

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

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Recently, an undescribed marine ciliate was isolated from China. Investigation of its morphology and infraciliature revealed it as an undescribed species representing a new genus, *Eurystomatella* n. gen., the type of the new family Eurystomatellidae n. fam. The new family is defined by close-set, apically positioned oral membranelles and a dominant buccal field that is surrounded by an almost completely circular paroral membrane. The new genus is defined by having a small oral membranelle 1 (M1), bipartite M2 and well-developed M3, a body surface faintly sculptured with a silverline system in a quadrangular, reticulate pattern and a cytostome located at the anterior third of a large buccal field. The type species of the new genus, *Eurystomatella sinica* n. sp., is a morphologically unique form that is defined mainly by the combination of a conspicuously flattened body, several caudal cilia, extremely long cilia associated with the buccal apparatus and a contractile vacuole located subcaudally. According to phylogenetic analyses of small-subunit (SSU) rRNA gene sequences, *Eurystomatella* clusters with the genus *Cyclidium*, as a sister group to the family Pleuronematidae. The great divergence in both buccal and somatic ciliature between *Eurystomatella* and all other known scuticociliates supports the establishment of a new family for *Eurystomatella*.

INTRODUCTION

Free-living scuticociliates have been revealed during the last few decades as a highly diverse assemblage with many more undescribed taxa than expected, especially in marine habitats, where they may be abundant (Dragesco, 1968; Fernandez-Leborans & Novillo, 1994; Grolière & Detcheva, 1974; Small & Lynn, 1985; Song, 1995, 2000; Song & Wilbert, 2000, 2002; Wang *et al.*, 2008a). As noted previously (Foissner *et al.*, 1994; Pérez-Uz & Song, 1995), living individuals of most scuticociliates are morphologically similar, making a combination of *in vivo* observations and description of the infraciliature and other structures

revealed by silver impregnations necessary for accurate identification and description.

In the present paper, we describe an unknown ciliate that was isolated from a coastal area of the Yellow Sea in northern China. Its unique infraciliature, especially that of the oral apparatus, marks it as belonging to a new taxon at both the genus and family levels. In addition, its small-subunit (SSU) rRNA gene was sequenced to analyse its phylogenetic position.

METHODS

Sample collection, observation and identification. Ciliates were isolated on 5 June 2008 from the coast of the Yellow Sea near Qingdao, China (36° 08' N 120° 43' E) (water conditions 18 °C, pH 8.0, salinity 31‰). Microscope observations and silver impregnation were done according to Wilbert (1975), and Corliss (1979) was taken as the authority for terminology.

Abbreviations: BI, Bayesian inference; MP, maximum-parsimony; NJ, neighbour-joining; PM, paroral membrane; SSU, small subunit.

The GenBank/EMBL/DDBJ accession number for the SSU rRNA sequence of *Eurystomatella sinica* is FJ012143.

DNA extraction, PCR amplification and sequencing. Total genomic DNA was extracted from cells using the REDEExtract-N-Amp Tissue PCR kit (Sigma) as described by Gong *et al.* (2007). PCR amplification of the SSU rRNA gene was performed as described by Miao *et al.* (2007) with primers Euk A (5'-AACCTGGTTGATCCTGCCAGT-3') and Euk B (5'-TGATCCTCTGCAGGTTCACCTAC-3') (Medlin *et al.*, 1988). A blunt-ended PCR product was prepared with T4 nucleotide polymerase and T4 kinase and then inserted into the pUCm-T vector (Takara). Transformations were accomplished using *Escherichia coli* JM109 competent cells. A mini-prep spin column kit (Sangon) was used to harvest and purify the plasmid DNA from confirmed clones for sequencing. Sequencing reactions were performed in both directions with an ABI Prism 377 automated DNA sequencer. For sequencing primers, we used three internal universal SSU rRNA gene primers (forward and reverse) as described by Elwood *et al.* (1985) together with primers M13F and M13R.

Phylogenetic analyses inferred from sequences of the SSU rRNA gene. Other than the SSU rRNA gene sequence of the new species, sequences of other ciliates used in the phylogenetic analysis were obtained from the GenBank/EMBL databases (Table 1). Two species of *Protocruzia* were selected as the outgroup for all analyses. The SSU rRNA gene sequence of one other pleuronematid, *Cyclidium porcatum*, was removed from the alignment after preliminary analysis because its inclusion produced unstable topologies that interfered with analyses and eliminated any benefit from its inclusion.

Sequences were aligned using CLUSTAL W version 1.83 (Thompson *et al.*, 1994) and the alignment was refined using BioEdit (Hall, 1999) by considering features of secondary structure. The final alignment included 50 taxa and 1703 nucleotide positions. Bayesian inference (BI) was performed with MrBayes version 3.1.2 (Huelsenbeck *et al.*, 2001). Two Markov chain Monte Carlo (MCMC) simulations were run for 1 000 000 generations, each with four simultaneous chains, using a sample frequency of 100 and a burn-in of 2500 trees. MrModeltest (Nylander, 2004) was used to identify the GTR + Γ_4 model set for among-site rate variation and allowing all substitution rates of the aligned sequence to be different as the best model for the BI analysis. A maximum-parsimony (MP) tree incorporating 1000 bootstrap replicates was constructed using PAUP* version 4.0b10 (Swofford, 2002) by means of a heuristic search with all characters coded as unordered. The PHYLIP package version 3.66 (Felsenstein, 2006) was used to calculate similarity and evolutionary distances between pairs of nucleotide sequences using the two-parameter model of Kimura (1980). A distance-matrix tree incorporating 1000 bootstrap replicates was then constructed with the neighbour-joining (NJ) method (Saitou & Nei, 1987).

The approximately unbiased (AU) test was carried out using the CONSEL package (Shimodaira & Hasegawa, 2001). The hypothesis (monophyly of Philasterida, Pleuronematida and Loxocephalida) was used to generate the constraint tree. The resulting trees were compared with the unconstrained Bayesian result in PAUP* 4.0b10.

RESULTS

Systematic treatment

Eurystomatellidae n. fam.

Diagnosis. Scuticociliates with close-set, well-developed and apically positioned oral membranelles; buccal field covering approximately two-thirds of the ventral surface

Table 1. SSU rRNA gene nucleotide sequences used in the present work

Species	GenBank accession no.	Length (bp)
<i>Anophryoides haemophila</i>	U51554	1763
<i>Bresslaua vorax</i>	AF060453	1780
<i>Cardiostomatella vermiformis</i>	AY881632	1769
<i>Chilodonella uncinata</i>	AF300281	1662
<i>Cohnilembus verminus</i>	Z22878	1717
<i>Coleps hirtus</i>	U97109	1772
<i>Colpoda inflata</i>	M97908	1788
<i>Cyclidium glaucoma</i>	Z22879	1718
<i>Cyclidium plouneouri</i>	U27816	1708
<i>Dexiotrichides pangi</i>	AY212805	1764
<i>Entodiscus borealis</i>	AY541687	1650
<i>Entorhipidium tenue</i>	AY541688	1759
<i>Epistylis wenrichi</i>	AF335515	1723
<i>Furgasonia blochmanni</i>	X65150	1765
<i>Glaucomides bromelicola</i>	AJ810077	1745
<i>Homalogastra setosa</i>	EF158848	1764
<i>Ichthyophthirius multifiliis</i>	U17354	1747
<i>Lembadion bullinum</i>	AF255358	1746
<i>Lechriopyla mystax</i>	AF527757	1712
<i>Mesanoophrys carcini</i>	AY103189	1759
<i>Metanoophrys similis</i>	AY314803	1763
<i>Miamiensis avidus</i>	AY550080	1759
<i>Obertrumia georgiana</i>	X65149	1765
<i>Paramecium bursaria</i>	AF100314	1648
<i>Paranoophrys magna</i>	AY103191	1759
<i>Parauronema longum</i>	AY212807	1759
<i>Philasterides dicentrarchi</i>	AY642280	1760
<i>Plagiopyla frontata</i>	Z29440	1704
<i>Plagiopyliella pacifica</i>	AY541685	1759
<i>Platyophrya vorax</i>	AF060454	1753
<i>Pleuronema czapikae</i>	EF486863	1674
<i>Pleuronema coronatum</i>	AY103188	1759
<i>Pleuronema</i> sp. WYG051211	EF486864	1676
<i>Prorodon teres</i>	X71140	1768
<i>Protocruzia contrax</i>	DQ190467	1788
<i>Protocruzia adherens</i>	AY217727	1752
<i>Pseudocohnilembus hargisi</i>	AY212806	1753
<i>Schizocalyptra aeschtae</i>	DQ777744	1763
<i>Schizocalyptra similis</i>	EU744177	1683
<i>Schizocalyptra sinica</i>	FJ156106	1683
<i>Schizocaryum dogieli</i>	AF527756	1760
<i>Tetrahymena canadensis</i>	M98022	1752
<i>Thyrophylax vorax</i>	AY541686	1758
<i>Trithigmostoma steini</i>	X71134	1715
<i>Trichodina nobilis</i>	AY102172	1698
<i>Urocentrum turbo</i>	AF255357	1754
<i>Uronema elegans</i>	AY103190	1757
<i>Uronemella filificum</i>	EF486866	1676
<i>Vorticella fusca</i>	DQ190468	1733

and surrounded by an almost completely circular paroral membrane.

Type genus. *Eurystomatella* n. gen.

***Eurystomatella* n. gen.**

Diagnosis. Eurystomatellids with closely arranged oral membranelles including small oral membranelle 1 (M1), bipartite M2 and well-developed M3. Body dorsoventrally flattened. Surface of cell faintly sculptured with silverline system in quadrangular mesh pattern. Cytostome located at anterior third of large buccal field.

Type species. *Eurystomatella sinica* n. sp.

Etymology. *Eury-*, Greek adjective, large; *-stoma*, Greek noun, mouth; *-ella*, Latin feminine diminutive suffix; feminine gender. Referring to the large buccal field.

***Eurystomatella sinica* n. sp. (Figs 1, 2 and 3; Table 2)**

Diagnosis. Living cell dorsoventrally flattened with proportions of length to width of approximately 2:1 (50–80 × 30–40 µm) and possessing several elongate caudal cilia. Buccal field occupying approximately three-quarters of the body length; M1 of buccal apparatus consisting of a single row of kinetosomes and M2a, M2b and M3 with multiple rows. Somatic kineties numbering 13–15 and composed of approximately 40 kinetids each. Contractile vacuole subcaudal; usually with one globular macronucleus.

Type locality. Sandy beach in Qingdao, Shandong Province, China (36° 08' N 120° 43' E); marine habitat.

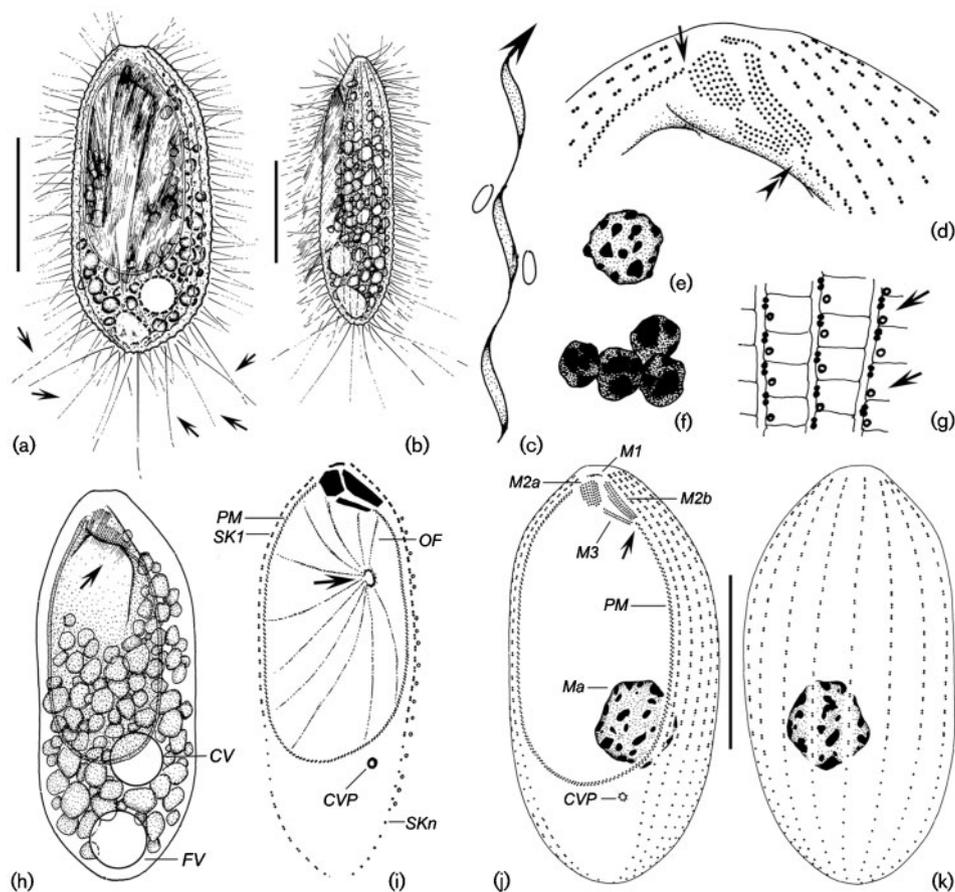


Fig. 1. Morphology and infraciliature of *Eurystomatella sinica* n. sp. from life (a–c, h) and after staining with silver nitrate (i) and protargol impregnation (d–g, j, k). (a) Ventral view of typical individual; note the elongate caudal cilia (arrows). (b) Lateral view. (c) Pattern of movement. (d) Ventral view of anterior portion of body, showing the buccal apparatus; arrow indicates the 'anterior' end of the paroral membrane (PM) and double arrowheads mark its 'posterior' end. (e, f) Nucleoli in the macronucleus. (g) Silverline system; arrows indicate extrusomes. (h) Ventral view of a slender specimen, showing the deeply excavated buccal area where the cytotome is located (arrow). (i) Buccal field; arrow marks the cytotome. Note the highly developed oral fibres (OF). (j, k) Ventral and dorsal views showing the general infraciliature; arrow in (j) indicates posterior end of PM. CV, Contractile vacuole; CVP, contractile vacuole pore; FV, food vacuole; M1, M2a, M2b and M3, oral membranelles 1–3; Ma, macronucleus; PM, paroral membrane; SK1 and SKn, first and last somatic kineties. Bars, 25 µm.

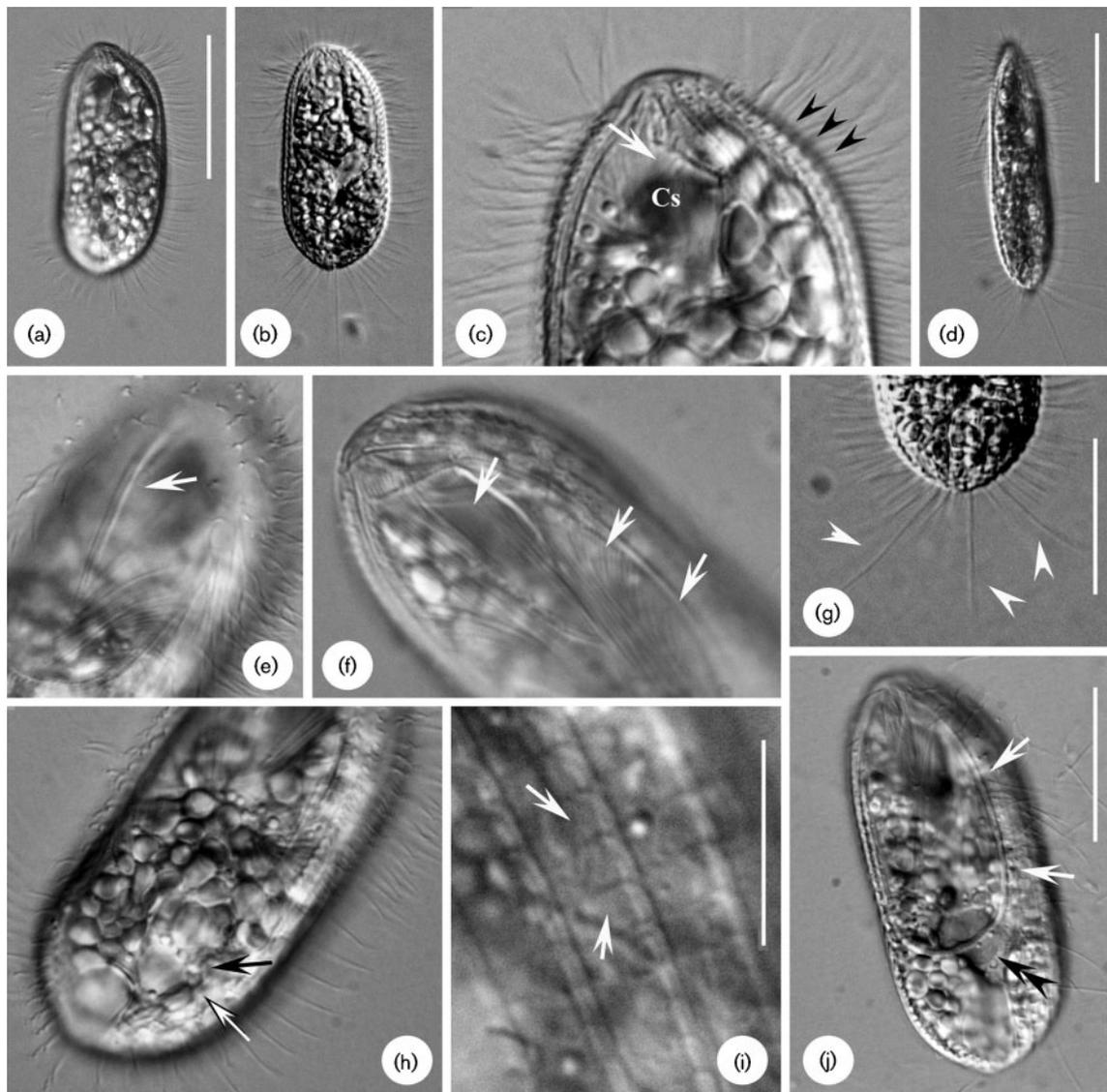


Fig. 2. Photomicrographs of living individuals of *Eurystomatella sinica* n. sp. (a, b) Ventral views of two typically oval individuals. (c) Ventral view of anterior part, showing the deeply excavated buccal area (arrow) where the cytostome (Cs) is located; arrowheads mark the notched pellicular outline. (d) Lateral view, showing the flattened body. (e, f) Ventral views, showing the cilia of the buccal apparatus (arrows). (g) Caudal portion, showing the elongate caudal cilia (arrowheads). (h) Ventral view, showing the contractile vacuole (arrows). (i) Detail of cell surface showing the ridged structure (arrows). (j) Ventral view, showing the contractile vacuole (double-arrowheads) and the ridge around the buccal field where the paroral membrane is located (arrows). Bars, 30 μm (a, d), 10 μm (i) and 20 μm (j).

Type specimens. One holotype slide with protargol-impregnated specimens has been deposited in the Natural History Museum, London, UK, with registration no. 2009:3:24:1. Two paratype slides (no. MM-2008-08060503) have been deposited in the collection of the Laboratory of Protozoology, OUC, China.

Etymology. The name *sinica* (Latin feminine adjective) refers to the fact that this species was first described from Chinese coastal waters.

Description. Living individuals usually measuring approximately $60 \times 30 \mu\text{m}$, with elongate to slender oval shape, broadly rounded at both ends, with anterior end slightly truncated (Fig. 1a, h; Fig. 2b, j). Body dorso-ventrally flattened, with height/width ratio of approximately 1:2. Buccal field comprising approximately 75% of the body length and 70% of the body width (Fig. 2j), surrounded by a ridged ring (or base of paroral membrane), surface hardly invaginated except for upper third where the cytostome is located (Fig. 1h; Fig. 2c,

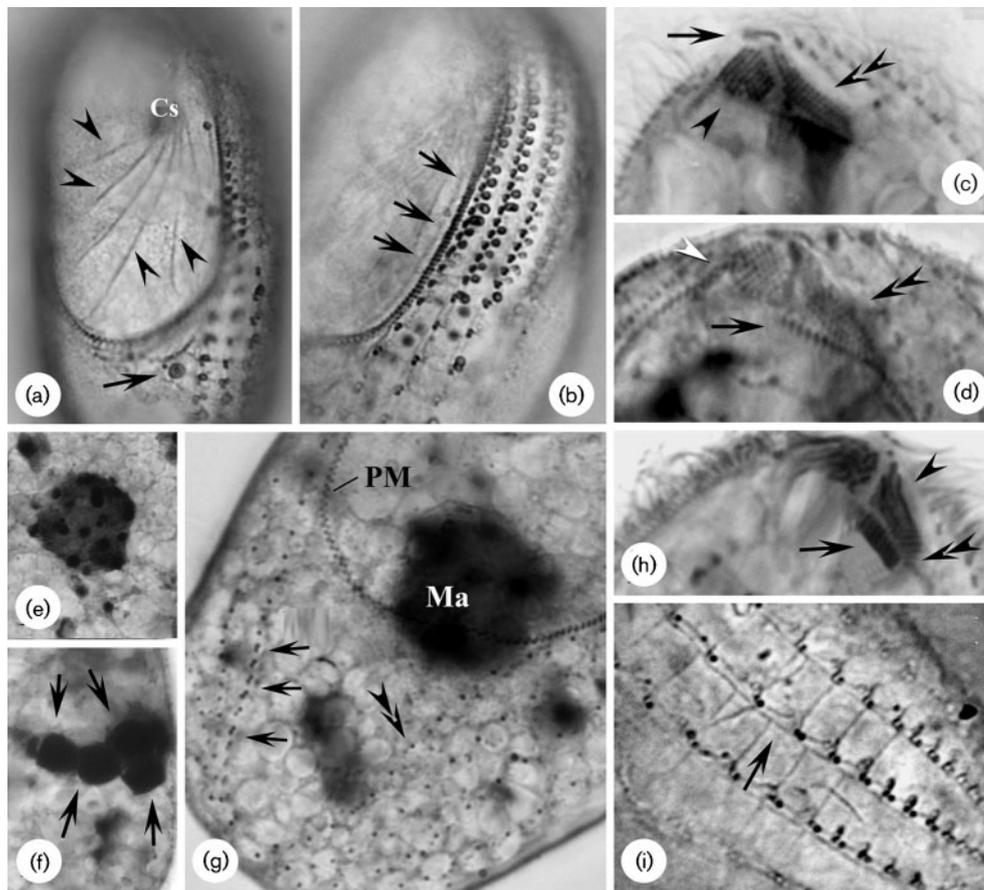


Fig. 3. Photomicrographs of *Eurystomatella sinica* n. sp. after staining with silver nitrate (a, b, i) and protargol impregnation (c–h). (a, b) Ventral views, showing the buccal area; arrowheads in (a) mark the oral fibres and the arrow indicates the contractile vacuole pore. Arrows in (b) mark the PM. (c, d, h) Ventral views of the anterior portion at different focal planes showing the buccal apparatus: (c) M1 (arrow), M2a (arrowhead) and M2b (double-arrowheads); (d) M2b (double-arrowheads) and M3 (arrow); arrowhead marks anterior end of PM; (h) M2b (arrowhead) and M3 (arrow); note the posterior end of the PM (double-arrowheads). (e, f) Nucleoli in the macronucleus. (g) Ventral view of caudal portion of cell; note the segment of somatic kinety 1 consisting of dikinetids (arrows) in contrast with other kineties composed of monokinetids (double-arrowheads). (i) Detail of the silverline system; arrow marks the interkinetal space. Cs, Cytostome; Ma, macronucleus; PM, paroral membrane.

arrow). Pellicle ridged, characteristically notched in profile (readily visible only under high magnification; Fig. 2c). No extrusomes detectable in living cell, but revealed by silver nitrate impregnation to be densely arranged along somatic kineties (Fig. 1g, arrows; Fig. 3b). Cytoplasm colourless to slightly greyish, often packed with numerous large granules (Fig. 1a, b, h; Fig. 2b, h, j). Usually one oval macronucleus with many globular nucleoli present (Fig. 1e, f; Fig. 3e), but several macronuclei (up to six) in some cells. Contractile vacuole located approximately one-fifth of the body length from posterior end, located left of the mid-line, measuring approximately 8 μm in diameter (Fig. 1a, b, h). No pulsation of contractile vacuole detected. One large food vacuole, often irregular in shape, frequently located at posterior end of cell (Fig. 1a, b, h).

Cilia of buccal apparatus conspicuously long (about 30–40 μm), never extending out strongly like those of some

other scuticociliates (e.g. *Pleuronema*), lying flat and pointing toward the posterior within the buccal field (Fig. 1a; Fig. 2e). Cell with approximately ten elongate (30 μm long) caudal cilia radiating conspicuously away from body (Fig. 1a, b; Fig. 2b, d, g). Somatic cilia approximately 10 μm in length. Very characteristic movement as follows: frequently lying completely stationary on the bottom for long periods (> 1 min) with all cilia stiffly spread, then crawling actively with jerking motion; when swimming (observed infrequently), moving moderately fast with rotation around the main axis of body (Fig. 1c), then remaining very quietly suspended in the water at the same spot for long periods.

Somatic kineties numbering 13–15 and terminating anteriorly at small, non-ciliated apical plate; large, non-ciliated, post-oral field on ventral side (Fig. 1i, j; Fig. 3g). Scutula not observed. Somatic kineties composed of dikinetids in

Table 2. Morphometric data for *Eurystomatella sinica* n. sp.

All data are based on protargol-impregnated specimens and measurements are given in μm . Max., Maximum; Min., minimum; Mean, arithmetic mean; SD, standard deviation; CV, coefficient of variation expressed as a percentage; *n*, number of specimens investigated; BB, basal bodies; BF, buccal field; KR, kinety rows; M1–3, membranelles 1–3; SK, somatic kinety.

Character	Max.	Min.	Mean	SD	CV	<i>n</i>
Body length	76	49	57.5	6.3	11.0	30
Body width	52	25	34.1	6.8	20.0	30
Length of BF	59	37	41.8	4.1	9.9	30
Width of BF	25	18	21.3	1.8	8.6	30
Number of:						
SK	15	13	14.1	0.5	3.6	30
Macronuclei	6	1	1.8	1.6	89.6	24
KR in M2a	9	5	7.3	0.9	12.6	26
KR in M2b	4	4	4.0	0	0	30
KR in M3	4	2	2.4	0.7	27.9	30
BB in SK1	47	34	41.2	3.7	9.0	18
BB in SKn	46	32	40.2	4.1	10.1	14

anterior half of body and monokinetids in posterior half (Fig. 1j, k). Segment of kinety 1 posterior to buccal field invariably composed of dikinetids (Fig. 3g, arrows).

Buccal apparatus as shown in Figs 1(d, i, j) and 3(c, d, h). Paroral membrane (PM) composed of closely spaced dikinetids, consisting of an almost completely closed ring except at anterior end where membranelles are located. M1 usually consisting of one row of kinetosomes but sometimes two. M2 in two parts: a shorter right part (M2a) consisting of approximately seven rows of kinetosomes and a longer left part (M2b) usually consisting of four rows, one of which is shorter. M3 consisting of two to four rows of kinetosomes, often difficult to see because of their location close to the posterior end of the PM.

Silverline system composed of rectangular, transversely orientated units with interkinetal space to the right of each kinety (Fig. 1g; Fig. 3i). Approximately ten oral fibres (oral ribs) converging on cytostome (Fig. 1i; Fig. 3a, arrowheads). Single contractile vacuole pore (CVP) located on the left side immediately posterior to the buccal field (Fig. 1i, j; Fig. 3a).

Sequence alignment and phylogenetic analysis

The complete SSU rRNA gene sequence of *E. sinica* includes 1757 bp, has a G + C content of 42.8 mol% and does not contain any conspicuous insertions or deletions. Similarities of the SSU rRNA gene sequence of *E. sinica* to those of the most closely related species in other genera of scuticociliates were as follows: *Cyclidium* (*C. plouneouri*, *C. glaucoma*), 87.5–91.2%; *Pleuronema* (*P. czapikae*, *Pleuronema* sp. WYG051211, *P. coronatum*), 81.3–87.1%; *Schizocalyptra* (*S. aeschtae*, *S. sinica*, *S. similis*), 82.3–87.0%.

Phylogenetic analyses of SSU rRNA gene sequences revealed three distinct clades of the Scuticociliates: the

orders Philasterida, Pleuronematida and Loxocephalida (Fig. 4). In all three trees, philasterid and pleuronematid clades were strongly supported to be sister groups (1.00 in BI, 73% in MP, 93% in NJ), while the loxocephalids were distant from both of them, associating with penicoline ciliates (Fig. 4). Monophyly of the scuticociliates was not supported in any of our trees regardless of alignment parameters, types of phylogenetic analysis or models of evolution used. However, the result of the AU test suggested that there were not significant differences when the three orders (Philasterida, Pleuronematida and Loxocephalida) were constrained to be a monophyletic clade. This indicates that the alternative hypothesis testing the monophyly of the Scuticociliatia was not rejected.

Monophyly of the order Pleuronematida was always retrieved (1.00 in BI, 100% in MP and NJ) in all trees. *E. sinica* was sister to species of *Cyclidium* in a small, fully supported (1.00 in BI, 100% in MP and NJ) clade divergent from other pleuronematids (Fig. 4). Within this clade, the two species of *Cyclidium* clustered together, separately from *E. sinica*, with high bootstrap support (1.00 in BI, 100% in MP and NJ).

DISCUSSION

The separation of families within the scuticociliates has generally been controversial because no widely acceptable criteria have been presented (Corliss, 1979; Foissner *et al.*, 1994; Lynn, 2008; Lynn & Small, 2002; de Puytorac *et al.*, 1994; Ma *et al.*, 2005; Song, 2000). Similarly, we cannot assign *Eurystomatella* to a known family with confidence, although molecular data indicate that it is related to *Cyclidium* in the well-known pleuronematid assemblage (Fig. 4).

The widely accepted systems of both Corliss (1979) and Lynn (2008) assigned *Cyclidium* and *Pleuronema* to

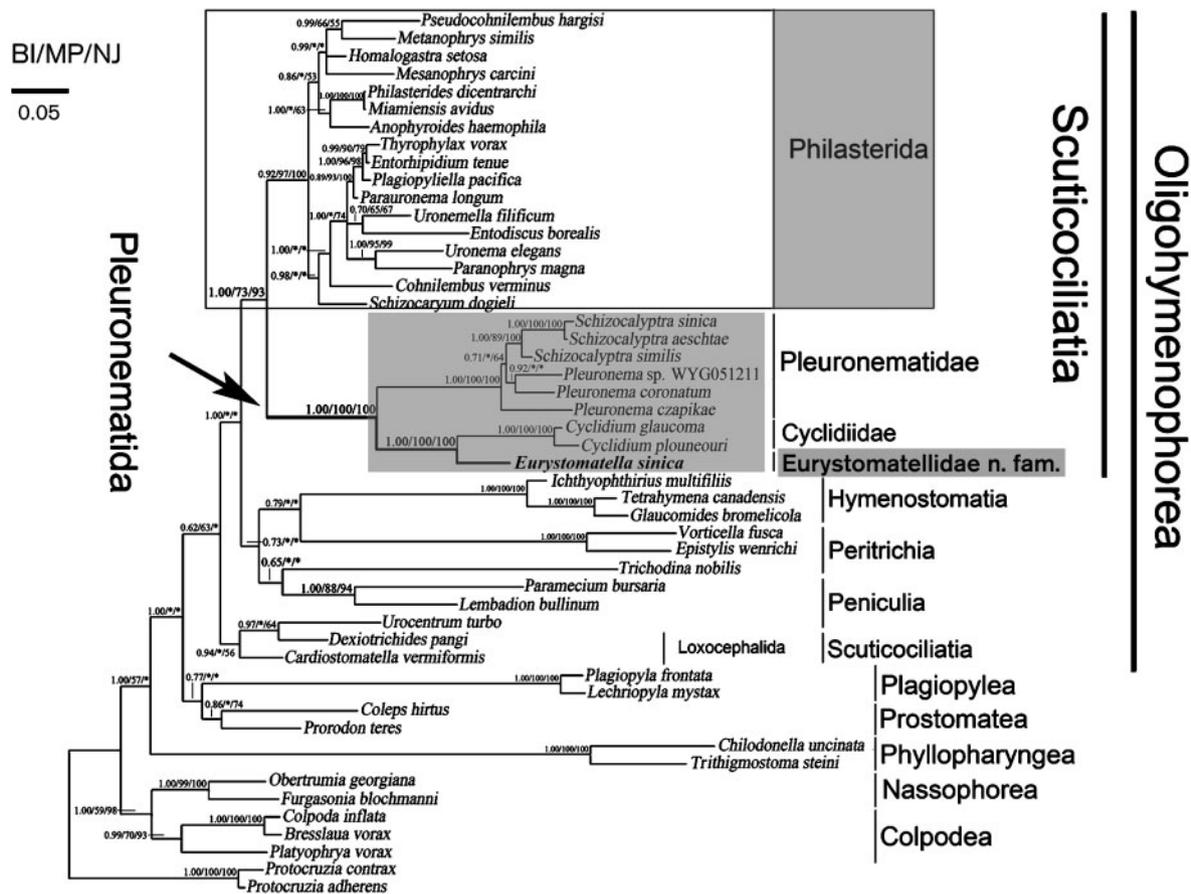


Fig. 4. Bayesian tree inferred from SSU rRNA gene sequences. Numbers at nodes represent Bayesian posterior probabilities and bootstrap percentages for MP and NJ analyses (both from 1000 replicates). Asterisks indicate bootstrap values less than 50% and disagreement between a method and the reference Bayesian tree at a given node. Evolutionary distance is represented by the branch length separating the species in the figure. Bar, 5 substitutions per 100 nucleotide positions.

different families (Cyclidiidae and Pleuronematidae, respectively). Clearly, the infraciliature and buccal apparatus of *E. sinica* show little affinity with those of either of these taxa. In cyclidiids, oral membranelles are characteristically fragmented, poorly organized or ungrouped and arranged longitudinally along the length of the buccal field. In addition, their PM is hook-like and has a conspicuous, posteriorly located scutica composed of paired basal bodies (Table 3). All of these characteristics exclude *Eurystomatella* from the family Cyclidiidae. The buccal morphology and infraciliature of taxa in the family Pleuronematidae are highly specialized, with an asymmetrical, hook-shaped PM, thread-like oral membranelles arranged along the longitudinal axis of the PM and several shortened 'paroral' somatic kineties (Long *et al.*, 2007a; Wang *et al.*, 2008a); therefore, there is no justification for assigning *Eurystomatella* to this family either.

It should be emphasized that the appearance of the buccal field and PM are unique among all known scuticociliates. The PM is almost completely closed in most of them, and

all the oral membranelles are packed together at the 'corner' of a huge buccal field. Additionally, the cytostome is always positioned at or near the 'bottom' of the buccal field in all known members of the order Pleuronematida. By contrast, the cytostome is located very far towards the 'top' of the buccal field (anterior to middle) in *E. sinica*, another very rare feature amongst other scuticociliates.

In conclusion, the unique buccal structures and general morphology of *E. sinica* indicate that it should be assigned to a new family Eurystomatellidae distinct from the families Cyclidiidae and Pleuronematidae (Table 3), but related to them by molecular information. The closely packed oral membranelles of *E. sinica* composed of multiple rows may represent a primitive stage of evolution within the eurystomatellid–cyclidiid–pleuronematid lineage, because they show similarities to the later stages in stomatogenesis of other scuticociliates (Ma & Song, 2003; Ma *et al.*, 2004). If this hypothesis is correct, the more substantial oral membranelles of *Eurystomatella* may represent the form that was ancestral to the relatively

Table 3. Morphological comparison of the families Eurystomatellidae, Cyclidiidae and Pleuronematidae

Unique features of the new family are in bold. Data were obtained by the present authors.

Character	Eurystomatellidae	Cyclidiidae	Pleuronematidae
Paroral membrane	Circular, almost completely closed	Hook-like, on right margin of buccal field	As in Cyclidiidae
Oral membranelles	All together and close-set, well-developed; anteriorly positioned	Non-differentiated, not clearly defined; longitudinally arranged	Highly differentiated, all parts clearly separated; longitudinally arranged
Cytostome position	Top of buccal field	Near bottom of buccal field	As in Cyclidiidae
Post-oral cilia-free field	Present	Absent	Present
Pre-oral kineties	Absent	Absent	Present
Anterior/posterior sutures	Absent	Absent	Present
Scutica	Absent	Present	Absent

narrow oral membranelles of cyclidiids and pleuronematids, explaining the divergent position of *Eurystomatella* in our phylogenetic trees.

At present, only a few scuticociliates (e.g. *Eurystomatella*, pleurostomatids and *Philasterides*) are known to have a buccal apparatus with four or more membranelles; almost all other scuticociliates have three isolated membranelles in their buccal apparatus (Corliss, 1979; Long *et al.*, 2007b; Lynn & Small, 2002; Ma & Song, 2003; Miao *et al.*, 2008; Shang *et al.*, 2006; Song, 2000; Wang *et al.*, 2008a, b). We described the oral structure in *Eurystomatella* according to the pattern in pleurostomatids. However, it is almost impossible to define the oral membranelles in our new organism with confidence because no stomatogenetic data are available at the moment. Thus, our numbering of the membranelles 1 to 3 in the description is provisional. M3 could actually be part of M2b and M2a might be, in fact, the real M3. Final definition will await further morphogenetic studies.

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