10.0	1.3	12.5	20
	Pl	18	24	20.3	21.0	3.8	16.9	21
	Ph	12	14	13.2	12.0	2.5	18.9	21
Buccal field, length	Cv	59	68	64.2	65.0	5.7	8.9	23
	Pl	24	38	31.7	29.0	4.6	14.5	23
	Ph	20	29	25.3	26.0	3.1	8.8	20
Somatic kinety 1, number of basal bodies	Cv	28	31	30.1	29.0	3.1	10.2	23
	Pl	20	24	22.1	21.0	1.1	4.9	23
	Ph	17	18	17.8	17.0	3.7	22.3	20
Macronucleus, length	Cv	4	5	4.2	4.0	0.3	7.1	24
	Pl	11	13	11.5	12.0	2.4	20.9	25
	Ph	8	11	8.9	9.0	1.2	13.5	24
Macronucleus, width	Cv	3	5	4.4	4.0	0.6	13.6	24
	Pl	10	12	11.4	11.0	2.1	18.4	25
	Ph	6	10	8.5	8.0	1.2	14.1	24

Data based on protargol-impregnated specimens. Measurements in µm. CV – coefficient of variation in %; M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of specimens investigated; SD – standard deviation.

and *P. salinus* by its body shape and size (cylindrical, body length 130–150 µm vs. ovoid, body length < 100 µm).

Compared with other four congeners, our new species, *Philaster sinensis* can be distinguished from *P. digitiformis* by: (1) short and uniform buccal cilia in *P. sinensis* spec. nov. vs. long buccal cilia and a sail-like PM in *P. digitiformis*; (2) fewer somatic kineties in *P. sinensis* spec. nov. (19–22 vs. 25–40); (3) M1 in *P. sinensis* spec. nov. is much wider than that in *P. digitiformis* (Fig. 6G–I; Fabre-Domergue 1885; Grolière 1974; Kahl 1931; Mugard 1948; Small and Lynn 1985; Thompson 1964; Tucolesco 1962).

Philaster sinensis spec. nov. differs from *P. apodigitiformis* in: (1) body cylindrical, asymmetrical in outline when viewed from ventral side with anterior end slightly curved sideways in *P. sinensis* vs. body ovoid and bilaterally flattened in *P. apodigitiformis*; (2) fewer somatic kineties (19–22 in *P. sinensis* vs. approximately 40 in *P. apodigitiformis*); (3) fewer longitudinal rows in M2 in *P. sinensis* (10–12 vs. approximately 25); (4) free-living in *P. sinensis* vs. parasitic lifestyle in *P. apodigitiformis* (Fig. 6J–L; Miao et al. 2009).

Compared to *Philaster hiatti* (Fig. 6C, D), *P. sinensis* spec. nov. can be recognized by: (1) the posterior part of M2 being conspicuously branched and thus Y-shaped in *P. sinensis* vs. M2 almost unbranched and having a digitation in *P. hiatti*; (2) fewer somatic kineties (19–22 in *P. sinensis* vs. 33 in *P. hiatti*) (Coats and Small 1976; Fan et al. 2011b; Small and Lynn 1985; Thompson 1969).

Philaster bergeri differs from *P. sinensis* in body shape (conspicuously slender vs. cylindrical) and number of kinetal rows in M1 (Fig. 6E, F; Grolière 1980).

Pseudocohnilembus hargisi Evans and Thompson, 1964 (Fig. 3A–H; Table 2)

Some characters e.g. smaller body size, fewer somatic kineties and a comparatively larger buccal field were found in the Zhuhai population. Description of Zhuhai population as well as comparison among different populations are presented (Evans and Thompson 1964; Gong et al. 2002; Ma et al. 2003; Shang et al. 2006; Song and Wilbert 2002).

Description of the Qingdao population: Size in vivo approximately 25–40 × 10–15 µm, body elongate-elliptical in outline becoming wider toward posterior end (Fig. 3A–C). Buccal cavity large, approximately 23–42 µm in length, corresponding about 60–70% of body length (Fig. 3D, F). Cytoplasm colourless to greyish, containing several shining granules. Pellicle thin and rigid, with longitudinal grooves along ciliary rows. No extrusomes distinguishable. One caudal cilium approximately 15 µm long (Fig. 3A). Macronucleus large and rounded, centrally located (Fig. 3H). CV approximately 5 µm in diameter, caudally positioned; contracting interval very short, less than 5 s (Fig. 3C). Somatic cilia approximately 7 µm long (Fig. 3E). Oral apparatus comprising three membranelles and one PM (Fig. 3G). Twelve to

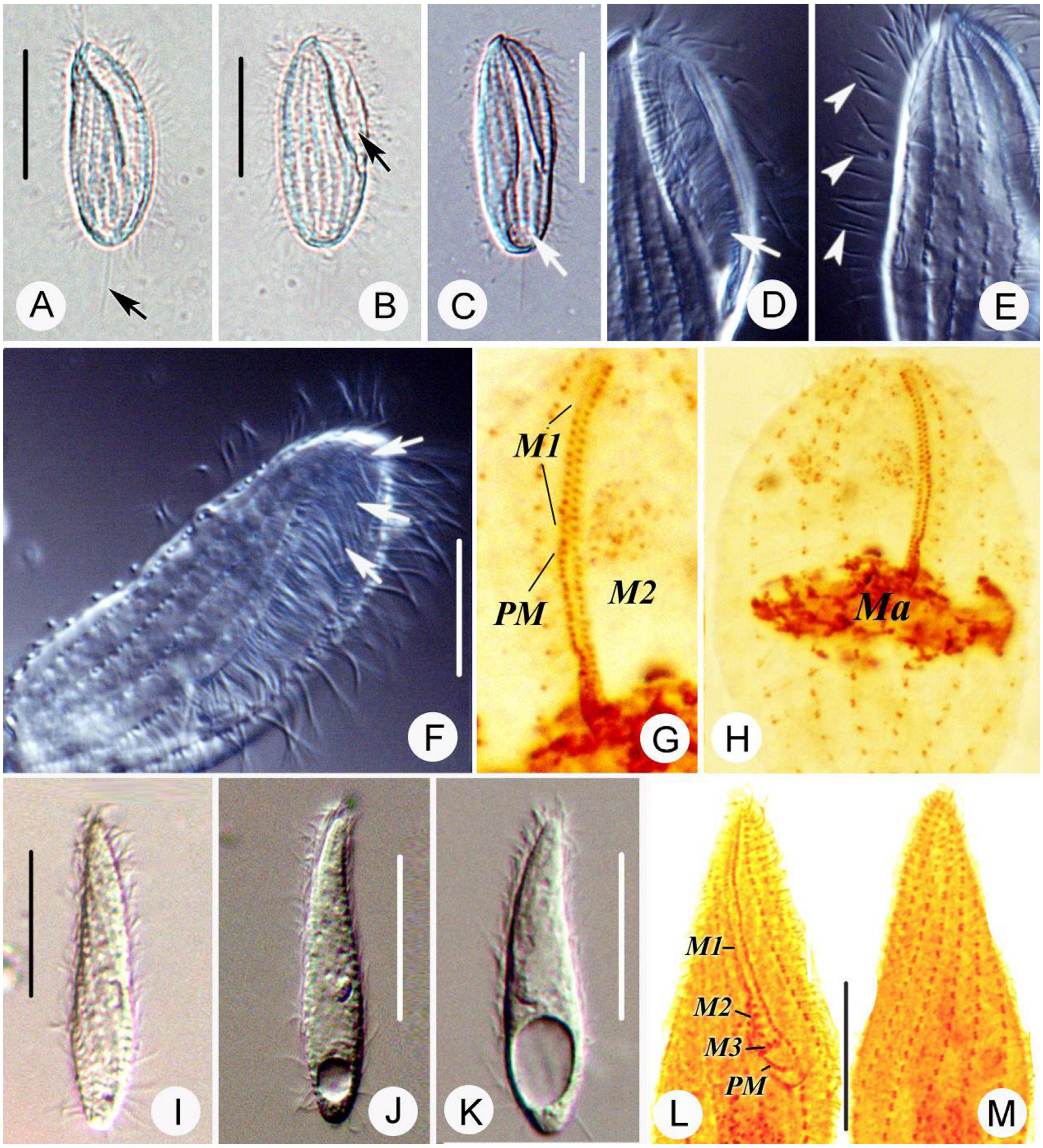

Fig. 3. *Pseudocohnilembus hargisi* (A–H) and *Cohnilembus verminus* (I–M) in vivo (A–F, I–K) and after protargol staining (G, H, L, M). (A, I) Ventral views of typical individuals, arrow in (A) shows caudal cilium. (B, C, J, K) Ventral views of four individuals, arrow in (B) marks buccal area, arrow in (C) points to CV. (D) Ventral view, arrow exhibits PM. (E) Anterior region, arrowheads show somatic cilia. (F) Ventral view, arrows show PMs. (G, L) Detailed structure of buccal area. (H) Partial infraciliature, showing somatic kineties. (M) Dorsal view, showing somatic kineties. M1, 2, 3, membranelle 1, 2, 3; Ma, macronucleus; PM, paroral membrane. Bars, 15 μm (F), 40 μm (A, B, C, L, M), 70 μm (I, J, K).

fourteen somatic kineties. M1 and M2 lie parallel to the cell's longitudinal axis, each being formed by a single row of kinetosomes (Fig. 3G). M2 starts anteriorly near second paired kinetids of SK1 and terminates posteriorly near mid-body region. Length of M2 approximately 80% of buccal field; M3 consists of three rows of kinetids (Fig. 3G).

PM with paired basal bodies organized in a zigzag pattern, lies immediately posterior to M1. Scutica comprising ca. seven kinetosomes aligned in a "Y" configuration at posterior end of PM.

Remarks and comparison

The morphological characteristics of the present population are similar to those described before (Evans and Thompson 1964; Gong et al. 2002; Ma et al. 2003; Shang et al. 2006; Song and Wilbert 2002), except that the Chinese population has: (1) a smaller body size 25–40 × 10–15 μm in vivo vs. 35–55 × 10–18 μm in previous studies; (2) 12–14 somatic kineties vs. 13–14 somatic kineties in previous studies; (3) a buccal field occupying approximately

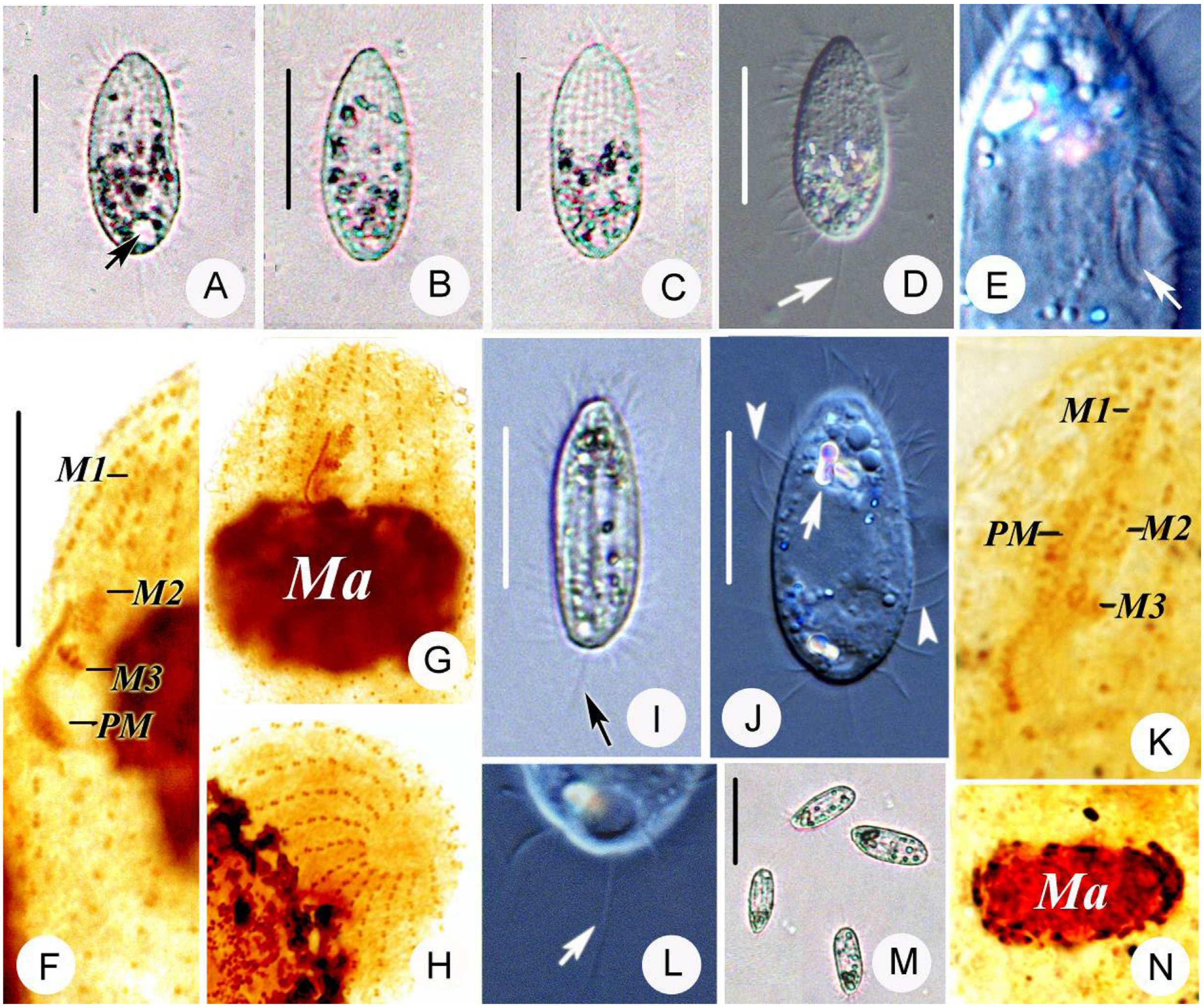

Fig. 4. *Paraurornema longum* (A–H) and *Paraurornema virginianum* (I–N) in vivo (A–E, I–M) and after protargol staining (F–H, K, N). (A, I) Ventral views of typical individuals, arrow in (A) shows CV, arrow in (I) marks caudal cilium. (B–D, J) Ventral views of four individuals, arrow in (D) marks caudal cilia, arrow in (J) points to dumbbell-shaped crystals and arrowheads in (J) refer to somatic cilium. (E) Ventral view, arrow exhibits buccal field. (F, G, K) Detailed structure of the buccal area. (H) Anterior part, to show somatic kineties. (L) Posterior part, arrow marks caudal cilium. (M) To show different body shapes. (N) Macronucleus. M1, 2, 3, membranelle 1, 2, 3; Ma, macronucleus; PM, paroral membrane. Bars, 10 µm (F), 30 µm (A–D), 40 µm (I, J), 70 µm (M).

60–70% of body length vs. approximately 50–60%. We believe, however, that these dissimilarities are population-dependent.

***Cohnilembus verminus* Kahl, 1931 (Fig. 3I–M; Table 2)**

This species has been described several times since the original report (Borror 1963a; Didier and Detcheva 1974; Hu et al. 1996; Kahl 1931; Song 2000; Thompson 1968). The Rizhao population of *Cohnilembus verminus* corresponds well with previous studies in terms of the body shape, buccal apparatus and behaviour. Description of Rizhao population as well as comparison among different populations are supplied herein.

Description of Rizhao population: Body 120–140 × 15–20 µm in vivo. Body slender, spindle-shaped, anterior end extremely narrowed and tapering, posterior end slightly pointed (Fig. 3I–K). Buccal field narrow, length approximately 45% of body length. Pellicle thin and slightly notched; extrusomes spindle-like and sparsely distributed. Cytoplasm colourless and hyaline, containing several shining, irregular-shaped crystal granules (Fig. 3I–K). One oval to spherical macronucleus centrally located. CV approximately 8 µm across, caudally located near ventral side (Fig. 3J). Eleven somatic kineties, with cilia approximately 8 µm long. Caudal cilium 10–15 µm long.

Somatic kineties composed of dikinetids in anterior 60% of body and monokinetids in posterior 40%, extending almost entire length of cell (Fig. 3M). Buccal apparatus highly characteristic; M1 one-rowed, occupying approximately 80% of buccal field; posterior portion divided into three groups, each with three basal bodies (Fig. 3L). M2 and M3 each with approximately six or seven basal bodies (Fig. 3L). PM short, extending anteriorly to about level of M2, anterior half one-rowed, posterior with zigzag structure. Scutica Y-shaped, consisting of approximately three pairs of basal bodies.

Remarks and comparison

Differences exist among various populations of *Cohnilembus verminus*. Our Rizhao population has constantly 11 somatic kineties, while there are 10 or 11 in Qingdao populations of Hu et al. (1996) and Song (2000), nine or 10 in Borror (1963a), nine in Didier and Detcheva (1974) as well as Thompson (1968); body size also varies: 120–140 × 15–20 µm in our current population, while 67–117 × 8–11 µm in Borror (1963a) (maybe data from stained specimens?) and 57–75 × 11–15 µm in Thompson (1968).

Parauronema longum Song, 1995 (Fig. 4A–H; Table 2)

Although, since its first report, this species has been redescribed by Song and Wilbert (2000), Fan et al. (2011a), Pan et al. (2011) based on their Qingdao populations, in the present work for the first time we discovered it from southern China seas located in subtropical region. Based on all these data an improved diagnosis is provided.

Improved diagnosis: Body approximately 30–55 × 12–25 µm in vivo; normally elongated and cylindrical with a large, truncated apical plate; posterior end rounded, ventral side straight while dorsal side convex. Length of buccal field approximately 40% of body length. Extrusomes bar-shaped, ca. 2–4 µm long. 16–24 somatic kineties. M1 long, consisting of two longitudinal rows of kinetids each with 7–11 kinetosomes; M2 well-separated from M1, 2- or 3-rowed, each row containing five or six kinetosomes. Marine habitat.

Description of Zhanjiang population: Body 40–45 × 20–25 µm in vivo, elongated oval with a large, truncated apical plate (Fig. 4A–C). Posterior end rounded, ventral side straight while dorsal side convex (Fig. 4A–C). Length of buccal field 40% of body length (Fig. 4E). Pellicle slightly indented at bases of cilia (Fig. 1E). Extrusomes bar-shaped, ca. 2–3 µm long. Cytoplasm colourless to greyish, containing several to many large (ca. 5 µm across) food vacuoles and dumbbell-shaped crystals (ca. 1–2 µm long) often concentrated at anterior and posterior end of body (Fig. 4A–C). Macronucleus ellipsoidal to spherical (about 10 µm across), no micronucleus observed (Fig. 4G). Single CV caudally located, approximately 5 µm across when fully extended (Fig. 4A). Somatic cilia approximately 6 µm long, densely arranged; caudal cilium about 10 µm long (Fig. 4D).

Locomotion by moderately fast swimming, although stationary for rather long periods when feeding, or by slow crawling on substrate with occasional jumps.

18–24 somatic kineties, SK1 with ca. 32 basal bodies (Fig. 4H). Somatic cilia ca. 5–7 µm long. M1 long, consisting of two longitudinal rows of kinetids, each with 7–10 kinetosomes (Fig. 4F, G). M2 well-separated from M1 and composed of three rows of kinetosomes, each row containing approximately six kinetosomes (Fig. 4F, G). M3 much shorter than M1 and M2 and composed of two or three short, irregularly arranged rows of kinetosomes (Fig. 4F, G). PM to the right of buccal cavity, terminating anteriorly at level of mid-region of M2. Scutica Y-shaped and comprising four pairs of kinetosomes (Fig. 4F).

Remarks and comparison

Song (1995) established this species based on populations from mariculture ponds in Qingdao, China, then Fan et al. (2011b) and Pan et al. (2011) redescribed it based on their Qingdao populations. Among Zhanjiang population and populations mentioned above, the population of Fan et al. (2011b) shows a much larger body size: 60–100 × 25–45 µm in Fan et al. (2011b) vs. 40–45 × 20–25 µm in the present study, 30–55 × 12–25 µm in Song (1995) and (45)–60 × 20–30 in Pan et al. (2011); the discrepancy perhaps attribute to the living environment.

The only differences between our Zhangjiang isolate and the previously described morphotypes found from other geographical areas, are the slightly greater range of number of somatic kineties (18–24 vs. 19–20) and the ratio of buccal field to body length is less [40% vs. 40–50% in Pan et al. (2011), (60)% in Song (1995) and Fan et al. (2011a,b)]. Nevertheless, we believe them to be conspecific.

Paraurонema virginianum Thompson, 1967 (Fig. 4I–N; Table 1)

Hitherto this species has been described many times (Foissner 1971; Grolière 1974; Song and Wilbert 2000; Thompson 1967; Wilbert and Kahan 1981; Xu and Song 1999). A brief description of Jiaozhou population as well as comparisons among all the previous populations, are presented.

Description of Jiaozhou population: Body 45–70 × 20–35 µm in vivo, elongate with a small, truncated apical plate (Fig. 4I, M). Body asymmetric when viewed ventrally (Fig. 4I, M). Buccal field extending to 35–40% of body length with buccal cilia about 5 µm long. Pellicle forming longitudinal ridges between somatic kineties. Extrusomes bar-shaped, about 4 µm long. Endoplasm containing numerous (ca 3 µm across) food vacuoles and dumbbell-shaped crystals (Fig. 4J). Single ovoid macronucleus located near body centre (Fig. 4N). CV located at posterior end of cell, about 10 µm across when fully extended (Fig. 4L). Somatic cilia 6 µm long in vivo, usually undetectable in middle portion of body (Fig. 4J). Caudal cilium approximately 20 µm long (Fig. 4L). Locomotion by moving slowly on substrate or by swimming moderately fast.

Eleven somatic kineties arranged longitudinally usually with dikinetids in anterior quarter to third of each row and monokinetids positioned posteriorly. Buccal apparatus as shown in Fig. 4K: M1 distinctly sub-apically positioned, separated from other membranelles and consisting of two rows of kinetids; M2 slightly shorter than M1, consisting of three longitudinal rows of basal bodies; M3 comprising three longitudinal rows. PM positioned on right of buccal cavity, terminating anteriorly to M2. Scutica Y-shaped with 5–7 basal bodies.

Remarks and comparison

The present species is the type of *Parauronema*. The Jiaozhou population studied here corresponds well with the original population (Thompson 1967) as well as the population of Song and Wilbert (2000), with only small differences in body size: 45–70 × 20–35 µm vs. 60–100 × 25–45 µm in the original population vs. 25–40 × 10–15 µm in Song and Wilbert (2000). The cell shape of the Jiaozhou population is rather similar with that described in Wilbert and Kahan (1981): body asymmetric when viewed ventrally with a small, truncated apical plate (Fig. 4I, M). Xu and Song (1999) described a Jiaozhou population in which a single longitudinal row with eight or nine basal bodies in M1 is present. The character is also consistent with the population studied by of Foissner (1971), in which he synonymized *Parauronema* with *Uronema*, based on *Parauronema* having a very similar morphology to *Uronema* and the only exception being a two-rowed M1 (vs. one-rowed). Considering *P. acutum* and *P. longum* were reported also with two longitudinal rows in M1, more evidence is needed to validate whether *Parauronema* is a junior synonym of *Uronema*.

Uronemella filificum (Kahl, 1931) Song and Wilbert, 2002 (Fig. 5A–G; Table 1)

Since first reported, this species has been redescribed on many occasions (Alekperov 2005; Borror 1963b; Fenchel 1968; Kahl 1931; Ma et al. 2002; Pérez-Uz et al. 1996; Song et al. 2002; Song and Wilbert 2002; Thompson and Kaneshiro 1968; Wilbert and Kahan 1981). On the basis of the previous descriptions as well as the data from Zhanjiang population, an improved diagnosis is added.

Improved diagnosis: Body approximately 25–40 × 12–30 µm in vivo; kidney-shaped with large, conspicuous apical plate; length of buccal field approximately 60% of body; 15–23 somatic kineties. M1 single-rowed, almost apically positioned; M2 and M3 short, each consisting of three rows of basal bodies. Marine habitat.

Description of Zhanjing population: Body 25–35 × 10–15 µm in vivo, kidney-shaped with large, conspicuous apical plate (Fig. 5A–C). Length of buccal field about 60% of body length. Pellicle thin and slightly notched. Extrusomes unrecognizable. Cytoplasm colourless to greyish, containing several (ca. 3 µm across) food vacuoles and dumbbell-shaped crystals (ca. 1–2 µm long) often concentrated at the anterior end of body (Fig. 5D). Caudal cilium 10–15 µm (Fig. 5E). CV located at posterior end of cell, 5 µm in diameter when fully extended (Fig. 5D). Locomotion by fast swimming, sometimes rotating with help of a caudal-cilium–associated sticky thread attached to substratum.

15–18 somatic kineties (Fig. 5F). M1 single-rowed with five or six kinetosomes; M2 triple-rowed; M3 small (Fig. 5F). PM starting anteriorly near right posterior of M2 and close to M3 (Fig. 5F). Scutica consisting of three or four basal body pairs (Fig. 5M). Macronucleus located in anterior half of body (Fig. 5G).

Remarks and comparison

Jankowski (1980) proposed the new genus *Uronemita* with *U. filificum* as its type species. However, *Uronemita* is an invalid name since neither clear definition nor detailed description was provided for it.

The Zhanjiang population corresponds well with morphotypes of Borror (1963b), Thompson and Kaneshiro (1968), Wilbert and Kahan (1981), Fenchel (1968) and Pérez-Uz et al. (1996) in body shape (pear-shaped), general feature of infraciliature (e.g. number of somatic kineties) and position of CVP (end of SK2). The characteristics of the Zhanjiang population are different from population reported by Song and Wilbert (2002) in having a distinct body shape (pear-shaped vs. D-like shape) and fewer somatic kineties (15–18 vs. 22 or 23) and no extrusomes (vs. extrusomes spindle-shaped, approximately 1 µm long and sparsely distributed beneath pellicle). We consider these variations to be population-dependent.

Glauconema trihymene Thompson, 1966 (Fig. 6E–G, I–N; Table 1)

Glauconema trihymene is the type species of the genus *Glauconema*. Though this species was isolated from south China seas for the first time, similar morphological characters (e.g. infraciliature and behaviour) were found in Guangdong population (Ma et al. 2006; Thompson 1966). Hence only morphometric characterization and important features are listed.

Description of Guangdong population: Trophont 30–35 × 15–20 µm in vivo, tomite 30–40 × 10–15 µm in vivo. In trophont, body kidney-shaped, asymmetrical; in tomite, body slender, elongated oval to spindle-shaped with inconspicuous frontal plate (Fig. 5H–J). Buccal cavity spacious in trophont while narrow in tomite.

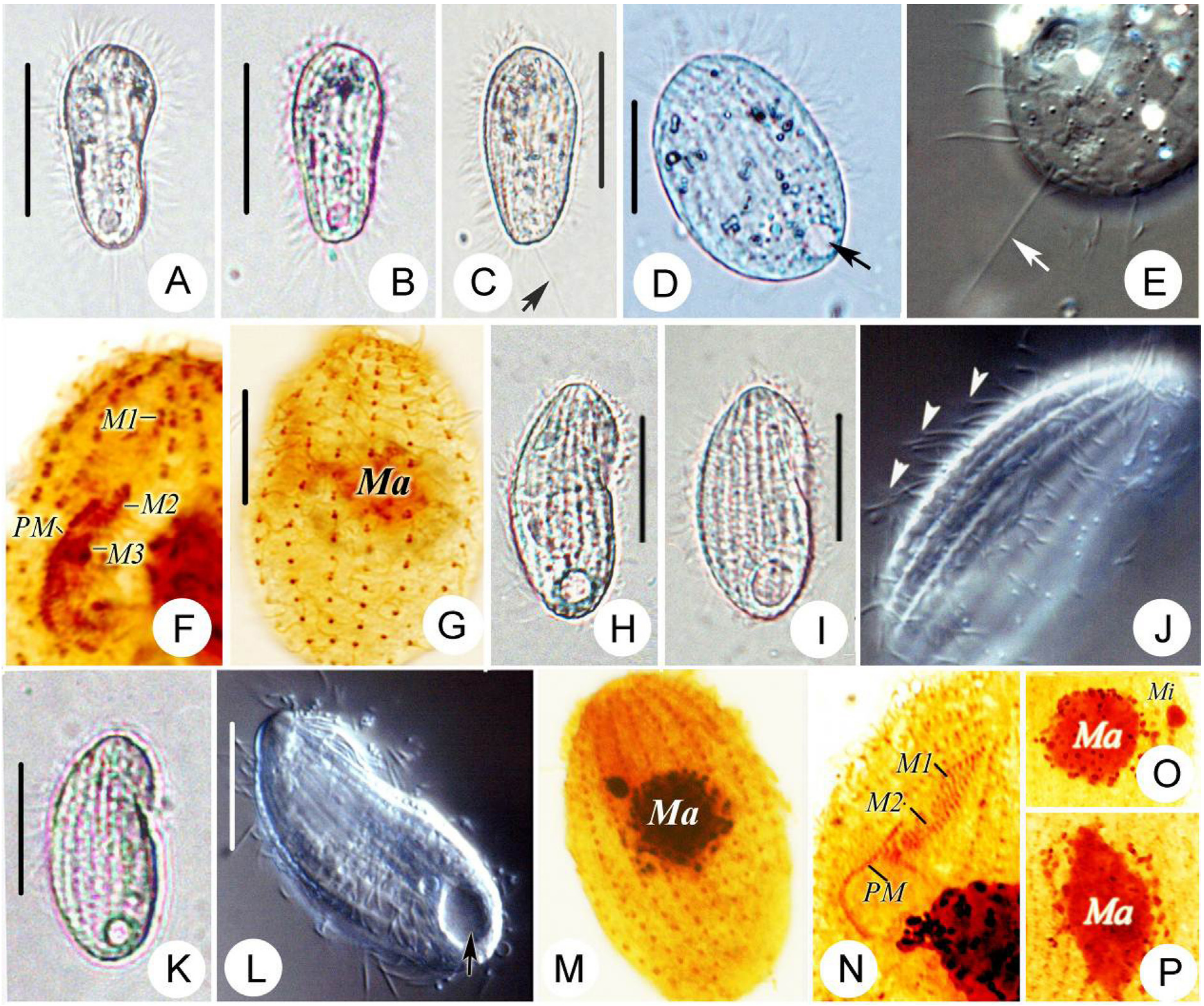

Fig. 5. *Uronemella filificum* (A–G) and *Glauconema trihymene* (H–P) in vivo (A–E, H–L) and after protargol staining (F, G, M–P). (A, H) Ventral views of typical individuals. (B–C, I, K) Ventral views of four individuals, arrows in (C) mark caudal cilium. (D, L) Ventral view, arrows show CV. (E) Posterior region, to show caudal cilium. (F, N) Detailed structure of the buccal area. (G, M) General infraciliature, showing somatic kineties. (J) Dorsal view, arrowheads marks somatic cilia. (O, P) Macronucleus of different shapes. M1, 2, 3, membranelle 1, 2, 3; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane. Bars, 10 µm (D, G, L), 20 µm (A–C, H–I, K).

Pellicle thin, slightly notched, no extrusomes distinguishable. CV located at posterior end of cell, about 6 µm in diameter (Fig. 5H, I). Macronucleus centrally located, micronucleus spherical (Fig. 5M, O, P). 16 or 17 somatic kineties, with cilia about 8 µm long (Fig. 5J). Caudal cilium approximately 15 µm long. In trophont, locomotion mostly by gliding back and forth on substrate, sometimes pausing; in tomite, swimming moderately fast.

Somatic kineties comprising mostly of dikinetids with only a few monokinetids in trophont (Fig. 5M). In trophont, M1 triple-rowed, terminating close to second pair of kinetids of SK1 (Fig. 5N); while in tomite, M1 double-rowed, extending to frontal margin; M2 three-rowed in trophont (Fig. 5N), while two-rowed in tomite; M3 located close to M2, composed of three short rows of basal bodies both in trophont and tomite. Scutica arranged as a short line, with several pairs of kinetosomes.

Remarks and comparison

Glauconema pacificum Small and Lynn, 1985 is very similar with *G. trihymene* in living morphology and habitat, but differs from *G. trihymene* in oral structure of the trophont and the number of somatic kineties: (1) M1 is conspicuously larger than M2 and the two membranelles are distinctly separated in *G. pacificum* while M1 is almost equal to M2, and the posterior end of M1 is barely separated from the anterior end of M2 in *G. trihymene*; (2) 14 somatic kineties in *G. pacificum* vs. 17 in *G. trihymene* (Small and Lynn 1985; Thompson 1966).

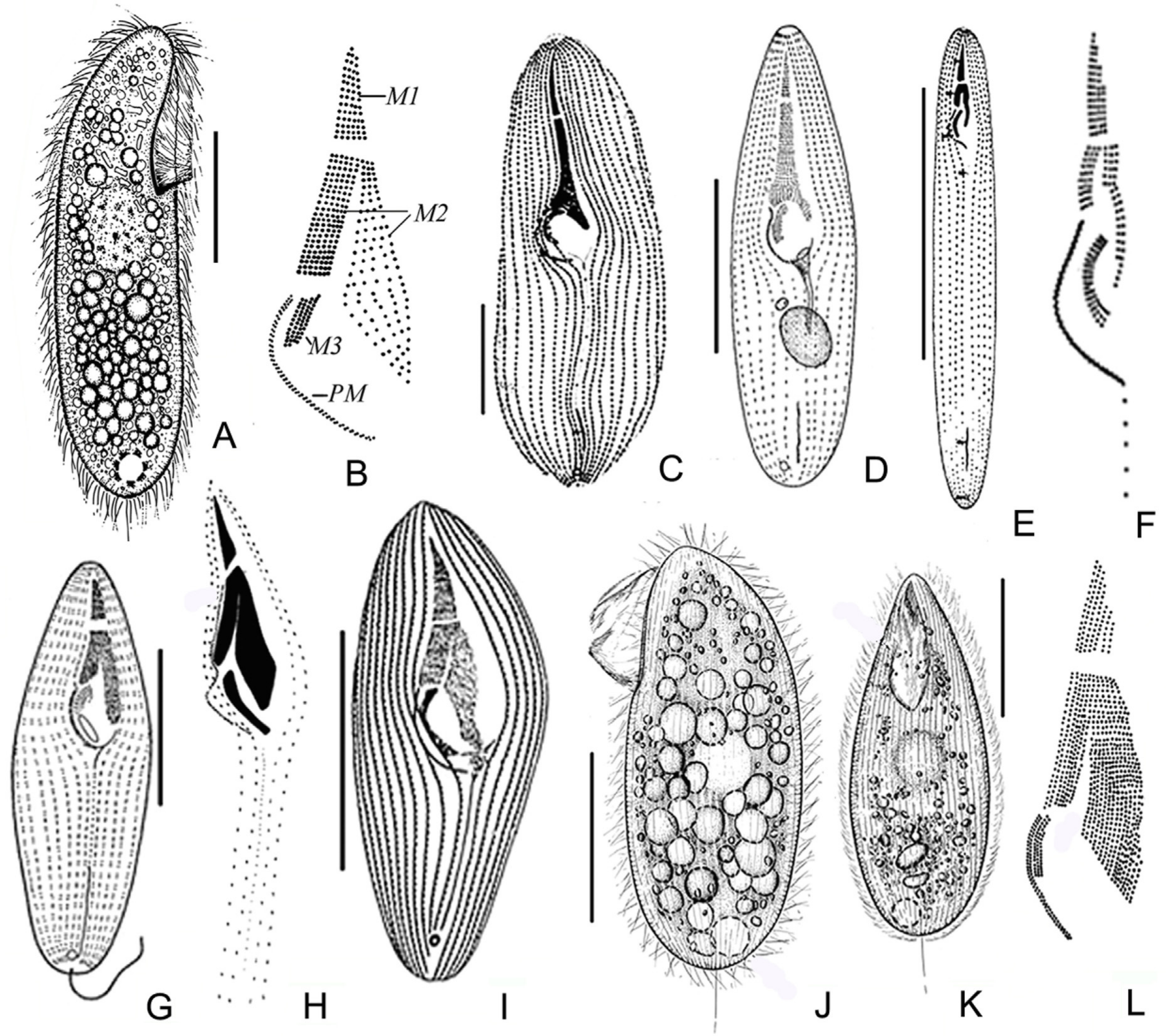

Fig. 6. Morphology and infraciliature of *Philaster sinensis* spec. nov. (A, B, from present work) and four congeners: *P. hiatti* (C, from Thompson 1969; D, from Small and Lynn 1985); *P. bergeri* (E, F, from Grolière et al. 1980); *P. digitiformis* (G, from Small and Lynn 1985; H, from Grolière 1974; I, from Mugard 1948), *P. apodigitiformis* (J, K, L, from Miao et al. 2009). M1, 2, 3, membranelle 1, 2, 3; PM, paroral membrane. Bars, 40 μm.

Molecular phylogeny based on sequences of SSU rDNA gene (Fig. 7)

Length (bp), GC content and GenBank accession number of SSU rDNA sequence for *Philaster sinensis* spec. nov. are as follows: 1690 bp, 43.8% and KJ815049.

The topologies of the SSU rDNA trees constructed using Bayesian inference and maximum-likelihood analyses are similar, therefore only the BI tree is presented here with support values from both algorithms (Fig. 7). The tree shows the 40 sequences of 18 genera, representing eight families, in the order Philasterida plus several out-group taxa. The clade Philasteridae, formed by *Philasterides armatalis, Philaster sinensis* spec. nov., *P. apodigitiformis* and *Philaster* sp., is sister to the clade comprising Uronematidae, Entodiscidae, Thyrophylacidae, Entorhipidiidae, Orchitophryidae and Pseudocohnilembidae with moderate support (67% ML/0.64 BI). Three *Philaster* species form a monophyletic assemblage with full statistical support which clusters with *Philasterides armatalis. Philaster sinensis* spec. nov. clusters with *P. apodigitiformis* in a fully supported clade. The reason that *Philaster* and *Philasterides* are assigned to the Philasteridae in Lynn (2008) is probably that the two genera have a similar buccal morphology, i.e. the large size of M1 and M2 compared with M3 (Lynn and Small 2002; Small and Lynn 1985; Song 2000).

Our phylogenetic analyses support the contention that both the genus *Philaster* and the family Philasteridae are monophyletic. As revealed in previous molecular phylogenetic analyses (Gao et al. 2012a,b), *Parauronema virginianum* falls into the *Uronema* clade, and *P. longum* is sister to *Plagiopyliella*, *Thyrophylax* and *Entorhipidium*. Considering the

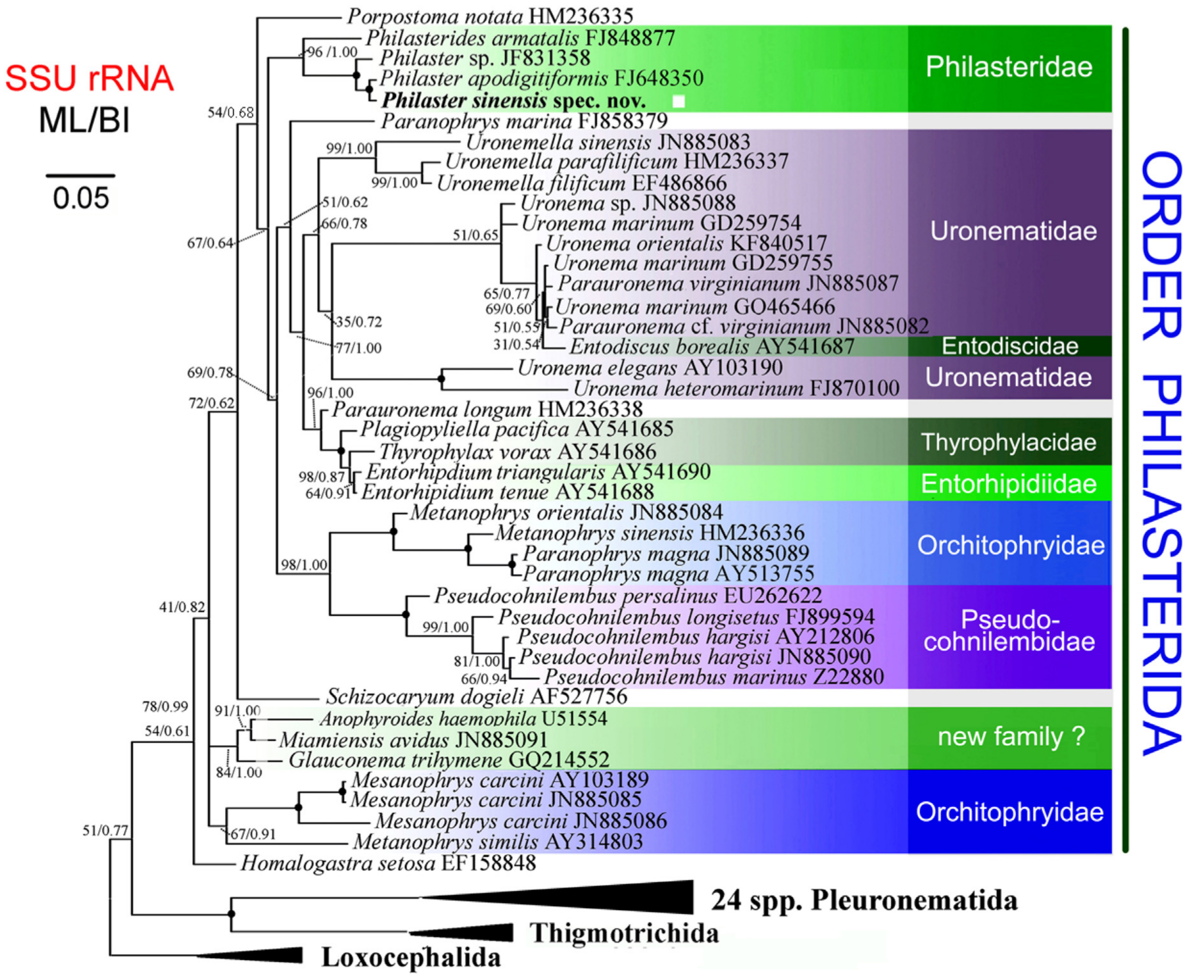

Fig. 7. Phylogenetic tree inferred from SSU rDNA sequences, showing the position of *Philaster sinensis* spec. nov. (in bold). Numbers at nodes represent the bootstrap values of maximum likelihood (ML) out of 1000 replicates and the posterior probability of Bayesian analysis (BI). The scale bar corresponds to five substitutions per 100 nucleotide positions.

similar morphological characters among *P. virginianum* and *Uronema* species (e.g. body usually elliptical with anterior end truncated, cytostome positioned in 1/3–1/2 of the body and PM extending anteriorly to anterior end of M2), we agree with Foissner (1971) that *P. virginianum* might be a member of *Uronema*. However, more evidence is needed to validate whether *Parauronema* is a synonym of *Uronema*, because *P. acutum* and *P. longum* Song, 1995 were reported since then, and *P. longum* is only distantly related to *Uronema* in both our and previous SSU rDNA trees (Fig. 7; Gao et al. 2012b).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (project numbers: 31272285, 31030059, 3147193), Research Fund for the Outstanding Young Teachers Program of Higher Education in Guangdong (Yq201352), the International Research Group Program (IRG14-22), Deanship of Scientific Research, King Saud University, and King Saud University Deanship for Scientific Research, Prolific Research Group (PRG-1436-01). Many thanks are given to Dr. Feng Gao and Prof. Weibo Song for their help suggestions on this paper.

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