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ORIGINAL ARTICLE

Molecular Phylogeny and Taxonomy of a New Freshwater Hymenostomatid from Northeastern China, with the Establishment of a New Genus *Anteglaucoma* gen. n. (Protista, Ciliophora, Oligohymenophorea)

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ABSTRACT

The morphology, infraciliature and SSU rDNA sequence of a new freshwater hymenostomatid ciliate, *Anteglaucoma harbinensis* gen. nov., spec. nov., collected from a farmland pond in Harbin, China, were investigated. The new genus *Anteglaucoma* is characterized as follows: small to medium-sized Glaucomidae with oral apparatus in anterior one-third of cell; paroral membrane composed of almost longitudinally arranged dikinetids; three adoral membranelles nearly equal in length and arranged almost longitudinally in parallel; silverline pattern tetrahymenid. The improved diagnosis of family Glaucomidae Corliss 1971 is provided based on the previous and present work. The type species *Anteglaucoma harbinensis* spec. nov. is defined by having 32–35 somatic kineties; four or five postoral kineties; membranelle 1 and membranelle 2 having five or six kinetosomal rows, membranelle 3 having three kinetosomal rows; single macronuclear nodule; contractile vacuole on average 15% from posterior body end; locomotion characterized by crawling with a rather hectic jerking motion; freshwater habitat. Phylogenetic analyses show that *Anteglaucoma* clusters in the family Glaucomidae and groups with the genera *Glaucoma*. The molecular and morphological data indicate that Glaucomidae is related to the family Bromeliophryidae in the phylogenetic trees.

CILIATES of subclass Hymenostomatia are common inhabitants of terrestrial, fresh, and brackish water environments (Chantangsi et al. 2007; Chung and Yao 2012; Corliss 1954, 1971; Foissner et al. 1994; Fried and Foissner 2007; Kher et al. 2011; Klug 1968; Liu et al. 2007; Mochizuki et al. 2002; Peck 1978). They show similar living features, relatively simple body plans and relatively few morphological characters for species circumscription (Borden et al. 1977; Corliss 1952, 1959; Frankel 1960; Liu et al. 2016a; McCoy 1975). Despite these problems, research on hymenostomatids is comparatively advanced with more than half of the genera recognized by Jankowski (2007) and Lynn (2008) investigated or reinvestigated with modern methods (Chen et al. 2016; Zhao et al. 2016). Recent discovery of new taxa has highlighted the necessity to direct further attention to the ciliates in class Oligohymenophora (Foissner 2003a, 2013; Foissner and Stoeck 2013; Gao et al. 2013, 2016; Liu et al. 2016b; Pan et al. 2015a,b, 2016; Quintela-Alonso et al. 2013; Zhao et al. 2016).

In the present work, a new hymenostomatid is described which was isolated from a farmland pond in Harbin, northeastern China. Based on its unique infraciliature, especially with respect to the oral apparatus, this form is regarded as a new taxon at the generic level. Small subunit ribosomal DNA (SSU rDNA) sequence data for this new species was also analyzed to determine its phylogenetic position within the Hymenostomatia.

MATERIAL AND METHODS

Sample collection and identification

Anteglaucoma harbinensis spec. nov. was collected on 26 Oct 2015 from a farmland pond (44°87′14.7″N; 127°09′12.0″E) in Harbin, Heilongjiang province, northeastern China (water temperature 14 °C and pH 7.3). About 0.4 L water was collected from 0.1 to 0.5 m below the water surface using a sampling bottle (Fig. S1). Ciliates were maintained in habitat water in Petri dishes as raw cultures at room temperature (ca. 25 °C) with rice grains added to enrich the growth of bacteria as food.

Isolated cells were observed and photographed in vivo using differential interference contrast microscopy. The protargol (Wilbert 1975) and silver carbonate (Ma et al. 2003) methods were used to reveal the infraciliature, and protargol was made according to Pan et al. (2013). Silver nitrate after Chatton-Lwoff and Klein-Foissner), all described in Foissner (1991). Supra-vital staining with methyl green-pyronin was also used to reveal the nuclear and extrusomes temporarily (Foissner 1979). Counts and measurements of stained specimens were performed at magnifications of 100–1250×. Drawings were made with the help of a drawing device. Samples for scanning electron microscopy (SEM) were prepared as described by Gu and Ni (1993) and were observed in a Hitachi S-4800 scanning electron microscope with accelerating voltage 10.0 kV. Systematics and terminology are mainly according to Lynn (2008).

DNA extraction, PCR amplification and sequencing

Genomic DNA of *Anteglaucoma harbinensis* spec. nov. was extracted, using the DNeasy Tissue kit (Qiagen, Valencia, CA), from five cells that had been starved overnight. The amplification of SSU rDNA was performed with universal primers, Euk A and Euk B (Medlin et al. 1988). REPLI-g Mini DNA polymerase (Neb, M0494L) was also used to perform PCR amplification. Purified PCR products of the appropriate size were inserted into the pMD™18-T vector (Takara Biotechnology, Dalian Co., Ltd., Dalian, Liaoning Province, China), transformed into *E. coli* competent cells and products from transformed clones were sequenced on an ABI-PRISM 3730 automatic sequencer (Applied Biosystems, Qingdao, Shandong Province, China) using M13 forward and reverse primers. The SSU rDNA sequence has been deposited in the GenBank database with accession number KY204069. Other sequences used in the study were obtained from the GenBank database (accession numbers shown in Fig. 4).

Phylogenetic analyses

Sequences were aligned using Clustal W implemented in BioEdit 7.0 (Hall 1999) enabling pairwise analysis. *Coleps nolandi* and *Tiarina fusa* were used as the outgroup. The maximum likelihodd (ML) analysis was conducted using RAxML-HPC2 on XSEDE (8.1.11) (Stamatakis 2006; Stamatakis et al. 2008) via the CIPRES Science Gateway website (http://www.phylo.org/sub_sections/portal), using the GTR + I + G model as selected by Modeltest v.3.4 (Posada and Crandall 1998). The reliability of internal branches was estimated by bootstrapping with 1,000 replicates. The Bayesian inference (BI) analysis was performed with MrBayes v3.2.3 (Ronquist and Huelsenbeck 2003) via the CIPRES Science Gateway using the GTR + I + G model selected by MrModeltest v.2.0 (Nylander 2004). The chain length of Markov chain Monte Carlo simulations was 1,000,000 generations with a sampling frequency of 100 generations. The first 25% of sampled trees was discarded as burn-in. Phylogenetic trees were visualized with TreeView v.1.6.6 (Page 1996) and MEGA v.4 (Tamura et al. 2007). To test the monophyly of Glaucomidae, the site-wise likelihoods for the resulting constrained topologies and the nonconstrained ML topologies were calculated using PAUP (Swofford 2002) and were then subjected to the AU test (Shimodaira 2002) as implemented in CONSEL (Shimodaira and Hasegawa 2001).

RESULTS

Morphological description of *Anteglaucoma harbinensis* gen. nov. spec. nov.

Description of living morphology

Body size about 60–70 × 35–40 μm in vivo, oval body with slightly pointed anterior and posterior ends (Fig. 1A, 2A–D). Ratio of length to width about 1.8:1 (Fig. 1A, 2A–

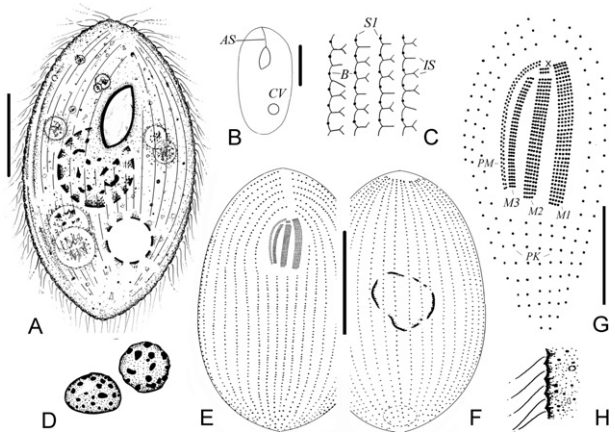

Figure 1 Morphology and infraciliature of *Anteglaucoma harbinensis* spec. nov. from life (**A**, **B**, **D**, **H**), after protargol (**E**–**G**) and Chatton-Lwoff silver nitrate staining (**C**). (A) Ventral view of a representative individual. (B) Ventral view, to show contractile vacuole and anterior suture. (C) Detail of silverline system. (D) Different shapes of macronucleus. (E, F) Infraciliature in ventral (E) and dorsal view (F) of holotype specimen. (G) Oral ciliature of the holotype specimen. (H) Part of pellicle, to show extrusomes. AS, anterior suture; B, basal bodies; CV, contractile vacuole; IS, intramerididonal cross-silverlines; M1–3, membranelles 1, 2 and 3; PK, postoral kineties; PM, paroral membrane; S1, silverline meridians. x = X-body. Bars, 10 μm (G), 20 μm (A, B), 30 μm (E, F).

Table 1. Morphometric data based on protargol-impregnated specimens (saturated mercuric chlorite as fixative) of *Anteglaucoma harbinensis* spec. nov.

Character	Min	Max	Mean	M	SD	CV	*n*
Body length, μm	66	73	69.4	70	3.4	4.9	15
Body width, μm	29	35	32.7	32	1.9	5.9	15
Buccal field length, μm	14	17	15.2	15	1.3	8.4	15
Buccal field width, μm	4	5	4.6	4	3.2	8.0	15
Somatic kineties, number	32	35	34.7	35	0.7	2.0	14
Macronucleus, number	1	1	1	1	0	0	15
Macronucleus length, μm	17	20	18.8	18	1.3	7.2	15
Macronucleus width, μm	16	19	18.2	18	1.1	6.1	15
Kinety rows in membranelle 1	5	6	5.4	5	0.4	7.4	11
Kinety rows in membranelle 2	5	5	5	5	0	0	11
Kinety rows in membranelle 3	3	3	3	3	0	0	11
Membranelle 1 length, μm	13	16	14.2	15	1.4	9.3	11
Membranelle 2 length, μm	12	13	12.3	12	0.9	7.5	11
Membranelle 3 length, μm	11	12	11.4	11	0.9	8.2	11
Kinetofragments at left mouth margin, number	0	0	0	0	0	0	12
Number of basal bodies in somatic kinety 1	49	53	50.4	50	2.4	4.8	15
Number of basal bodies in somatic kinety *n*	47	51	48.4	48	0.9	23.0	15

CV, coefficient of variation (%); M, Median; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of specimens; SD, standard deviation.

C). Dorsoventrally flattened about 3:1 to 4:1. Buccal field broadly elliptical, approximately 15–18% of body length, and oriented slightly obliquely to the main body axis (Fig. 1A, 2A, B, F, G). Oral ciliature rather inconspicuous due to the lack of paroral cilia and comparatively small, about 15 μm long adoral membranelles lying more or less flat within buccal field when motionless (Fig. 1A, 2N). Pellicle thin and slightly notched, with dense, spindle-shaped (2–3 μm) subcortical extrusomes (Fig. 1A, H). Cytoplasm hyaline to greyish, containing several to many large (approximately 10 μm) bacteria-filled food vacuoles and variably sized (3–5 μm) refringent granules (Fig. 1A, 2A–D, G). Single ellipsoid to spherical macronucleus, approximately 18 μm across, no micronucleus observed even after Supra-vital staining with methyl green-pyronin (Fig. 1A, D, 2H, J). Contractile vacuole on average 15% from posterior body end, approximately 12 μm diameter in diastole, pulsating at intervals of approximately 1 min (Fig. 1A, B, 2E). Somatic cilia approximately 10 μm long, densely arranged (Fig. 1A and 2F, L), no nonciliated dorsolateral area observed, all the cilia in dorsolateral arranged as shown in Fig. 2K. Locomotion characteristic, usually stationary on bottom of the Petri dish for long periods (> 5 min), then crawling with a rather hectic jerking motion; swimming moderately fast while rotating about main body axis then remaining motionless suspended for long periods.

Infraciliature

Thirty-two to 35 meridional somatic kineties, extending entire length of body and consisting of monokinetids throughout (Fig. 1E, F, 2H, J, K). Distinct preoral suture (Fig. 1B). Kinety 1 commencing right of the buccal cavity, having approximately 50 basal bodies. Right and left ventral ciliary rows abut preoral suture, approximately four or five postoral kineties (Fig. 1E, F, 2I). Dorsal kineties originate subapically, leaving a small, bare obovate frontal field (Fig. 1E, F). Buccal apparatus: PM (paroral membrane) extending along the right buccal margin, slightly curved left anteriorly, comprising barren, longitudinally arranged dikinetids (Fig. 1G, 2M, P). Three elongate adoral membranelles (approximately 15 μm in length) slightly decreasing in length from M1 (membranelle 1) to M3 (membranelle 3) and arranged longitudinally almost in parallel (Fig. 1G, 2M, P). An uninterrupted kinety but no kinetofragment presented at the left margin of the oral opening (Fig. 2O, arrow). Membranelle 1 composed of five or six longitudinal rows of kinetids (Fig. 1G, 2M, P). Membranelle 2 slightly shorter than M1, comprising five longitudinally rows of kinetids, with a patch ("X-group") of basal bodies (consisting of four, rarely five, basal bodies) at its anterior end. Membranelle 3 shorter than M1 and M2 (membranelle 2), slightly concave to left, composed of only three rows (Fig. 1G, 2M, P).

Silverline system

Silverline pattern tetrahymenid (Fig. 1C, 2Q, R). The basal bodies of a ciliary row connected by a silverline (primary silverline meridians) containing the basal bodies and producing many minute, transversely oriented cross-fibers (Fig. 1C, 2Q, R). Secondary silverline meridians absent (Fig. 1C). All sized specimens having two type transversely arranged intermeridional cross-fibers: one stick-shaped and the other having a shape like letter "Y" rotated 90 degrees clockwise (Fig. 1C, 2Q). Many granules presented in silverlines belonging to the extrusomes (Fig. 2R). Silverlines in the buccal cavity not studied in detail (Fig. 2R).

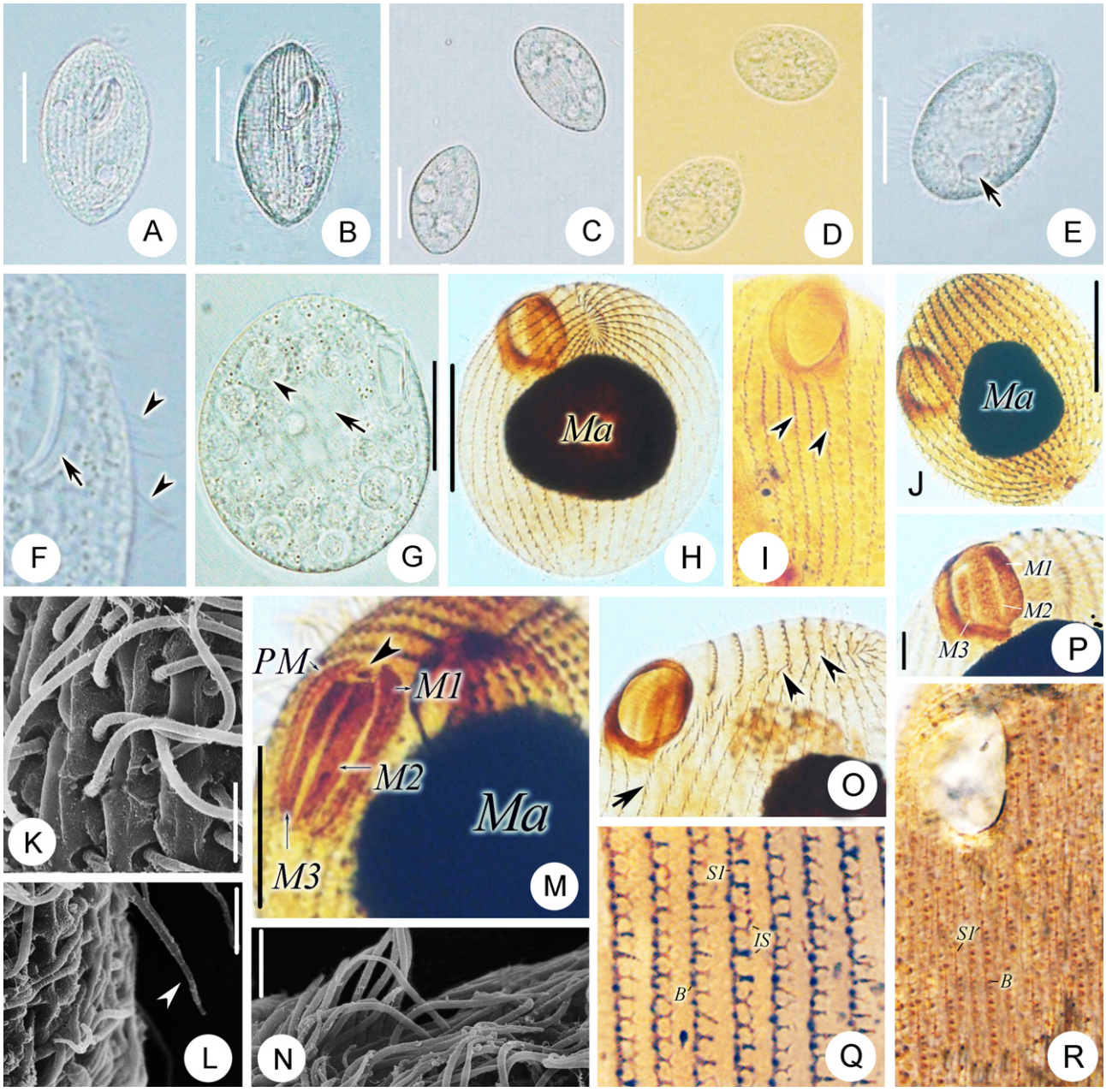

Figure 2 *Anteglaucoma harbinensis* spec. nov. from life (**A–I**), after silver carbonate (**H–J**, **M**, **O**, **P**) and Chatton-Lwoff silver nitrate staining (**Q**, **R**) and after SEM preparation (**K**, **L**, **N**). (A). Ventral view of a representative individual. (B–E) Different body shapes, arrow in (E) shows contractile vacuole. (F) Ventral view, to show buccal field (arrow) and somatic cilia (arrowheads). (G) Ventral views, to show food vacuoles (arrowheads) and macronucleus (arrows). (H, J) Ventral views, to show somatic kineties and macronucleus. (I) Ventral view, to mark postoral kineties (arrowheads). (K, L) Somatic cilia (arrowhead in L). (M, P) Detailed infraciliature of buccal area, arrowhead shows X-body. (N) Adoral membranelles. (Q, R) Detail of silverline system. B, basal bodies; IS, intrameridional cross-silverlines; M1–3, membranelles 1, 2 and 3; Ma, macronucleus; PM, paroral membrane; S1, silverline meridians. Bars, 2 μm (K), 5 μm (L, N, Q), 15 μm (M, P), 30 μm (A–E, G, H, J).

Gene sequence data

The SSU rDNA sequence of *Anteglaucoma harbinensis* spec. nov. has been deposited in the GenBank database with the accession number, length and G+C content as follows: NHMUK 2016.10.26.1, 1630 bp (not including Euk A and Euk B primers), 43.19%.

DISCUSSION

Remarks and comparisons with similar species

Lynn (2008) synonymized the Bromeliophryidae Foissner 2003a with the Glaucomidae Corliss 1971 without any

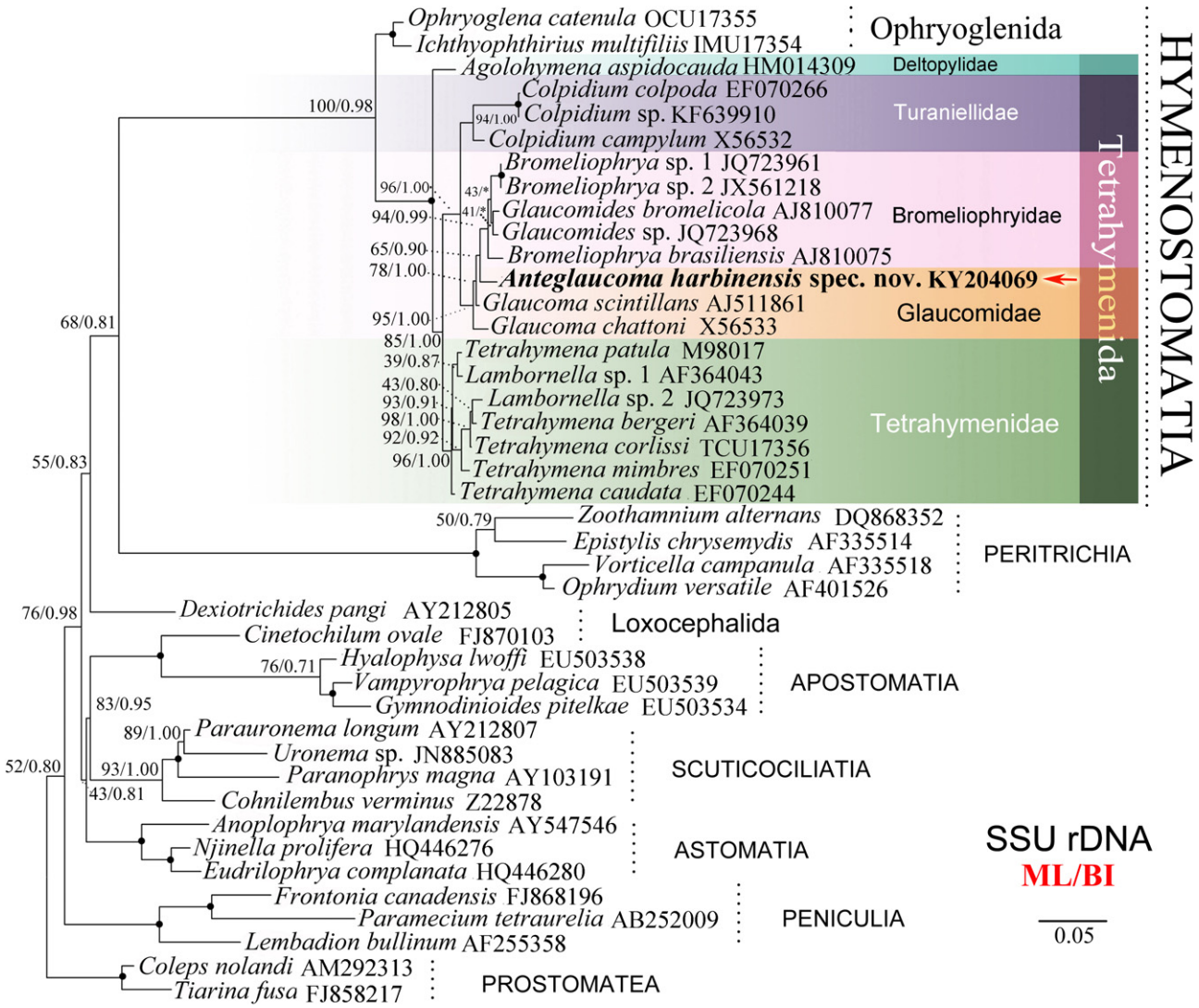

Figure 3 Phylogenetic tree inferred from SSU rDNA sequences, showing the position of *Anteglaucoma harbinensis* spec. nov. (in bold, arrow). Numbers at nodes represent the bootstrap values of maximum likelihood (ML) out of 1,000 replicates and the posterior probability of Bayesian analysis (BI). '*' indicates topologies that differ between the ML and BI phylogenies. Fully supported (100%/1.00) branches are marked with solid circles. The scale bar corresponds to five substitutions per 100 nucleotide positions.

supporting evidence (Lynn 2008). Comparatively, Foissner (2013) strongly supported family rank for Bromeliophrya and Glaucomides based on the molecular trees, besides, he also provided a morphological and an ecological reason: (1) bromeliophryid ciliates have large, bare area on left and/or dorsal side and split of somatic kineties to provide migrating kinetofragments at left mouth margin; (2) ciliates in Bromeliophryidae have been found only in phytotelmata, indicating that they underwent an independent evolution there while glaucomids occur globally in ordinary standing and running waters (Foissner 2003b). We could not agree with the synonymization of Bromeliophryidae with Glaucomidae in Lynn (2008) but support the establishment of family rank in Foissner (2013).

In addition, Foissner (2013) provided the improved diagnosis for the family Bromeliophryidae in his work: partially reduced somatic ciliature and a patch ("X-group") of basal bodies; life cycle monophasic or biphasic with rapacious macrostomes; silverline pattern tetrahymenid; stomatogenesis glaucomid, one or several left side kineties split and the anterior fragments migrate to the left margin of the oral opening; inhabit neotropic phytotelmata (usually tank bromeliads) (Foissner 2003b).

Compared with ciliates in Bromeliophryidae, *Anteglaucoma harbinensis* spec. nov. can be distinguished by (1) lack the ability to produce macrostomes; (2) occur in ordinary farmland pond (vs. inhabit neotropic phytotelmata); (3) the absence of a kinety fragment left of the oral opening; (4) the ciliature (holotrichous vs. a large, nonciliated area dorsolaterally), although *Anteglaucoma* has a completely barren PM which is only shared by *Bromeliophrya brasiliensis* (assigned to Bromeliophryidae), the reason

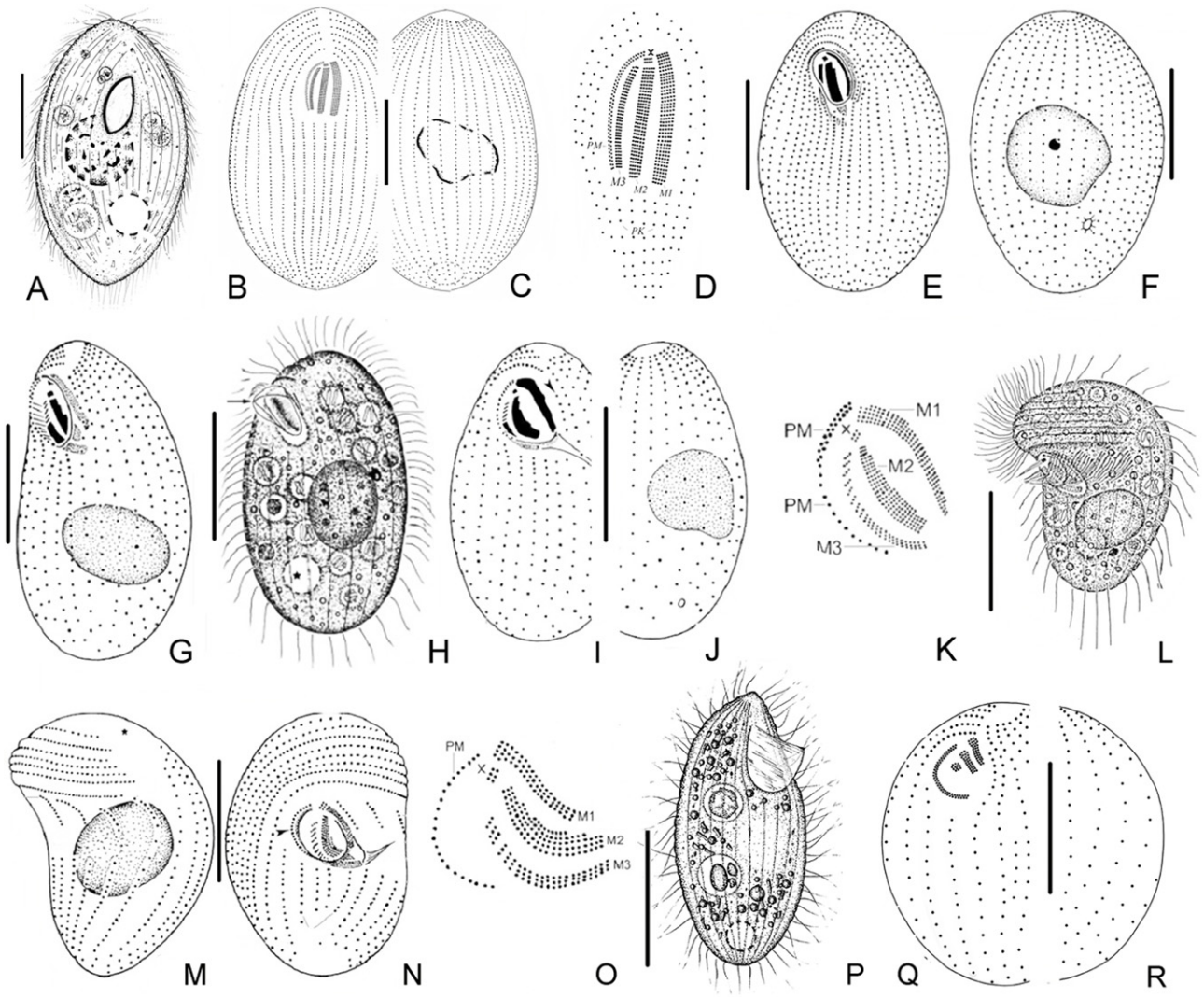

Figure 4 Morphological comparisons among *Anteglaucoma* gen. nov. and some similar genera. (**A**–**D**) *Anteglaucoma harbinensis* spec. nov. (from present work). (**E**, **F**) *Glaucoma scintillans* Ehrenberg, 1830 (from Foissner, 2013). (**G**) *Glaucoma reniformis* Schewiakoff, 1892 (from Foissner 2013). (**H**–**K**) *Glaucomides bromelicola* Foissner, 2013 (from Foissner 2013). (**L**–**O**) *Bromeliophrya quadristicha* Foissner and Stoeck 2013 (from Foissner and Stoeck 2013). (**P**–**R**) *Tetrahymena australis* (from Liu et al. 2016a,b). M1–3, membranelles 1, 2 and 3; PK, postoral kineties; PM, paroral membrane; x, X-body. Bars, 20 μm.

perhaps is that barren PM is not a stable feature which is also absent in *Glaucomides* spp. (bromeliophryid ciliates) (Foissner 2013). Based on the above reasons, *Anteglaucoma* should be assigned to the family Glaucomidae but not Bromeliophryidae (Table 1 and Fig. 2).

This placement is also supported by the molecular data (Fig. 3). The topologies of the SSU rDNA trees constructed using Bayesian inference and maximum likelihood analyses were almost identical, therefore, only the maximum likelihood tree is shown in Fig. 3. As the phylogenetic tree shows, within the family *Glaucomidae*, three *Bromeliophrya* species and two *Glaucomides* species form a highly supported clade (94% ML, 0.99 BI), which then clusters with *Anteglaucoma harbinensis* with moderate supports (65% ML, 0.90 BI). The two *Glaucoma* species occupied basal positions within the Glaucomidae clade. The phylogenetic analysis indicates that: (1) *Anteglaucoma* clusters in the Glaucomidae clade, which reinforces the assignment of *Anteglaucoma* into the family Glaucomidae; (2) with one more genus added, the family Glaucomidae is still monophyletic, which is also supported by the results of AU test ($p = 0.856$).

Except for the completely barren PM, *Anteglaucoma* differs from the related genera *Glaucoma* and *Glaucomides* by the arrangement of adoral membranelles (three long membranelles arranged almost longitudinally in parallel in *Anteglaucoma* vs. three membranelles distinctly concave) and presence of micronucleus (absence in *Anteglaucoma* vs. present in *Glaucomides)* (Foissner 2013; Fig. 4A-K).

Anteglaucoma can be separated from *Bromeliophrya* by the following features: (1) lack the ability to produce macrostomes; (2) occur in ordinary farmland pond (vs.

inhabit neotropic phytotelmata); (3) the absence of a kinety fragment left of the oral opening; (4) holotrichous (vs. a large, nonciliated area dorsolaterally); (5) right side ciliary rows normally presented (vs. some right side ciliary rows rectangularly curved to abut on dorsal side rows preorally in *Bromeliophrya*); (6) oral apparatus in the anterior one-third of cell (vs. in second quarter in *Bromeliophrya*); (7) M1 five or six rowed, long and almost longitudinally parallel with M2 and M3 (vs. M1 three rowed, short and concave, forms ring-like pattern with the large, C-shaped M3 in *Bromeliophrya*) (Fig. 4A-D, L-O; Foissner 2003b, Foissner and Stoeck 2013).

Anteglaucoma harbinensis spec. nov. has a very distinct identity due to the structure of the buccal apparatus, the distinctive locomotion by crawling with a rather hectic jerking motion and a special silverline pattern (primary silverline meridians with two type transversely arranged intermeridional cross-fibers: one stick-shaped and the other having a shape like letter "Y" rotated 90 degrees clockwise):. This combination of features clearly separates it from all known hymenostomatids at the species level (Fig. 4; Corliss 1954, 1959; Foissner 2003b; Foissner and Stoeck 2013; Liu et al. 2016a,b).

TAXONOMIC SUMMARY

Subclass Hymenostomatia Delage & Hérouard, 1896
Order Tetrahymenida Fauré-Fremiet in Corliss, 1956
Family Glaucomidae Corliss 1971

Improved diagnosis. Size, small to medium; shape, ovoid to ellipsoid; somatic ciliation, holotrichous; oral cavity, relatively large; with either or both oral polykinetids 2 and 3 having > 3 kinetosomal rows; a small group of kinetosomes (X group) typically anterior to the enlarged oral polykinetid 2; macronucleus, globular to ellipsoid; silverline pattern Glaucomid or Tetrahymenid; Macrostomes usually absent; ordinary freshwater and occasionally terrestrial habitats.

Type genus. *Glaucoma* Ehrenberg, 1830

Taxa assignable. Presently, the family comprises *Glaucoma* Ehrenberg, 1830, *Glaucomella* Groliere, 1977, *Anteglaucoma* gen. nov., *Chasmatostoma* Engelmann, 1862, *Dapedophrya* Foissner, 1995, *Dichilum* Schewiakoff, 1893, *Epenardia* Corliss 1971, *Espejoia* Burger, 1908, *Jaocorlissia* Small & Lynn, 1985, *Monochilum* Schewiakoff, 1893 and *Physalophrya* Kahl, 1931.

Remarks. With the addition of *Anteglaucoma* (present work) and the deletion of *Glaucomides* and *Bromeliophrya* [assigned to Bromeliophryidae according to Foissner (2013)], improved diagnosis is provided herein. Unfortunately, the Glaucomidae clade consists of only two *Glaucoma* and one *Anteglaucoma* species, although the family contains about 11 genera and many species not yet sequenced (Foissner 2013, Lynn 2008).

Genus *Anteglaucoma* gen. nov.

Diagnosis. Small to medium-sized Glaucomidae with a suture in anterior part of body; oral apparatus in anterior one-third of cell; paroral membrane (PM) composed of almost longitudinally arranged dikinetids; three adoral membranelles of nearly the same length, arranged almost longitudinally in parallel; PM unciliated; silverline pattern tetrahymenid.

Type species. *Anteglaucoma harbinensis* spec. nov.

Etymology. The name "*Anteglaucoma*" refers to its oral apparatus being different from that of the genus *Glaucoma.* Feminine gender.

Anteglaucoma harbinensis spec. nov.

Diagnosis. Size in vivo about 65 × 30 μm, oval body with slightly pointed anterior and posterior ends; dorsoventrally flattened about 3:1 to 4:1; buccal field approximately 15% of body length; extrusomes present; 32 to 35 somatic kineties; four or five postoral kineties; adoral membranelle 1 (M1) and membranelle 2 (M2) having five or six kinetosomal rows, membranelle 3 (M3) having three kinetosomal rows; single macronuclear nodule; contractile vacuole on average 15% from posterior body end; locomotion by crawling with a rather hectic jerking motion; freshwater habitat.

Type location. A freshwater pond at Harbin (44°87′14.7″ N; 127°09′12.0″E), northeastern China.

Type specimens. The holotype slide with protargol-impregnated specimens is deposited in the Natural History Museum, London, UK with registration NHMUK 2016.10.26.1.

Etymology. The species-group name *harbinensis* indicates that this species was isolated from a sampling site in Harbin.

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LITERATURE CITED

Borden, D., Miller, E. T., Whitt, G. S. & Nanney, D. L. 1977. Electrophoretic analysis of evolutionary relationships in *Tetrahymena*. *Evolution*, 31:91–102.

Chantangsi, C., Lynn, D. H., Brandl, M. T., Cole, J. C., Hetrick, N. & Ikonomi, P. 2007. Barcoding ciliates: a comprehensive study of 75 isolates of the genus *Tetrahymena*. *Int. J. Syst. Evol. Microbiol.*, 57:2412–2423.

Chen, X., Gao, S., Liu, Y., Wang, Y. Y., Wang, Y. R. & Song, W. 2016. Enzymatic and chemical mapping of nucleosome distribution in purified micro- and macronuclei of the ciliated model organism, *Tetrahymena thermophila*. *Sci. Chin. Life Sci.*, 59:909–919.

Chung, P. & Yao, M. 2012. *Tetrahymena thermophila* JMJD3 homolog regulates H3K27 methylation and nuclear differentiation. *Eukaryot. Cell*, 11:601–614.

Corliss, J. O. 1952. Comparative studies on holotrichous ciliates in the *Colpidium-Glaucoma-Leucophrys-Tetrahymena* group. I. General considerations and history of strains in pure culture. *Trans. Am. Microsc. Soc.*, 71:159–184.

Corliss, J. O. 1954. Review of the genus *Glaucoma* Ehrenberg, 1830 (family Tetrahymenidae). *J. Protozool.*, 1(Suppl.):1.

Corliss, J. O. 1959. *Glaucoma chattoni* n. sp., an experimental organism often confused with the type species of the genus, *G. scintillans* Ehrbg., 1830. *J. Protozool.*, 6:24.

Corliss, J. O. 1971. Establishment of a new family (Glaucomidae n. fam.) in the holotrich hymenostome ciliate suborder Tetrahymenina, and description of a new genus (*Epenardia* n. g.) and a new species (*Glaucoma dragescui* n. sp.) contained therein. *Trans. Am. Microsc. Soc.*, 90:344–362.

Foissner, W. 1979. Methyl green-pyronin: its aptitude for supravital staining of protozoa, especially of their mucocysts (author's transl). *Mikroskopie*, 35:108–115.

Foissner, W. 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Eur. J. Protistol.*, 27:313–330.

Foissner, W. 2003a. Morphology and ontogenesis of *Bromeliophrya brasiliensis* gen. n., sp. n., a new ciliate (Protozoa: Ciliophora) from Brazilian tank bromeliads (Bromeliaceae). *Acta Protozool.*, 42:55–70.

Foissner, W. 2003b. Morphology and ontogenesis of Lambornella trichoglossa nov. spec., a new tetrahymenid ciliate (Protozoa, Ciliophora) from Brasilian tank bromeliads (Bromeliaceae). *Eur. J. Protistol.*, 39:63–82.

Foissner, W. 2013. Description of *Glaucomides bromelicola* n. gen., n. spec. (Ciliophora, Tetrahymenida), a macrostome forming inhabitant of bromeliads (Bromeliaceae), including redescriptions of *Glaucoma scintillans* and *G. reniformis*. *J. Eukaryot. Microbiol.*, 60:137–157.

Foissner, W., Berger, H. & Kohmann, F. 1994. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. – Band III: Hymenostomata, Prostomatida, Nassulida. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Heft 1/94. p. 1–548.

Foissner, W. & Stoeck, T. 2013. Morphology of *Bromeliophrya quadristicha* n. spec., an inhabitant of tank bromeliads (Bromeliaceae), and phylogeny of the Bromeliophryidae (Ciliophora, Tetrahymenida). *J. Eukaryot. Microbiol.*, 60:223–234.

Frankel, J. 1960. Morphogenesis in *Glaucoma chattoni*. *J. Protozool.*, 7:362–376.

Fried, J. & Foissner, W. 2007. Differentiation of two very similar glaucomid ciliate morphospecies (Ciliophora, Tetrahymenida) by fluorescence in situ hybridization with 18S rRNA targeted oligonucleotide probes. *J. Eukaryot. Microbiol.*, 54:381–387.

Gao, F., Warren, A., Zhang, Q., Gong, J., Miao, M., Sun, P., Xu, D., Huang, J., Yi, Z. & Song, W. 2016. The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). *Sci. Rep.*, 6:24874.

Gao, S., Xiong, J., Zhang, C., Berquist, B. R., Yang, R., Zhao, M., Molascon, A. J., Kwiatkowski, S. Y., Yuan, D., Qin, Z., Wen, J., Kapler, G. M., Andrews, P. C., Miao, W. & Liu, Y. 2013. Impaired replication elongation in *Tetrahymena* mutants deficient in histone H3 Lys 27 monomethylation. *Genes Dev.*, 27:1662–1679.

Gu, F. & Ni, B. 1993. The exploration of preparing protozoan specimens. *J. Electron Microsc.*, 12:525–529.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.*, 41:95–98.

Jankowski, A. W. 2007. Phylum Ciliophora Doflein, 1901. In: Alimov, A. F. (ed.), Protista Part 2. Nauka, St. Petersburg. p. 415–993.

Kher, C. P., Doerder, F. P., Cooper, J., Ikonomi, P., Achilles-Day, U., Küpper, F. C. & Lynn, D. H. 2011. Barcoding *Tetrahymena*: discriminating species and identifying unknowns using the cytochrome coxidase subunit I (cox-1) barcode. *Protist*, 162:2–13.

Klug, S. H. 1968. Cortical studies on *Glaucoma*. *J. Protozool.*, 15:321–327.

Liu, M., Fan, X., Gao, F., Gao, S., Yu, Y., Warren, A. & Huang, J. 2016a. *Tetrahymena australis* (Protozoa, Ciliophora): a well-known but "Non-Existing" taxon - consideration of its identification, definition and systematic position. *J. Eukaryot. Microbiol.*, 63:760–770.

Liu, M., Gao, F., Al-Farraj, S. A. & Hu, X. 2016b. Morphology and small subunit rRNA gene sequence of *Uronemita parabinucleata* n. sp. (Ciliophora, Uronematidae), with an improved generic diagnosis and the key to species of Uronemita. *Eur. J. Protistol.*, 54:1–10.

Liu, Y., Taverna, S. D., Muratore, T. L., Shabanowitz, J., Hunt, D. F. & Allis, C. D. 2007. RNAi-dependent H3K27 methylation is required for heterochromatin formation and DNA elimination in *Tetrahymena*. *Genes Dev.*, 21:1530–1545.

Lynn, D. H. 2008. The ciliated protozoa: characterization, classification and guide to the literature. 3rd ed. Springer, Dordrecht. p. 1–605.

Ma, H., Choi, J. K. & Song, W. 2003. An improved silver carbonate impregnation for marine ciliated protozoa. *Acta Protozool.*, 42:161–164.

McCoy, J. W. 1975. Updating the tetrahymenids IV. Cortical properties of Glaucoma. *Protistologica*, 11:149–158.

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71:491–499.

Mochizuki, K., Fine, N. A., Fujisawa, T. & Gorovsky, M. A. 2002. Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in *Tetrahymena*. *Cell*, 110:689–699.

Nylander, J. A. A. 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Uppsala.

Page, R. D. M. 1996. TREEVIEW: an application to view phylogenetic trees on personal computers. *Comput. Appl. Biosci.*, 12:357–358.

Pan, X., Bourland, W. & Song, W. 2013. Protargol synthesis: an in-house protocol. *J. Eukaryot. Microbiol.*, 60:609–614.

Pan, H., Hu, J., Jiang, J., Wang, L. & Hu, X. 2016. Morphology and phylogeny of three *Pleuronema* species (Ciliophora, Scuticociliatia) from Hangzhou Bay, China, with description of two new species, *P. binucleatum* n. sp. and *P. parawiackowskii* n. sp. *J. Eukaryot. Microbiol.*, 63:287–298.

Pan, X., Huang, J., Fan, X., Ma, H., Al-Rasheid, K. A. S., Miao, M. & Gao, F. 2015a. Morphology and phylogeny of four marine scuticociliates (Protista, Ciliophora), with descriptions of two new species: *Pleuronema elegans* spec. nov. and *Uronema orientalis* spec. nov. *Acta Protozool.*, 54:31–43.

Pan, X., Yi, Z., Li, J., Ma, H., Al-Farraj, S. A. & Al-Rasheid, K. A. S. 2015b. Biodiversity of marine scuticociliates (Protozoa, Ciliophora) from China: description of nine morphotypes including a new species, *Philaster sinensis* spec. nov. *Eur. J. Protistol.*, 51:142–157.

Peck, R. K. 1978. Ultrastructure of the somatic and buccal cortex of the tetrahymenine hymenostome *Glaucoma chattoni*. *J. Protozool.*, 25:186–198.

Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14:817–818.

Quintela-Alonso, P., Nitsche, F., Wylezich, C., Arndt, H. & Foissner, W. 2013. A new *Tetrahymena* (Ciliophora, Oligohymenophorea) from groundwater of Cape Town, South Africa. *J. Eukaryot. Microbiol.*, 60:235–246.

Ronquist, F. & Huelsenbeck, J. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19:1572–1574.
Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.*, 51:492–508.
Shimodaira, H. & Hasegawa, M. 2001. Consel: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, 17:1246–1247.
Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22:2688–2690.
Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.*, 57:758–771.
Swofford, D. L. 2002. Phylogenetic analysis using parsimony (*and othermethods). Version 4, Sunderland, MA.
Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software Ver. 4.0. *Mol. Biol. Evol.*, 24:1596–1599.
Wilbert, N. 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. *Mikrokosmos*, 64:171–179.
Zhao, Y., Yi, Z., Gentekaki, E., Zhan, A., Al-Farraj, S. A. & Song, W. 2016. Utility of combining morphological characters, nuclear and mitochondrial genes: an attempt to resolve the conflicts of species identification for ciliated protists. *Mol. Phylogenet. Evol.*, 94:718–729.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Sampling map and sites. (**A**–**C**) Three photographs showing the same farmland pond in Harbin, Heilongjiang province, northeastern China (44°87′14.7″N; 127°09′12.0″E).