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European Journal of Protistology 54 (2016) 1–10

European Journal of PROTISTOLOGY

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Morphology and small subunit rRNA gene sequence of *Uronemita parabinucleata* n. sp. (Ciliophora, Uronematidae), with an improved generic diagnosis

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Received 10 December 2015; received in revised form 15 February 2016; accepted 18 February 2016
Available online 27 February 2016

Abstract

The morphology and infraciliature of a new species, *Uronemita parabinucleata* n. sp., isolated from intertidal sediments in a coastal region in northern China, were investigated using live observation and silver impregnation methods. The new species is characterized by an in vivo body size of about 20–50 × 10–25 μm, 22 or 23 somatic kineties, two macronuclear nodules, and one caudal cilium. Its small subunit ribosomal RNA gene (SSU rDNA) was sequenced and compared with those of other *Uronemita* species to reveal nucleotide differences. Phylogenetic analyses indicated that *Uronemita* is monophyletic and that the new species clusters with its congener *Uronemita filificum*, with full support provided by both Bayesian inference and maximum likelihood algorithms. Based on previous studies and the present study, an improved diagnosis of the genus *Uronemita* is supplied, which has been absent since the establishment of this genus. A key to the *Uronemita* species is also provided.

Keywords: Infraciliature; Phylogeny; Scuticociliates; SSU rDNA; *Uronemita parabinucleata* n. sp.

Introduction

Ciliates in the subclass Scuticociliatia Small, 1967 are usually very small (15–50 μm), distributed in various aquatic environments worldwide (Lynn 2008; Song et al. 2009), and exhibit great biological and morphological diversity (Foissner 1995; Foissner and Wilbert 1981; Foissner et al. 1982, 2009, 2014; Jankowski 2007; Lynn and Small 2002; Pan et al. 2011, 2015; Seo et al. 2013; Song and Wilbert 2002). Recent studies have demonstrated that the diversity of this group has not been well documented (de Castro et al. 2014; Foissner et al. 2014; Pan et al. 2015; Xu et al. 2015), which highlights the need to conduct further studies on this group.

In recent years, molecular phylogenetic analyses based on small subunit ribosomal RNA gene (SSU rDNA) sequences have increasingly been used to investigate the evolutionary relationships within Scuticociliatia (Gao and Katz 2014; Gao et al. 2012a,b, 2013, 2014).

In the present work, a novel species of the reactivated genus *Uronemita* Jankowski, 1980 (junior synonym *Uronemella* Song and Wilbert, 2002) is documented based on live observation and silver staining preparations, and

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http://dx.doi.org/10.1016/j.ejop.2016.02.005
0932-4739/

its SSU rDNA sequence was characterized and analyzed to determine the phylogenetic position of the new species within *Uronemita*. An improved diagnosis of *Uronemita* based on available data and an updated key to the species are provided.

Material and Methods

Sampling and cultivation

Samples were collected from the surface of sandy littoral sediments of the No. 1 Bathing Beach in Qingdao, China (36°03′18″ N; 120°20′22″ E), on 3 August 2015, when the water temperature was about 24 °C and the salinity was 28‰.

The sampling method was mainly that of Yan et al. (2015). At a slightly polluted location near a sewage outfall on the beach, 5 cm-deep holes were dug in the sand, into which seawater gradually seeped. Sandy sediments and seawater were collected from the bottoms of the holes. The specimens were isolated and maintained in non-clonal cultures in Petri dishes containing filtered seawater at room temperature (ca. 22 °C), with artificial fish food granules or rice grains to enrich the bacterial food (Qu et al. 2015).

Morphology

Living cells were isolated and observed using bright-field and differential interference contrast microscopy at 100–1000× magnification. The protargol staining method described by Wilbert (1975) and Chatton-Lwoff wet silver nitrate (Foissner 2014) were used to reveal the infraciliature, nuclear apparatus, and silverline system. Counts and measurements of the stained specimens were performed at a magnification of 1000×. Drawings of living cells were produced using free-hand sketches and photomicrographs, and drawings of silver-stained specimens were produced with the help of a camera lucida (Luo et al. 2014). The terminology used is mainly that of Jankowski (2007) and Song and Wilbert (2002).

DNA extraction, PCR amplification, and sequencing

Genomic DNA extraction, PCR amplification, and sequencing of the SSU rDNA from *Uronemita parabinucleata* n. sp. were carried out according to the methods of Huang et al. (2014). Genomic DNA was extracted from cleaned cells using a DNeasy Tissue Kit (Qiagen, CA, USA). Primers 18s-F (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and 18s-R (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin et al. 1988) were used for SSU rDNA amplification. To minimize the possibility of PCR amplification errors, Q5® Hot Start High-Fidelity DNA Polymerase (New England BioLabs, USA) was used. Purified PCR products in the appropriate size were inserted into the pEASY-T1 vector (TransGen Biotech, Beijing, China) and transformed into competent cells. Sequencing was performed bidirectionally on an ABI 3700 sequencer (GENEWIZ Biotechnology Co., Ltd., Beijing, China).

Phylogenetic analyses

The SSU rDNA sequences of *Uronemita parabinucleata* n. sp. and 59 other ciliates obtained from the NCBI GenBank database were used for the phylogenetic analyses. Ten pleuronematid species were treated as the outgroup taxa. The sequences were aligned with MUSCLE v3.7 (Edgar 2004), using the default parameters of the web server (http://www.phylogeny.fr/version2_cgi) (Dereeper et al. 2008, 2010). The resulting alignments were refined manually by trimming both ends, using the BioEdit 7.0.5.2 program (Hall 1999). The final alignment included 1744 characters and 60 taxa (available from the authors upon request).

Bayesian inference (BI) analysis was performed with MrBayes v.3.2.6 on XSEDE (Ronquist and Huelsenbeck 2003) via the CIPRES Science Gateway (CIPRES portal: http://www.phylo.org/sub_sections/portal) using the GTR + I + G evolutionary model as the best-fit model, which was selected by MrModeltest v.2 (Nylander 2004), according to the Akaike Information Criterion. Markov chain Monte Carlo simulations were run with two sets of four chains for 4,000,000 generations at a sampling frequency of 100 and a burn-in of 10,000 trees (25%). All the remaining trees were used to calculate the posterior probability using a 50% majority rule consensus. Maximum likelihood (ML) analyses were conducted online on the CIPRES Science Gateway with RAxML-HPC2 on XSEDE (8.2.3) (Stamatakis 2014), using the GTR + I + G evolutionary model as the best model according to the Akaike information criterion selected by the Modeltest v.3.4 program (Posada and Crandall 1998). Node support came from 1000 bootstrap replicates. TreeView v.1.6.6 (Page 1996) and MEGA 4.0 (Tamura et al. 2007) were used to visualize the tree topologies. Systematic classification was conducted primarily in accordance with Lynn (2008).

Comparison of the SSU rDNA sequences within the genus *Uronemita*

The SSU rDNA sequence of *Uronemita parabinucleata* n. sp., along with the sequences of three congeners obtained from the GenBank database, was aligned using BioEdit 7.0.5.2 (Hall 1999). After deleting both ends of the alignment, the numbers of unmatched sites and sequence similarities were calculated. The alignment was then modified manually by removing identical nucleotides with BioEdit 7.0.5.2 (Hall 1999), resulting in a nucleotide matrix.

Results and Discussion

Reactivation of *Uronemita* Jankowski (1980)

Remarks. Jankowski (1980) established a new genus, *Uronemita*, with *Uronema filificum* Kahl, 1931 as its type species. He provided a short statement for the new genus: "similar to *Uronema*, without argyrome." Song and Wilbert (2002) stated that *Uronemita* is invalid according to the International Commission on Zoological Nomenclature ICZN (1999), as no clear definition or detailed description was provided for it. They suggested the establishment of a new genus, *Uronemella*, with a definition, and they designated *Uronema filificum* Kahl, 1931 [which was transferred into the genus *Homalogastra* by Song (1993) and to *Urocyclon* by Small and Lynn (1985)] as its type species, with detailed descriptions. Two additional species were included in the genus: *Uronemella binucleata* (Song, 1993) Song and Wilbert, 2002 (basionym: *Homalogastra binucleata* Song, 1993) and *Uronemella cymruensis* (Pérez-Uz and Hope, 1997) Song and Wilbert, 2002 (basionym: *Urocyclon cymruensis* Pérez-Uz and Hope, 1997). According to the definition of *Uronemella*, two new species—*Uronemella parafilificum* and *U. sinensis*—were added (Gong et al. 2007; Pan et al. 2013) and the generic diagnosis was amended (Pan et al. 2013).

However, the genus *Uronemita* established by Jankowski (1980) is nomenclaturally valid according to ICZN 1964 (article 13, a.i.; this edition was essential for Jankowski 1980), as a type species was designated and a definition was provided (although the defintion is incorrect according to later data). Thus, the statement by Song and Wilbert (2002) that *Uronemita* is invalid is obviously incorrect. Jankowski (2007) also commented on this problem, and synonymized *Uronemella* Song and Wilbert, 2002 with *Uronemita* Jankowski, 1980. He provided a description of the type species, *Uronemita filificum* (Kahl, 1931) Jankowski, 1980, but without redefining the genus. We agree with Jankowski (2007) on the validity of *Uronemita*, and transfer four other species formerly assigned to *Uronemella* into the current genus.

Because the original definition of the genus *Uronemita* is incomplete and incorrect and no amended generic diagnosis has been provided, herein we provide an improved diagnosis of *Uronemita* based on previous and present data. The diagnosis is mainly from that of the synonym *Uronemella* with some emendations.

Improved diagnosis of genus *Uronemita* Jankowski, 1980. Thigmotactic Uronematidae with generally elongate-elliptical or inverted pear-shaped body; apical plate dominant; subequatorially positioned cytostome, about one-half to two-thirds of the total body length; *Uronema*-like oral apparatus with membranelle 1 consisting of one row of basal bodies and membranelles 2 and 3 consisting of two or more longitudinal rows of basal bodies; paroral membrane commencing anteriorly at about the mid portion of membranelle 2; one typical caudal cilium; somatic kineties comprising a mixture of dikinetids and monokinetids; locomotion in vivo exhibiting typical rotatory movement with help of a caudal-cilium-associated sticky thread; marine habitat.

Type species (by original designation). *Uronemita filificum* (Kahl, 1931) Jankowski, 1980 (basionym: *Uronema filificum* Kahl, 1931).

Species assignable. *Uronemita filificum* (type spiecies); *Uronemita binucleata* (Song, 1993) nov. comb. [Synonyms: *Homalogastra binucleata* Song, 1993; *Uronemella binucleata* (Song, 1993) Song and Wilbert, 2002]; *Uronemita cymruensis* (Pérez-Uz and Hope, 1997) nov. comb. [Synonyms: *Urocyclon cymruensis* Pérez-Uz and Hope, 1997; *Homalogastra cymruensis* (Pérez-Uz and Hope, 1997) Song and Wilbert, 2000; *Uronemella cymruensis* (Pérez-Uz and Hope, 1997) Song and Wilbert, 2002]; *Uronemita parabinucleata* n. sp.; *Uronemita parafilificum* (Gong et al., 2007) nov. comb. (Synonym: *Uronemella parafilificum* Gong et al., 2007) and *Uronemita sinensis* (Pan et al., 2013) nov. comb. (Synonym: *Uronemella sinensis* Pan et al., 2013).

Uronemita parabinucleata n. sp.

Diagnosis. Size in vivo about 20–50 × 10–25 μm. Twenty-two or 23 somatic kineties. Two macronuclear nodules.

Type locality. Marine water from sandy littoral sediments of the No. 1 Bathing Beach in Qingdao (36°03′18″ N; 120°20′22″ E), eastern China.

Deposition of type slides. One protargol preparation (registry no. LMJ2015080301) with holotype specimen (Fig. 2G, H) is deposited in the Laboratory of Protozoology, Ocean University of China. Two paratype slides containing protargol and silver nitrate stained specimens (registry no. NHMUK 2016.1.25.1 and NHMUK 2016.1.25.2, respectively) are deposited in the Natural History Museum, London.

Etymology. Composite of the Greek word *para* (beside) and the species-group name *binucleata*, indicating that this species is similar to *Uronemita binucleata* in having two macronuclear nodules.

Description (table* 1 and Figs 1A–G, 2A–L). Body size approximately 25–50 × 10–25 μm in vivo, with a length: width ratio of about 2:1. Inverted pear-shaped or elongated elliptical outline. Anterior end flat and slightly truncated, with a prominent apical plate covering about one-half to two-thirds of body width, and slightly narrow posterior part (Figs 1A, B, 2A–C). Ventral side slightly concave and dorsal side conspicuously convex (Fig. 2D). Buccal field about three-fifths of body length, with cytostome positioned slightly posterior to equatorial level (Figs 1A, B, 2A–C). Pellicle thin and conspicuously notched, with grooves located longitudinally along ciliary rows (Figs 1A, 2A, C, F). No extrusomes detected in vivo. Cytoplasm colorless to slightly grayish, containing several refractile globules and bar-like crystals distributed at in anterior and posterior portions (Figs 1A, 2A–D). A single contractile vacuole, positioned

Table 1. Morphometric data of *Uronemita parabinucleata* n. sp. from life and after protargol impregnation.

Characteristic[a]	Max	Min	Mean	Median	SD	CV	*n*
Body length (living cells)	40	30	35.00	35	3.34	9.54	15
Body width (living cells)	20	15	17.20	18	1.90	11.03	15
Body length (protargol-stained cells)	42	30	35.04	35	3.86	11.01	25
Body width (protargol-stained cells)	30	20	23.68	25	3.20	13.50	25
Width of apical plate	20	10	13.08	13	2.06	15.75	25
Length of paroral membrane	10	8	9.20	9	0.76	8.30	25
Number of somatic kineties	23	22	22.96	23	0.20	0.87	25
Number of kinetids in SK_1	27	19	22.20	22	1.66	7.47	25
Number of dikinetids in SK_1	20	9	14.00	14	2.84	20.31	25
Number of kinetids in SK_{mid}	17	11	14.28	14	1.40	9.80	25
Number of dikinetids in SK_{mid}	13	4	8.12	8	2.49	30.65	25
Number of macronuclear nodules	2	2	2.00	2	0	0	>100
Diameter of single macronuclear nodule	14	8	10.52	10	1.50	14.29	25

[a]Measurements in μm. CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of individuals examined; SD, standard deviation; SK_1, the kinety on right of buccal field; SK_{mid}, the middle kinety on dorsal side.

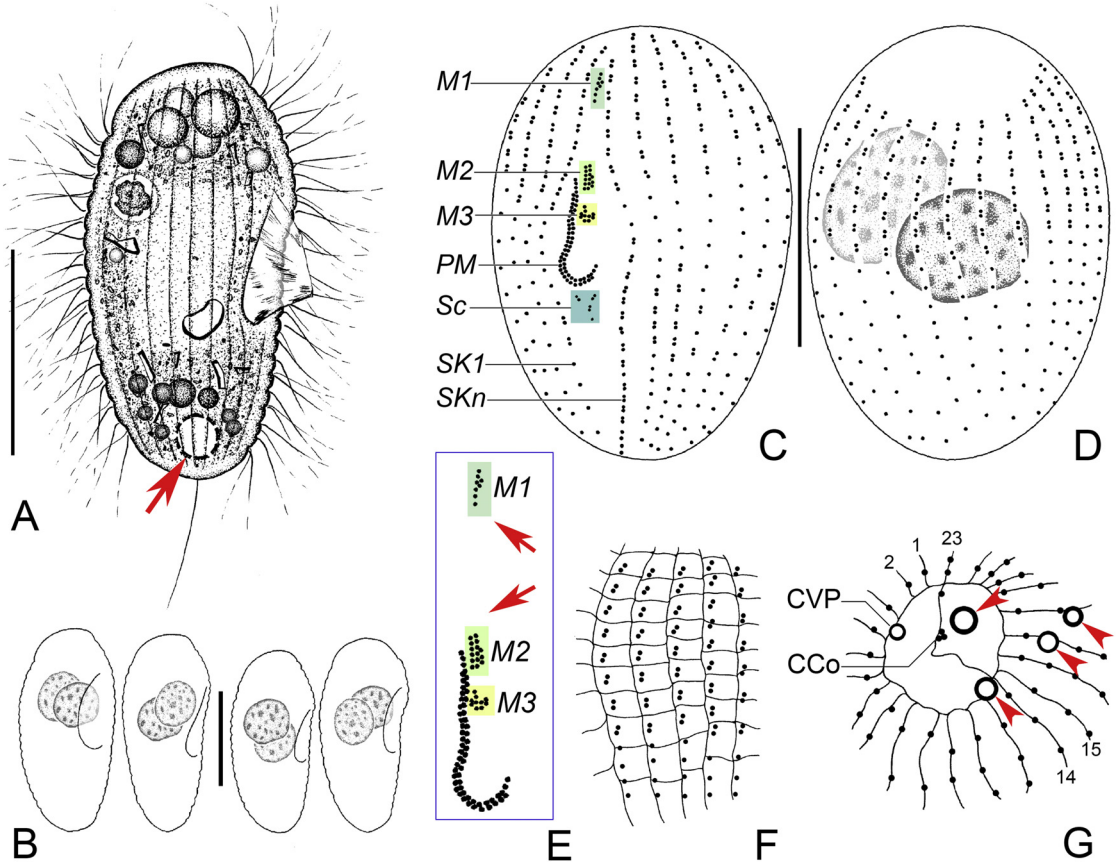

Fig. 1. (A–G) *Uronemita parabinucleata* n. sp. in vivo (A, B), after protargol (C–E) and silver nitrate (F, G) impregnation. (A) Right ventrolateral view of a representative individual, arrow shows contractile vacuole. (B) Right ventrolateral views of different body shapes. (C, D) Ventral (C) and dorsal (D) view of holotype specimen to show the infraciliature and nuclear apparatus. (E) Detail of buccal field, arrows indicate the gap between membranelles 1 and 2. (F) Portion of silverline system. (G) Caudal view, showing the caudal complex. Arrowheads very likely point to the vacuoles after the ejection of extrusomes, and this kind of vacuoles are usually distributed over silver nitrate stained cells. CCo, caudal complex; CVP, contractile vacuole pore; M1–3, membranelle 1–3; PM, paroral membrane; Sc, scutica; SK1, the somatic kinety right of buccal field; SKn, the somatic kinety left of buccal field. Scale bars = 15 μm.

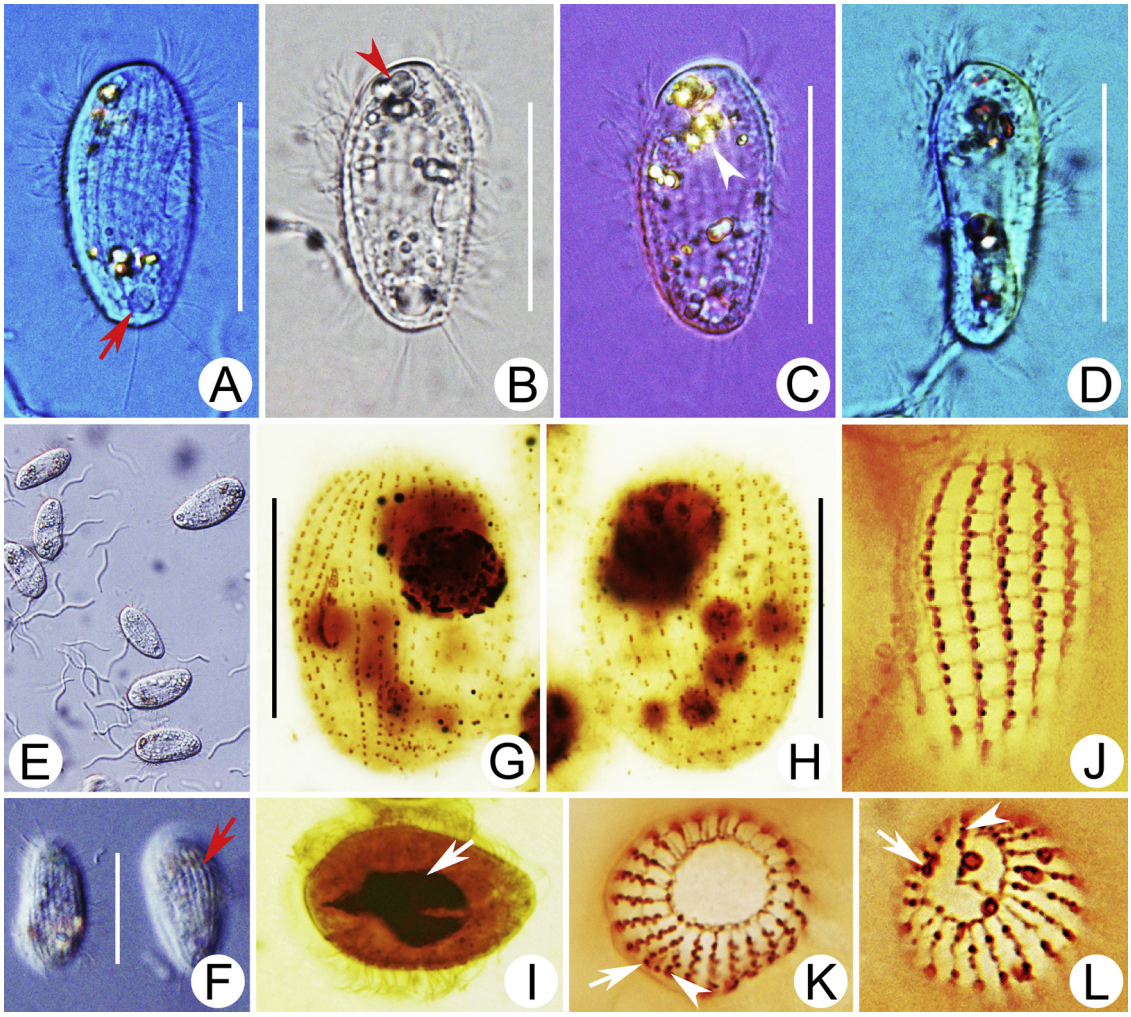

Fig. 2. (A–L) Photomicrographs of *Uronemita parabinucleata* n. sp. from life (A–F, with B in bright field illumination and others in DIC microscopy), after protargol (G–I) and silver nitrate (J–L) staining. (A–C) Right ventrolateral views of representative individuals, arrow in A shows contractile vacuole, arrowheads in B and C indicate the refractile globules and bar-like crystals. (D) Lateral view of another cell. (E) Different body shapes and sizes. (F) Arrow shows the longitudinal grooves along the kineties. (G, H) Left ventrolateral (G) and right dorsolateral (H) view of holotype specimen to show the infraciliature and nuclear apparatus. (I) View of a specimen in early stage of cell division, indicating the two macronuclear nodules do not fuse during division. Arrow points to one of the elongated macronuclear nodule. (J) Dorsal view, showing the silverline system. (K) Apical view, revealing the large apical plate. Arrow indicates somatic kinety 1, arrowhead denotes somatic kinety *n* (*n* = 23 in present case). (L) Caudal view, to demonstrate the caudal complex. Arrowhead shows the somatic kinety *n* and arrow points to the contractile vacuole pore. Scale bars = 30 μm.

caudally, up to 6 μm in diameter when fully expanded, pulsating at intervals of 25–35 s (Figs 1A, 2A–C).

Cilia approximately 6–8 μm long, densely arranged; a single caudal cilium about 12–17 μm in length (Figs 1A, 2A–C). Locomotion by 'rotatory movement': with help of a thread-like structure deriving from the caudal cilium, cells become attached temporarily to a substrate and rotate continuously; for more details, see Song and Wilbert (2002).

Ciliature as shown in Figs 1C, D, 2G, H. Most cells contain 23 (22 in only one of the 25 cells examined) almost bipolar somatic kineties. Each kinety composed of closely arranged dikinetids in anterior part and loosely arranged monokinetids posteriorly (Figs 1C, D, 2G, H), except for the SKn (kinety on left of buccal field), which located slightly posteriorly and consisting almost entirely of dikinetids (Figs 1C, F, 2G, L). The SK1 (kinety on right of buccal field) composed of 19–27 kinetids, of which 9–19 dikinetids. Caudal complex consisting of about three argentophilic granules (Fig. 1G). Invariably, two spherical to oval macronuclear nodules adjacent to each other, located anteriorly or centrally, each about 10 μm in diameter; they do not fuse during division (Figs 1D, 2G–I).

Oral apparatus typical of genus (Fig. 1E). Membranelle 1 located one-fifth of the body length from anterior end, composed of about six basal bodies arranged in one longitudinal row, with two additional basal bodies positioned closely, left of membranelle 1 (Figs 1C, E, 2G). Membranelle 2 slightly shorter than membranelle 1, comprising of three rows of

kinetids, and located approximately one-third of body length from anterior end (Figs 1C, E, 2G). Membranelle 3 composed of about 10 basal bodies, forming a small patch behind membranelle 2 (Fig. 1E). Paroral membrane right of buccal cavity, with basal bodies arranged in a zig-zag pattern, anterior end commenced at middle portion of membranelle 2, occupying one-fifth of body length (Figs 1C, 2G). Scutica consisting of three pairs of basal bodies, with an additional basal body positioned posteriorly (Figs 1C, 2G).

Silverline system as shown in Figs 1F, G, 2J–L. Silverline basically located in each somatic kinety row, with short transverse lines connecting two neighboring longitudinal lines (Figs 1F, 2J). At anterior end of cell, silverline forming a closed circle around the apical plate (Fig. 2K). Pore of contractile vacuole at posterior ends of somatic kineties 3 and 4. Silverline at somatic kinety *n* extending posteriorly across caudal complex, connecting with silverline at about somatic kinety 14 or 15 on dorsal side (Figs 1G, 2L).

Molecular data and phylogenetic analyses (Table 2 and Figs 3 and 4)

The SSU rDNA sequence of *Uronemita parabinucleata* n. sp. was deposited in GenBank with accession number KU199245. The length and GC content of the sequence (not including primers) were 1711 bp and 42.78%, respectively.

Phylogenetic trees based on the SSU rDNA sequences were constructed using both BI and ML algorithms. The topologies of the BI and ML trees are generally concordant, and the topology of ML analysis is presented, along with support values from both algorithms (Fig. 3). As shown in the phylogenetic trees, 10 pleuronematids (see Fig. 3 for accession numbers) are selected as an outgroup, and all available genera and families within the order Philasterida are included. The genus *Uronemita* is monophyletic with full support, and is sister to the clade comprising *Uronema*, *Parauronema*, and

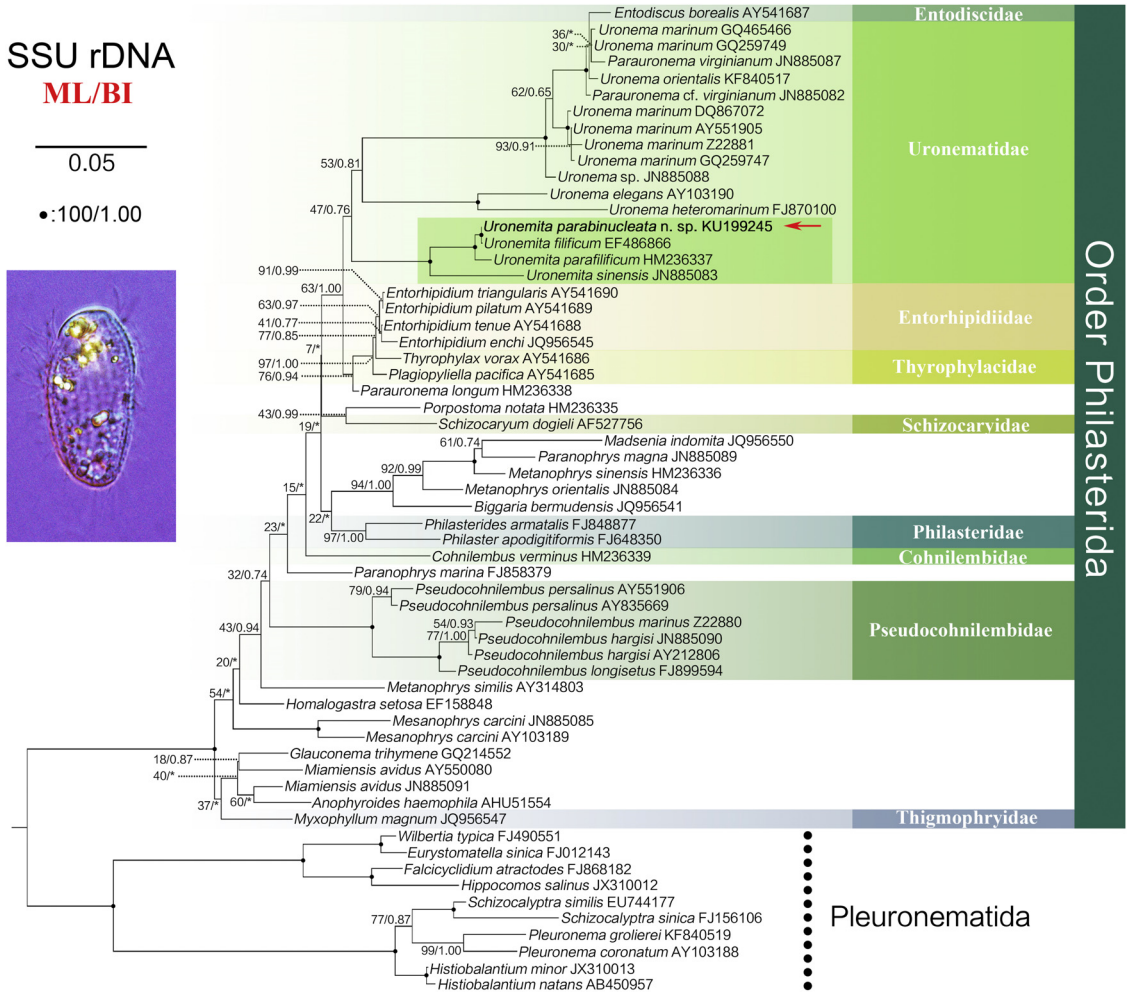

Fig. 3. Maximum likelihood (ML) tree inferred from SSU rDNA sequences, showing the position of *Uronemita parabinucleata* n. sp. Numbers near branches denote ML bootstrap value/BI posterior probability. Asterisk (*) indicates topologies that differ between the ML and BI analyses. Fully supported (100%/1.00) branches are marked with solid circles. The scale bar corresponds to 5 substitutions per 100 nucleotide positions. All branches are drawn to scale. Systematic classification mainly follows Lynn (2008).

Table 2. Sequence comparisons of the small subunit ribosomal DNA in the genus *Uronemita*, determined by BioEdit 7.0.5.2 (Hall 1999).

SSU rDNA sequence[a]	1	2	3	4
1 *U. parabinucleata*	ID	99.9	99.1	95.3
2 *U. filificum*	1	ID	99.0	95.3
3 *U. parafilificum*	14	15	ID	95.0
4 *U. sinensis*	76	77	82	ID

[a] Values below the diagonal are numbers of unmatched sites; those above the diagonal are sequence similarity percentages (%). GenBank accession numbers are given in Fig. 3.

Entodiscus (53% ML, 081 BI). Within *Uronemita*, *U. parabinucleata* n. sp. clusters with *U. filificum*, followed by *U. parafilificum* and *U. sinensis*.

The results of the sequence comparison are shown in Fig. 4 and Table 2. *Uronemita parabinucleata* KU199245 differs from *U. filificum* in one nucleotide, and from *U. parafilificum* and *U. sinensis* in 14 and 76 nucleotides, respectively.

Morphological comparison of *Uronemita parabinucleata* n. sp. with its congeners (Tables 3 and 4)

Thus far, *Uronemita* comprises five species that should be compared with *Uronemita parabinucleata* n. sp.

Uronemita parabinucleata n. sp. and *U. binucleata* are similar in body size and shape in vivo, as well as number of macronuclear nodules. However, the former can be distinguished from the latter mainly by the number of somatic kineties (22 or 23 vs. 16 or 17), the size of the macronuclear nodules (8–14 μm vs. 4–8 μm in diameter), the length of the caudal cilium (about 12–17 μm vs. 20–25 μm), and the structure of the infraciliature: (1) in *U. parabinucleata* n. sp., the distance between membranelle 1 and membranelle 2 is conspicuously longer than the length of membranelle 1; (2) membranelle 2 in the new species is relatively smaller than that of *U. binucleata* (membranelle 2 occupies 5.4% and 9% of the body length in *U. parabinucleata* and *U. binucleata*, respectively. Data measured from drawings and photomicrographs); (3) in the new species, somatic kinety 1 and somatic kinety *n* contain much more basal bodies; and (4) the basal bodies in the posterior part of somatic kinety *n* in *U. parabinucleata* n. sp. are more densely arranged (Song 1993).

Uronemita parabinucleata n. sp. resembles *U. filificum* in the structure of the buccal field. However, the new species can be separated from *U. filificum* by its different body features (inverted pear-shaped or elongated elliptical; length: width ca. 2:1; pellicle notched with grooves vs. slender D- or kidney-shaped; length: width ca. 3:2; pellicle slightly notched in *U. filificum*) and two macronuclear nodules (vs. one in *U. filificum*) (Pan et al. 2015; Song and Wilbert 2002).

Uronemita cymruensis differs from *U. parabinucleata* n. sp. in cell shape (slender oval vs. inverted pear-shaped or elongated elliptical), number of somatic kineties (24–27 vs.

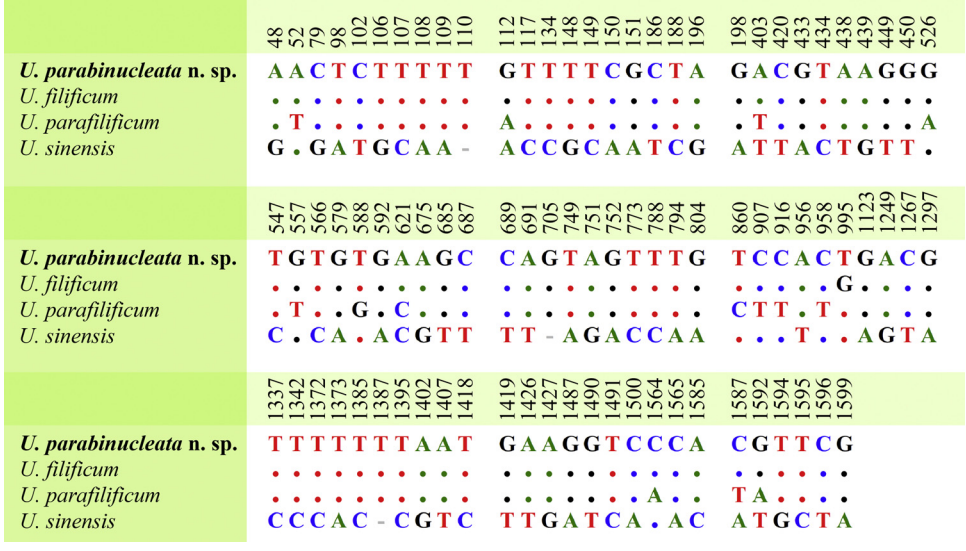

Fig. 4. Sequence comparison of the SSU rDNA showing the unmatched nucleotides between *Uronemita parabinucleata* n. sp. (KU199245) and congeners (for GenBank accession numbers, see Fig. 3). Nucleotide positions are given at the top of each column. Insertions and deletions are compensated by introducing alignment gaps (–). Matched sites are represented by dots (.).

Table 3. Morphological comparison of *Uronemita parabinucleata* n. sp. with its congeners.

Character[a]	*U. parabinucleata* n. sp.	*U. binucleata*	*U. filificum*	*U. parafilificum*	*U. sinensis*	*U. cymruensis*
Cell size in vivo	25–50 × 10–25 μm	25–40 × 15–20 μm	25–45 × 12–30 μm	20–35 × 10–20 μm	25–35 × 15–20 μm	ca. 29 × 16 μm
Size after protargol staining	30–42 × 19–30 μm	N/A	28–43 × 16–28 μm	21–30 × 11–17 μm	34–46 × 20–31 μm	22–37 × 12–23 μm
Body features	Inverted pear-shaped or elongated elliptical in outline; length: width ca. 2:1; pellicle notched with grooves	Inverted pear-shaped; length: width ca. 2:1; pellicle notched	Slender D-, inverted pear- or kidney-shaped; length: width ca. 3:2; pellicle slightly notched	Kidney- or inverted pear-shaped; length: width ca. 3:2; pellicle inconspicuously notched with ridges	Elongate-elliptical in outline; length: width ca. 2:1; pellicle inconspicuously notched without ridges	Inverted pear-shaped, slender oval; length: width ca. 2:1; pellicle slightly notched in some individuals
Caudal cilia	Single, about 12–17 μm	Single, about 20–25 μm	Single, ca. 10–20 μm long	At least four extremely long cilia (including one typical caudal cilium), ca. 18–25 μm	Single, about 15 μm long	Single, length data not provided
SK, No.	23, rarely 22	16 or 17	15–23	16 or 17	9 or 10	24–27
Ma, No.	2	2	1	1	1	1
Diameter of Ma	8–14 μm	4–8 μm	10–14 μm	5–10 μm	10–18 μm	12–20 μm
Data resource	Original	Song (1993)	Pan et al. (2015), Song and Wilbert (2002)	Fan et al. (2011), Gong et al. (2007)	Pan et al. (2013)	Pérez-Uz and Hope (1997), Song and Wilbert (2002)

[a] Ma, macronuclear nodule; N/A, non applicable; SK, somatic kineties.

Table 4. Infraciliature comparison of *Uronemita parabinucleata* n. sp. with congeners.

Character[a]	*U. binucleata*	*U. parabinucleata* n. sp.	*U. filificum*
Distance between M1 and M2	Approximately equals the length of M1	Conspicuously longer than the length of M1	Conspicuously longer than the length of M1
Percentage of M2/body length	ca. 9.0%	ca. 5.4%	ca. 5.5%
Number of basal bodies in SK1	17–20	19–27	18–23
Number of basal bodies in SKn	19–22	ca. 27	23–32
Denseness of posterior part of SKn	Loose-set	Densely arranged	Densely arranged
Commencing position of PM	Commencing anteriorly at the frontal end of M2	Commencing anteriorly at mid portion of M2	Commencing anteriorly at mid portion of M2
Data resource	Song (1993)	Original	Song and Wilbert (2002)

[a] M1 and M2, membranelle 1 and 2; PM, paroral membrane; SK1, the kinety on right of buccal field; SKn, the kinety on left of buccal field; percentage of M2/body length were measured from drawings and photomicrographs.

22 or 23) and number and size of macronuclear nodules (one, diameter 12–20 μm vs. two, each 8–14 μm in diameter) (Pérez-Uz and Hope 1997; Song and Wilbert 2002).

Uronemita parabinucleata n. sp. and *U. parafilificum* are similar in body size and shape. The former can be separated from the latter, however, by some live features (length: width ca. 2:1; pellicle notched with grooves vs. length: width ca. 3:2; pellicle inconspicuously notched with pellicular ridges between ciliary rows), number and length of caudal cilia (single cilium, about 12–17 μm vs. at least four extremely long cilia, including one typical caudal cilium, ca. 18–25 μm in *U. parafilificum*), number of somatic kineties (22 or 23 vs. 16 or 17) and number of macronuclear nodules (two vs. one) (Fan et al. 2011; Gong et al. 2007).

Uronemita sinensis has an elongated elliptical body outline with a slightly narrow anterior end (vs. inverted pear-shaped or elongated elliptical with a wider anterior), fewer somatic kineties (nine or ten vs. 22 or 23), and one (vs. two) macronuclear nodule. Thus, it cannot be confused with *Uronemita parabinucleata* n. sp. (Pan et al. 2013).

Comparison of *Uronemita parabinucleata* n. sp. with other morphologically similar species

Song and Wilbert (2000) re-established the genus *Urocyclon* mainly because no type species was fixed, and they described a new species: *Urocyclon ovatum*, whose cell size and shape resembles the size and shape of *Uronemita parabinucleata* n. sp.

However, the new species differs in the number of macronuclear nodules (two vs. one) and somatic kineties (22 or 23 vs. 23–28). Moreover, *Urocyclon ovatum* has gradually shortened somatic kineties 1–4 (vs. not shortened in the new species) and a highly reduced membranelle 1 (vs. membranelle 1 composed of six basal bodies arranged in one row), which is relatively close to membranelle 2 and membranelle 3 (vs. farther away in *U. parabinucleata* n. sp.). In addition, membranelle 2 is shorter and the scutica is composed of three pairs of kineties and a single basal body in the new species, but of two single or paired basal bodies in *Urocyclon ovatum* (Song and Wilbert 2000).

Phylogenetic analyses

According to the morphological data, *Uronemita parabinucleata* n. sp. resembles *U. binucleata* mainly in having two macronuclear nodules. However, there are no SSU rDNA sequence data on *U. binucleata*.

Based on the present phylogenetic analyses, the genus *Uronemita* is a monophyletic group within the family Uronematidae. The findings confirm the validity of *Uronemita* as a genus (Gao et al. 2012a,b; Gong et al. 2007; Song and Wilbert 2002; Yi et al. 2009). Phylogenetically, *Uronemita parabinucleata* n. sp. is most closely related to *U. filificum* (Fig. 3). However, *U. parabinucleata* n. sp. differs morphologically from *U. filificum* in many aspects, of which the most significant is the presence of two macronuclear nodules in *U. parabinucleata* n. sp. vs. only one in *U. filificum*. The explanation for their close phylogenetic relationship is that *U. parabinucleata* n. sp. and *U. filificum* are more similar in buccal field structure, somatic kinety 1, and somatic kinety *n* when compared with *U. parabinucleata* and *U. binucleata* (see Table 4) (Pan et al. 2015; Song 1993; Song and Wilbert 2002). The results of the present study indicate that aspects of the infraciliature seem to be more important diagnostic characteristics within the genus *Uronemita* than the number of macronuclear nodules or body shape.

Key to the identification of species of *Uronemita*

1	One macronuclear nodule	2
1′	More than one macronuclear nodule	3
2	A single caudal cilium	4
2′	More than one caudal cilium	*U. parafilificum*
3	Number of somatic kineties ≥ 20	*U. parabinucleata*
3′	Number of somatic kineties < 20	*U. binucleata*
4	Number of somatic kineties > 10	5
4′	Number of somatic kineties ≤ 10	*U. sinensis*
5	Body slender D- or kidney-shaped; length: width ca. 3:2	*U. filificum*
5′	Body inverted pear-shaped, slender oval; length: width ca. 2:1	*U. cymruensis*

Acknowledgements

We are grateful to Helmut Berger and two anonymous reviewers for their thoughtful comments that led to improvements in the manuscript. This work was supported by the Natural Science Foundation of China (project numbers: 31430077, 41576134, 31470064), the Deanship of Scientific Research at King Saud University (PRG-1436-24), China Postdoctoral Science Foundation (2015T80746, 2014M551958), and the postdoctoral foundation from Qingdao and Shandong Province. We thank Prof. Weibo Song for critical help with the drafting of this manuscript.

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