Morphology and small-subunit rRNA gene sequences of two novel marine ciliates, *Metanophrys orientalis* spec. nov. and *Uronemella sinensis* spec. nov. (Protista, Ciliophora, Scuticociliatia), with an improved diagnosis of the genus *Uronemella*

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The morphology and infraciliature of two novel marine scuticociliates, *Metanophrys orientalis* spec. nov. and *Uronemella sinensis* spec. nov., collected from sandy beaches at Qingdao, China, were investigated using live observation and protargol-staining methods. *Metanophrys orientalis* spec. nov. is distinguished by the following characteristics: marine habitat and a slender to elongate oval body with pointed anterior end and rounded caudal end, *in vivo* about 25–50 μm long; buccal field about a quarter to a third of body length; nine or ten somatic kineties with dikinetids approximately in anterior half of body, monokinetids in posterior half; membranelles 1 and 2 almost equal in length and composed of two and three longitudinal rows of kinetids respectively; paroral membrane with zigzag structure extending anteriorly to middle portion of membranelle 2; contractile vacuole pore located at posterior end of somatic kinety 1. The genus *Uronemella* is redefined as follows: marine form with an elongate-elliptical or inverted pear-shaped body; apical plate conspicuous; buccal field about two-thirds of body length, cytostome subequatorially located; oral apparatus *Uronema*-like; somatic kineties comprising a mixture of dikinetids and monokinetids. *Uronemella sinensis* spec. nov. is recognized by having an elongate-elliptical body with truncated apical frontal plate, size *in vivo* about 25–35×15–20 μm, nine or ten somatic kineties, membranelle 1 consisting of two or three basal bodies, contractile vacuole pore at posterior end of somatic kinety 1. This study also compared the small-subunit rRNA gene sequences of these two species with other closely related species to show the sequence divergence, which ranged from 3.53 to 9.60 %. Phylogenetic analyses support the contention that the genus *Uronemella* is monophyletic, while *Metanophrys* is non-monophyletic.

INTRODUCTION

As common members of ecosystems in habitats worldwide, the ciliates in the subclass Scuticociliatia exhibit great species richness and biological diversity and often act as symbionts or pathogens of aquatic animals (Fan *et al.*, 2011a; Gao *et al.*, 2012a, b; Lobban *et al.*, 2011; Pan *et al.*, 2010; Song & Wilbert, 2002; Song *et al.*, 2002; Whang *et al.*, 2013). Investigations of scuticociliates in intertidal sediments have demonstrated that this group is much more diverse than previously assumed (Fan *et al.*, 2011a, b; Foissner *et al.*, 1994; Foissner & Wilbert, 1981; Pan *et al.*, 2011; Song, 2000). Moreover, with the application of molecular techniques in taxonomy, species need to be compared not only at the morphological level but also at the molecular level, and there is a particular need for further descriptions and comparisons at the molecular level in this group (Gao *et al.*, 2010, 2013; Grolière *et al.*, 1978; Medlin *et al.*, 1988; Miao *et al.*, 2011; de Puytorac *et al.*,

Abbreviations: BI, Bayesian inference; ML, maximum-likelihood; SSU, small-subunit.

The GenBank/EMBL/DDBJ accession numbers for the SSU rRNA gene sequences of *Metanophrys orientalis* spec. nov. and *Uronemella sinensis* spec. nov. are JN885084 and JN885083, respectively.

Three supplementary figures are available with the online version of this paper.

1974; Small & Lynn, 1985; Song *et al.*, 2002; Song & Wilbert, 2000a; Zhang *et al.*, 2011).

The genus *Metanophrys* de Puytorac, 1974 was established by de Puytorac *et al.* (1974) with *Metanophrys durchoni* as the type species, and four species have since been added to the genus, namely *Metanophrys elongata* (Biggar & Wenrich, 1932) Grolière *et al.*, 1978, *Metanophrys echini* Small & Lynn, 1985, *Metanophrys sinensis* Song & Wilbert, 2000 and *Metanophrys similis* Song *et al.*, 2002. Among them, *Metanophrys sinensis* and *Metanophrys similis* have been found in Chinese seas and described several times (Song & Wilbert, 2000b; Song *et al.*, 2002, 2009). This genus is distinguished by the following features: body with pointed anterior end and no apical plate, cytostome above mid-body, membranelle 1 composed of two rows of kinetids, each with six kinetosomes; membranelle 2 equal to membranelle 1 in length, three-rowed, paroral membrane with zigzag structure extending anteriorly to middle portion of membranelle 2, single caudal cilium (Strüder & Wilbert, 1982).

The genus *Uronemella* Song & Wilbert, 2002, meanwhile, comprises three nominal species from marine habitats, *Uronemella filificum* Kahl, 1931, *U. binucleata* (Song, 1993) Song & Wilbert, 2002 and *U. parafilificum* Gong *et al.*, 2007 (see Fig. 4), and is generally recognized by having a prominent buccal field (which accounts for more than 50 % of body length), a dominant apical plate and a typical rotatory movement with the help of a sticky thread associated with the caudal cilium (Borror, 1963; Gong *et al.*, 2007; Pérez-Uz *et al.*, 1996; Pérez-Uz & Hope, 1997; Song & Wilbert, 2002; Thompson & Kaneshiro, 1968; Wilbert & Kahan, 1981).

In this paper, two novel species are described and the diagnosis of the genus *Uronemella* is emended based on current observations. Additionally, the paper contributes to the currently very limited molecular data relating to these two genera by comparing their small-subunit (SSU) rRNA gene sequences with those of closely related species.

METHODS

Metanophrys orientalis spec. nov. was collected on 13 October 2010 from Yangkou bathing beach (36° 14′ N 120° 40′ E) in Laoshan district, Qingdao, China (water temperature about 23 °C, pH 7.6 and salinity 30 ‰). *Uronemella sinensis* spec. nov. was isolated on 4 October 2010 from the Shilaoren bathing beach (36° 5′ N 120° 27′ E) in Laoshan district, Qingdao, China (water temperature 17.2 °C, pH 7.4 and salinity 35 ‰). In each case, the upper 15 cm layer of sand was collected together with some water from the site. Ciliates were maintained in glass Petri dishes (9–10 cm across) as raw cultures for 1 week at room temperature and then isolated by using a glass micropipette.

Isolated cells were observed and photographed *in vivo* using differential interference contrast microscopy. Protargol (Wilbert, 1975) and Chatton–Lwoff (Wilbert & Song, 2008) methods were used to reveal the infraciliature and argyrome, respectively. Counts and measurements of stained specimens were performed at magnifications of 100× to 1250×. Drawings were carried out with the help of a camera lucida (Foissner, 2006). Systematics and terminology are mainly according to Lynn (2008) and Small & Lynn (1985).

The SSU rRNA gene sequences of *Metanophrys orientalis* spec. nov. and *Uronemella sinensis* spec. nov. were deposited in the GenBank database with the accession numbers JN885084 and JN885083, respectively (Gao *et al.*, 2012a), but they were considered as unidentified forms at that time. In this study, these two sequences were compared with those of another 14 morphologically similar species as follows: *Uronema marinum* (GenBank accession no. GQ465466), *Uronema elegans* (AY103090), *Uronema heteromarinum* (FJ870100), *Uronemella filificum* (EF486866), *Uronemella parafilificum* (HM236337), *Metanophrys sinensis* (HM236336), *Metanophrys similis* (AY314803), *Paranophrys magna* (JN885089), *Mesanophrys carcini* (JN885085), *Parauronema virginianum* (JN885087), *Glauconema trihymene* (GQ214552), *Miamiensis avidus* (JN885091), *Anophyroides haemophila* (AF107779) and *Homalogastra setosa* (EF158844). Sequences were aligned using CLUSTAL W implemented in BioEdit 7.0 (Hall 1999) using pairwise analysis.

Bayesian inference (BI) analyses were performed with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) using the GTR+I+G model selected by MrModeltest version 2.2 (Nylander, 2004) according to the AIC criterion. Markov chain Monte Carlo simulations were run with two sets of four chains using the default settings: chain length 1 000 000–3 000 000 generations, with trees sampled every 100 generations. The first 25 % of sampled trees were discarded as burn-in. All remaining trees were used to calculate posterior probabilities using a majority rule consensus. Maximum-likelihood (ML) trees were reconstructed with PhyML version 2.4.4 (Guindon & Gascuel, 2003) using the best model according to the AIC criterion selected by Modeltest version 3.4 (Posada & Crandall, 1998). The reliability of internal branches was assessed using non-parametric bootstrapping with 1000 replicates. Phylogenetic trees were visualized with TreeView version 1.6.6 (Page, 1996) and MEGA version 4 (Tamura *et al.*, 2007).

RESULTS AND DISCUSSION

Metanophrys orientalis spec. nov. (Figs 1, 2 and S1; Tables 1 and 2) (subclass Scuticociliatia Small, 1967; order Philasterida Small, 1967; genus *Metanophrys* de Puytorac *et al.*, 1974)

Diagnosis. Medium-sized, slender to elongated oval, *in vivo* about 25–50 μm with pointed anterior end; buccal field about a quarter to a third of body length; nine or ten somatic kineties with dikinetids approximately in anterior half of body length; membranelle 1 (M1) composed of two rows of kinetids, each with six kinetosomes; membranelle 2 (M2) the same length as membranelle 1, three-rowed; contractile vacuole pore located at posterior end of somatic kinety 1; single caudal cilium present; marine habitat.

Type locality. Yangkou bathing beach, Laoshan district of Qingdao, northern China (36° 14′ N 120° 40′ E).

Type slides. The holotype slide (registration no. PXM-2010101301) and one paratype slide (registration no. NHMUK 2013.7.4.1) with protargol-stained specimens are deposited in the Laboratory of Protozoology, Ocean University of China, and the Natural History Museum, London, UK, respectively.

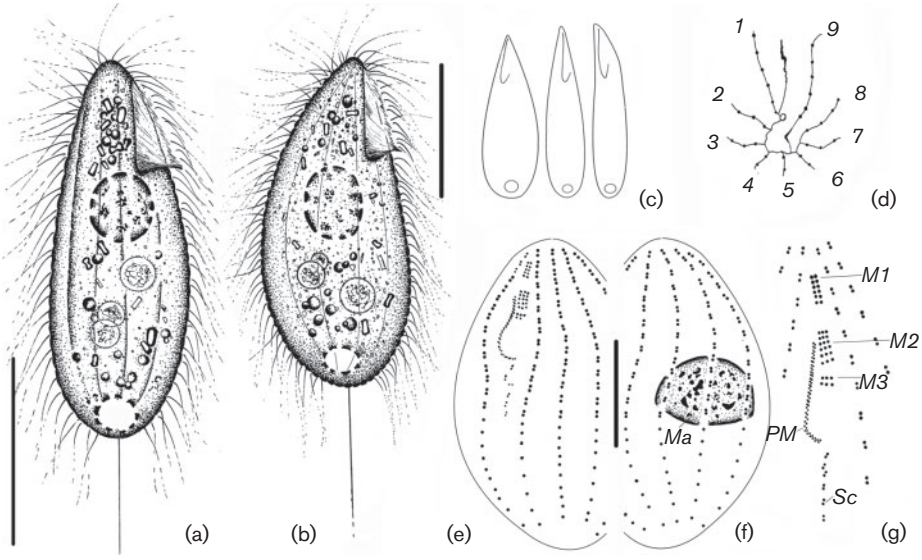

Fig. 1. *Metanophrys orientalis* spec. nov. from life (a–c) and after staining with protargol (e–g) and silver nitrate (d). (a) Ventral view of a representative individual. (b, c) To show different body shapes. (d) Caudal view to show silverline system. (e, f) Ventral (e) and dorsal (f) views of the same specimen, showing infraciliature and nuclear apparatus. (g) Detailed structure of the buccal area. M1–3, Membranelles 1, 2 and 3; Ma, macronucleus; PM, paroral membrane; Sc, scutica. Bars, 20 μm (a, b, e, f).

Etymology. The species-group name *orientalis* (eastern, of the Orient) refers to the fact that this species was first isolated from Chinese coastal waters.

Description. Cell size *in vivo* 25–50 × 12–20 μm. Body shape usually elongated oval to slender with anterior end distinctly pointed, posterior rounded (Figs 1a–c and 2a and Fig. S1a, available in IJSEM Online). Body asymmetrical in outline when viewed from ventral side with anterior end slightly curved sideways (Figs 2a–c and S1a–c). Ventral side slightly straightened, while dorsal side convex (Figs 1a, 2a, b, S1a and S2a). Buccal field a quarter to a third of body length, with buccal cilia about 8–10 μm long (Figs 2c, f and S1c, f). Somatic cilia densely arranged and about 7–8 μm

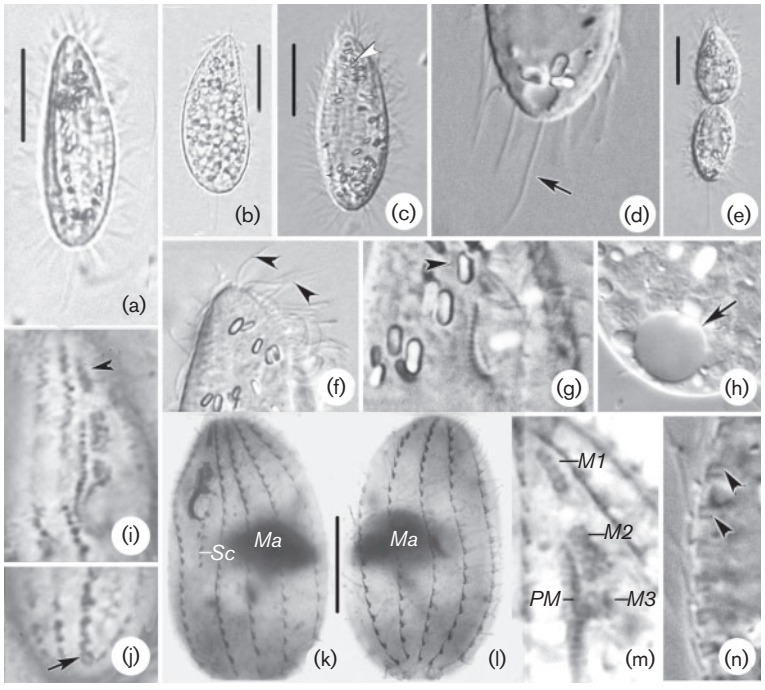

Fig. 2. *Metanophrys orientalis* spec. nov. from life (a–h, m) and after staining with protargol (k, l, m) and silver nitrate (i, j). (a–c) Different individuals; arrow in (c) indicates crystals. (d) Ventral view; arrow marks the single prolonged caudal cilium. (e) Individual undergoing binary fission. (f) Anterior region of cell; arrowheads mark buccal cilia. (g) Ventral view showing crystals (arrowhead). (h) To show contractile vacuole (arrow). (i) Ventral view, to show M1 (arrowhead). (j) Ventral view, showing contractile vacuole pore (arrowhead). (k, l) Ventral (k) and dorsal (l) views of the same specimen, showing infraciliature and nuclear apparatus. (m) Detailed infraciliature of buccal area. (n) Detailed view of cortex; arrowheads mark extrusomes. M1–3, Membranelles 1, 2 and 3; Ma, macronucleus; PM, paroral membrane; Sc, scutica. Bars, 20 μm (a–c, e) and 15 μm (k, l).

Table 1. Morphometric characterization of *Metanophrys orientalis* spec. nov. (upper rows) and *Uronemella sinensis* spec. nov. (lower rows)

Data are based on protargol-stained specimens.

Character	Min.	Max.	Mean	SD	CV (%)	*n*
Body length (μm)	40	58	49.2	4.9	9.9	20
	34	46	59.1	3.7	9.2	19
Body width (μm)	27	40	33.3	4.1	12.3	20
	20	31	25.2	2.8	11.0	19
Length of buccal field (μm)	11	16	13.4	1.7	12.6	15
	22	30	25.1	2.1	10.1	15
Number of somatic kineties	9	10	9.3	0.5	1.8	18
	9	10	9.6	1.0	4.8	19
Number of rows in membranelle 1	2	2	2	0	0	15
	1	1	1	0	0	14
Number of rows in membranelle 2	3	3	3	0	0	14
	2	2	2	0	0	14
Length of macronucleus (μm)	10	15	12.5	4.3	8.9	20
	12	18	15.3	3.2	5.2	19
Width of macronucleus (μm)	8	12	10.4	7.6	10.2	20
	10	15	13.6	7.2	13.2	19

long (Figs 2c and S1c). Single caudal cilium about 15 μm long (Figs 2d and S1d). Pellicle thin (Fig. 1a). Extrusomes arranged in rows between somatic kineties (Figs 1a, b, 2n and S1n). Endoplasm colourless to greyish, and containing several food vacuoles in middle portion of the body and many bar- or dumbbell-like crystals in the anterior and posterior regions (Figs 1a, 2c, f, g and S1c, f, g). One large spherical to ovoid macronucleus centrally located with many small nucleoli (Figs 1f, 2k, l and S1k, l). Contractile vacuole about 5 μm in diameter, caudally positioned near ventral side, pulsating at intervals of 5 s (Figs 1a, 2h and S1h). Locomotion by swimming moderately fast, sometimes continuously without pause, or crawling on substrates.

Nine or ten somatic kineties with dikinetids arranged in approximately in anterior half of each row and monokinetids positioned posteriorly (Figs 1e, f, 2k, l and S1k, l). M1 slightly away from apex and comprising two rows of kinetids with six basal bodies each (Figs 1g and 2i). M2, as long as M1, also composed of about six basal bodies in each row. Membranelle 3 (M3) located close to M2, and normally comprised three short arranged rows of basal bodies (Figs 1g, 2m and S1m). Paroral membrane (PM) extended to about anterior third of body. Contractile vacuole pore located at posterior end of kinety 2 (Figs 1d, 2j and S1j).

SSU rRNA gene sequence (Table 2). The SSU rRNA gene of *Metanophrys orientalis* is 1735 bp long with a G+C content of 44.78 mol%. It differs from those of morphologically similar species at 62–169 positions (Table 2).

Remarks and comparison. The novel form is similar to three nominal species, *Metanophrys elongata* (Biggar & Wenrich, 1932) Grolière *et al.*, 1978, *Metanophrys similis* Song *et al.*, 2002 and *Metanophrys sinensis* Song & Wilbert, 2000 (Fig. 4).

Compared with *Metanophrys orientalis* spec. nov., *Metanophrys elongata* has a larger body (100–120 μm vs

Table 2. SSU rRNA gene sequence dissimilarities among 16 scuticociliates

Values below the diagonal are pairwise distances (%); those above the diagonal are numbers of different sites. Species described in the present study are marked in bold.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. ***Uronemella sinensis* spec. nov.**	–	152	150	156	76	83	141	163	143	160	164	125	107	111	105	97
2. *Uronema marinum*	8.64	–	170	191	139	150	169	175	161	181	180	5	137	266	131	129
3. *Uronema elegans*	8.52	9.65	–	106	124	133	130	135	168	133	176	154	83	119	122	122
4. *Uronema heteromarinum*	8.85	10.83	6.01	–	147	155	159	171	172	174	190	163	131	143	134	138
5. *Uronemella filificum*	4.33	7.90	7.04	8.34	–	15	129	149	119	145	139	123	103	116	124	121
6. *Uronemella parafilificum*	4.73	8.52	7.56	8.79	0.85	–	129	150	124	150	139	131	112	123	116	124
7. ***Metanophrys orientalis* spec. nov.**	8.02	9.60	7.39	9.02	7.42	7.33	–	62	132	73	150	143	119	102	88	106
8. *Metanophrys sinensis*	9.28	9.94	7.67	9.69	8.48	8.53	3.53	–	150	48	156	142	121	140	117	114
9. *Metanophrys similis*	8.11	9.13	9.52	9.73	6.75	7.03	7.49	8.50	–	158	130	138	116	131	89	78
10. *Paranophrys magna*	9.10	10.29	7.56	9.87	8.24	8.53	4.16	2.73	8.96	–	163	147	115	120	119	129
11. *Mesanophrys carcini*	9.32	10.23	9.99	10.77	7.88	7.90	8.52	8.86	7.37	9.26	–	143	120	136	124	135
12. *Parauronema virginianum*	7.06	0.28	8.70	9.20	6.95	7.40	8.08	8.06	7.80	8.31	8.08	–	134	135	131	142
13. *Glauconema trihymene*	6.05	8.70	4.69	7.40	5.81	6.33	6.72	6.75	6.55	6.53	6.74	7.57	–	35	52	52
14. *Miamiensis avidus*	6.27	15.02	6.72	8.08	6.55	6.95	5.76	8.02	7.40	6.74	7.68	7.66	1.98	–	42	42
15. *Anophyroides haemophila*	6.04	7.40	6.89	7.57	6.94	6.55	4.97	6.57	5.00	6.62	6.94	7.40	2.94	2.37	–	60
16. *Homalogastra setosa*	5.48	7.28	6.89	7.00	6.87	7.01	5.99	6.44	4.41	7.29	7.66	8.02	2.94	2.37	3.39	–

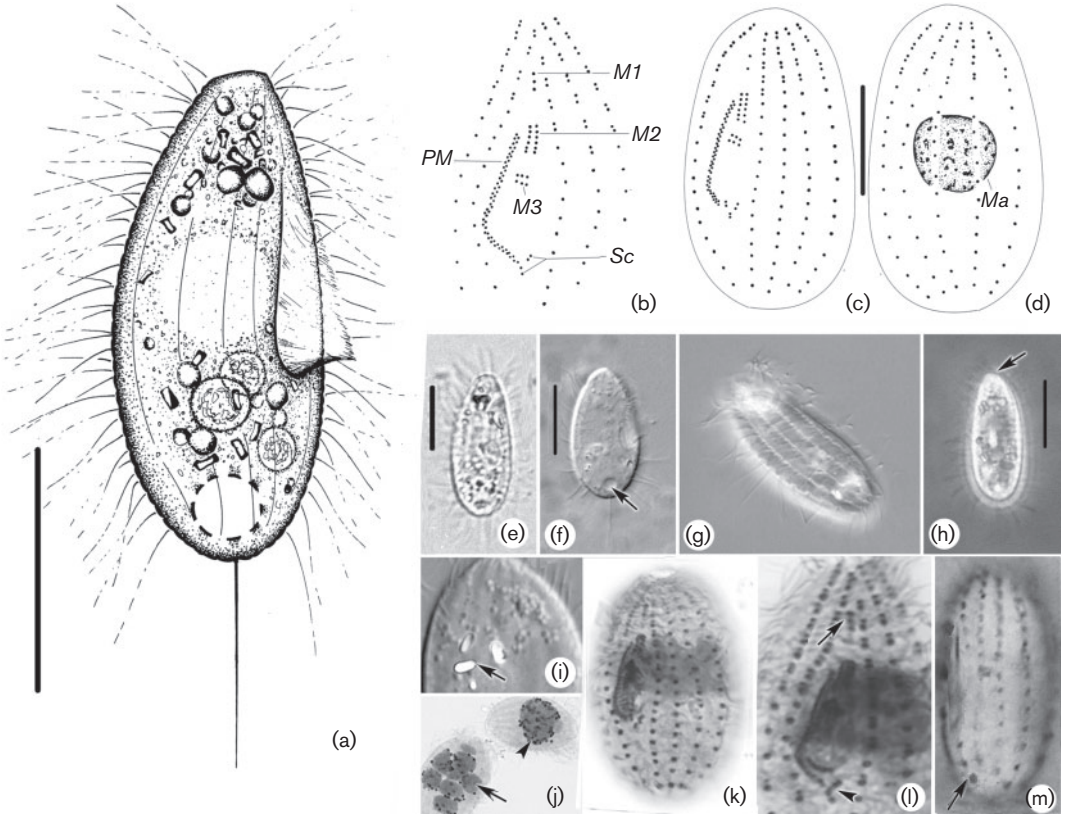

Fig. 3. *Uronemella sinensis* spec. nov. from life (a, e–i) and after staining with protargol (b–d, j–l) and silver nitrate (m). (a) Ventral view of typical cell. (b, l) Detailed infraciliature of buccal area; arrow shows M1 and arrowhead marks scutica. (c, d) Ventral and dorsal views of the same specimen. (e, f, h) Ventral views of different individual; arrow in (f) marks contractile vacuole and that in (h) marks the small apical plate. (g) Dorsal view. (i) Anterior region of cell; arrow marks crystals. (j) Different appearances of single (arrowhead) and multiple (arrow) macronuclei. (k) Ventral view, showing infraciliature. (m) Ventral view, to show contractile vacuole pore (arrow). M1–3, Membranelles 1, 2 and 3; Ma, macronucleus; PM, paroral membrane; Sc, scutica. Bars, 20 μm (a, e, f, h) and 15 μm (c, d).

25–50 μm), a larger number of somatic kineties (15–20 vs nine or ten) and highly developed, extremely long M1 and M2 (Grolière *et al.*, 1978, 1980).

Metanophrys similis can be separated easily from *Metanophrys orientalis* by the different arrangement of the scutica (basal bodies solitary and sparsely distributed in a long row in *Metanophrys similis* vs in pairs and closely packed in *Metanophrys orientalis*), the appearance of the pellicle (notched in the former vs smooth in the latter) and the different number of rows in M1 (two in *Metanophrys similis* vs one in *Metanophrys orientalis*) (Song *et al.* 2002) (Fig. 4g, h).

The novel species most closely resembles *Metanophrys sinensis* (Song & Wilbert, 2000b). The latter, however, can be distinguished from *Metanophrys orientalis* in (i) the presence or absence of extrusomes (absent in *Metanophrys sinensis* vs arranged in rows between somatic kineties in *Metanophrys orientalis*); (ii) distinctly longer M1 than M2 in *Metanophrys sinensis* (vs they are equal in length in *Metanophrys orientalis*); (iii) structure of M2 (two-rowed vs three-rowed); (iv) the length of the buccal field relative to the body length (half in *Metanophrys sinensis* vs a quarter to a third in *Metanophrys orientalis*); (v) the location of the contractile vacuole pore (at posterior end of kinety 2 in *Metanophrys sinensis* vs at posterior end of kinety 1 in *Metanophrys orientalis*) (Song & Wilbert, 2000b); (vi) dikinetids approximately in anterior two-thirds of each somatic kinety in *Metanophrys sinensis* (vs half in *Metanophrys orientalis*) (Fig. 4n).

Comparison of SSU rRNA sequences shows that the sequence of the novel species has differences in 62–132 gene sites compared with those of its congeners, but it differs from that of *Paranophrys magna* in 73 sites, indicating its closer relationship compared with *Metanophrys similis*, which is consistent with the phylogenetic analyses (Gao *et al.*, 2012a). The sequence difference of 88 sites between the novel form and *Anophyroides haemophila* indicates a possible close relationship between *Metanophrys* and *Anophyroides* (Table 2).

The topologies of the phylogenetic trees reconstructed using BI and ML analyses were similar; therefore, only the

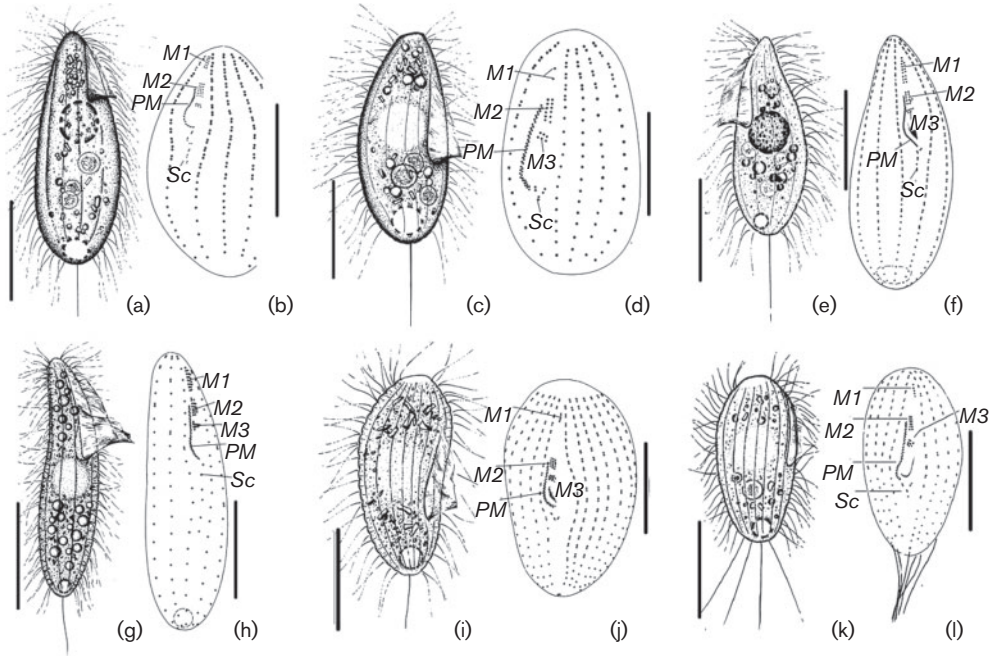

Fig. 4. Morphological comparisons among *Uronemella sinensis* spec. nov., *Metanophrys orientalis* spec. nov. and their congeners. Species from life (a, c, e, g, i, k) and after protargol impregnation (b, d, f, h, j, l). (a, b) *Metanophrys orientalis* spec. nov. (from present work). (c, d) *Uronemella sinensis* spec. nov. (e, f) *Metanophrys sinensis* Song & Wilbert, 2000 (from Song & Wilbert, 2000b). (g, h) *Metanophrys similis* Song *et al.*, 2002 (from Song *et al.* 2002). (i, j) *Uronemella filificum* Kahl, 1931 (from Song & Wilbert, 2002). (k, l) *Uronemella parafilificum* Gong *et al.*, 2007 (from Gong *et al.*, 2007). M1–3, Membranelles 1, 2 and 3; PM, paroral membrane; Sc, scutica. Bars, 15 μm.

BI tree is shown (Fig. 5). Our phylogenetic trees show that the three species of the genus *Metanophrys* included in the analyses are divided into two clades: *Metanophrys similis* clusters with members of the genus *Mesanophrys*. *Metanophrys sinensis* branches as a sister group with the *Paranophrys magna* clade (1.00 BI, 100 ML) and *Metanophrys orientalis* spec. nov. forms a sister taxon to the branch comprising the above three species (1.00 BI, 100 ML). *Metanophrys similis* is well characterized and, based on its morphology, its generic placement is not in doubt (Song *et al.*, 2002). Why *Metanophrys similis* clusters in a separate clade from its congeners is unclear, but may reflect the fact that phylogenetic relationships at this level cannot be resolved completely using SSU rRNA gene sequence data alone.

Uronemella sinensis spec. nov. (Figs 3 and S2; Tables 1 and 2) (subclass Scuticociliatia Small, 1967; order Philasterida Small, 1967; genus *Uronemella* Song & Wilbert, 2002)

Some novel characters were found in the novel species; hence, an improved diagnosis of the genus *Uronemella* is supplied here, based on both previous studies and the present study.

Improved diagnosis of genus *Uronemella*

Body generally elongate-elliptical or pear-shaped, with an apical plate; buccal field about two-thirds of the total body length; membranelle 1 (M1) consisting of one row of basal bodies, membranelles 2 and 3 (M2 and M3) with two or more longitudinal rows of basal bodies; paroral membrane (PM) extending anteriorly to about the mid-level of M2; somatic kineties comprising a mixture of dikinetids and monokinetids; marine habitat.

Uronemella sinensis spec. nov.

Diagnosis. Body elongate-elliptical with truncated apical frontal plate, *in vivo* about 25–35 × 15–20 μm, buccal field about 65 % of body length; nine or ten somatic kineties; membranelle 1 one-rowed with two or three basal bodies; contractile vacuole caudally positioned near ventral margin with its opening pore at posterior end of somatic kinety 1; marine habitat.

Type locality. A sandy beach named Shilaoren (salinity: 35‰) in Laoshan district of Qingdao (36° 5′ 30″ N 120° 27′ 54″ E), northern China.

Type slides. The holotype slide (registration no. PXM-20101004) and one paratype slide (registration no. NHMUK 2013.7.4.2) with protargol-stained specimens were deposited in the Laboratory of Protozoology, Ocean University of China, and the Natural History Museum, London, UK, respectively.

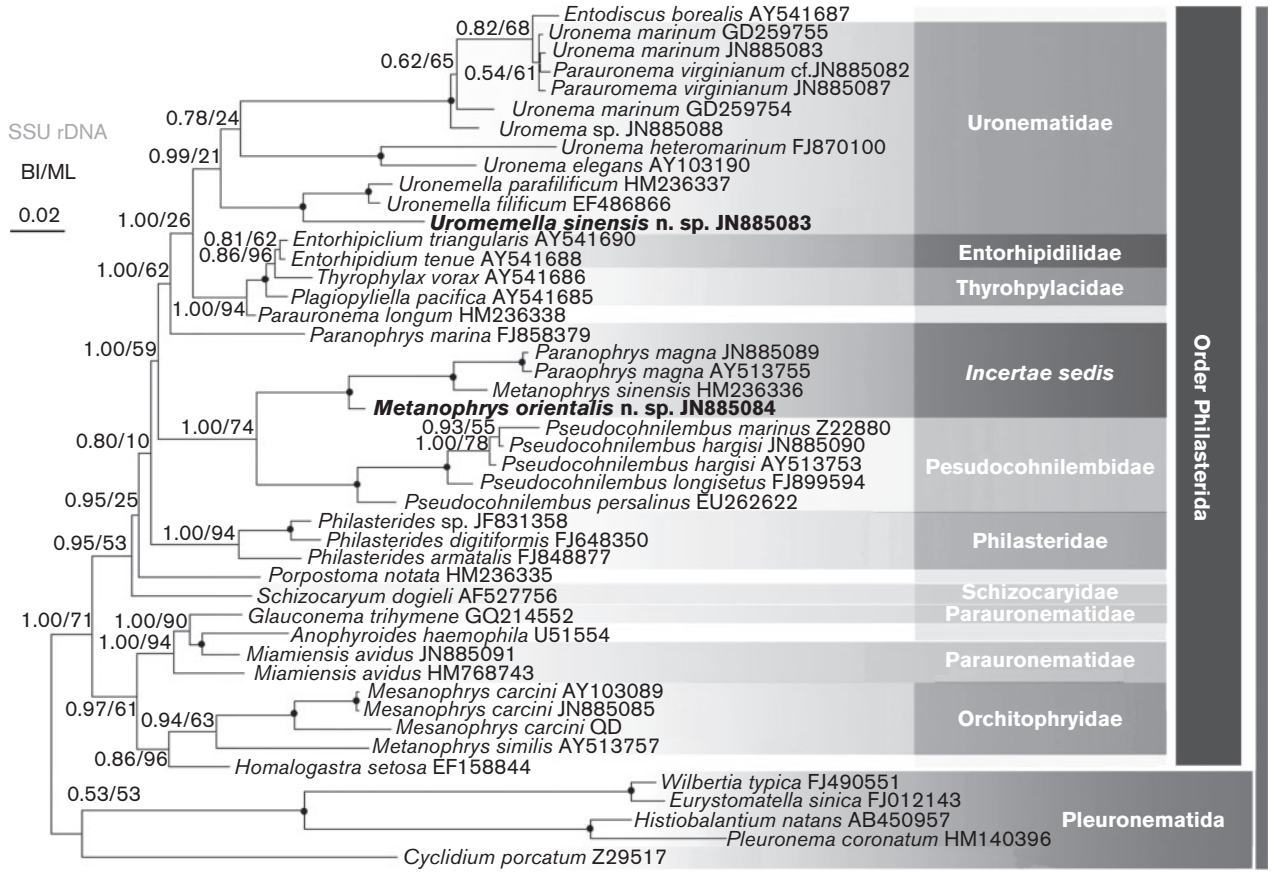

Fig. 5. Phylogenetic tree inferred from SSU rRNA gene sequences, showing the positions of *Metanophrys orientalis* spec. nov. and *Uronemella sinensis* spec. nov. (bold). Nodal support for branches in the BI and ML trees are marked in order. *Wilbertia typica*, *Eurystomatella sinica*, *Histiobalantium natans*, *Pleuronema coronatum* and *Cycliuium porcatum* are the outgroup taxa. Bar, 2 substitutions per 100 nucleotide positions.

Etymology. The species gets its name *sinensis* due to the locality where it was isolated (China).

Description. Size *in vivo* about 25–35 × 15–20 μm, elongate-elliptical in outline becoming wider toward posterior end (Figs 3a, e and S2a, e). Anterior end truncated, with a conspicuous apical plate, dorsal area broadly rounded (Figs 3a, h and S2a, h). Buccal field about 65 % of body length (Figs 3a, f and S2a, f). Surface of the cell smooth, without ridges (Figs 3f, g and S2f, g). Extrusomes rod-shaped, about 2 μm long, and tightly packed beneath cortex. Cytoplasm colourless to greyish, containing several to many large (about 5 μm across) food vacuoles and dumbbell-shaped crystals, usually 2 μm long (Figs 3a, e, h, i and S2a, e, h, j). Macronucleus large and spherical, located mostly at anterior region. Contractile vacuole about 5 μm in diameter, positioned caudally near ventral side (Figs 3f and S2f). Somatic cilia about 10 μm long, densely arranged; single caudal cilium approximately 15 μm long (Figs 3a and S2a). Swimming moderately fast while rotating about main body axis, sometimes quiet on the bottom.

Nine or ten somatic kineties (SK) arranged longitudinally, which usually have dikinetids in anterior quarter to a third of each row and monokinetids positioned posteriorly (Figs 3c, d, k and S2c, d, k). Buccal apparatus as shown in Figs 3b, i and S2b, i: M1 distinct subapically positioned, separated from other membranelles and consisting of two or three basal bodies in a short row; M2 relatively large and composed of two longitudinal rows of basal bodies; M3 comprising three longitudinal rows (Figs 3b, c, k, l and S2b, c, k, l). PM positioned on right of buccal cavity, terminating anteriorly to M2 (Figs 3b, c). Scutica consisting of three pairs of basal bodies (Figs 3c, l and S2c, l). Silverline system typical for genus, cytopyge (CyP) located subterminally as a thin argentophilic patch between SK1 and SKn. Contractile vacuole pore positioned at end of the first somatic kinety (Figs 3m and S2m).

SSU rRNA gene sequence (Table 2). The SSU rRNA gene is 1753 bp long with a G + C content of 43.18 mol%. The sequence differs from those of morphologically similar species at 76–164 positions (Table 2).

Remarks and comparison. In terms of live morphology, infraciliature and habitat, two morphologically similar species should be compared with the novel species, *Uronemella filificum* Kahl, 1931 and *U. parafilificum* Gong *et al.*, 2007 (Fig. 4).

U. filificum can be separated from the novel species through its body shape (pear-shaped vs elongate-ellipsoid), more somatic kineties (21–23 vs nine or ten), larger apical plate and behaviour (thigmotactic vs non-thigmotactic) (Song & Wilbert, 2002) (Fig. 4i, j).

U. parafilificum also differs from *U. sinensis* in having more somatic kineties (21–23 vs nine or ten) and more basal bodies in its membranelles (six vs two or three) and in its behaviour (thigmotactic vs non-thigmotactic) (Gong *et al.*, 2007) (Fig. 4l).

Results from the comparison of SSU rRNA sequences support the morphological identification of the novel form, which is also consistent with the phylogenetic analyses (Gao *et al.*, 2012a); the sequence of the novel species differs from those of *U. filificum* and *U. parafilificum* in 76 and 83 sites, respectively (Table 2).

In addition to *U. sinensis* spec. nov., SSU rRNA gene sequences of two congeners, *U. parafilificum* and *U. filificum*, are available. All of these sequences were included in the phylogenetic analyses. As shown in Figs 5 and S3, the three *Uronemella* species form a monophyletic assemblage, with full support (1.00 BI, 100 ML). Noticeably, *U. sinensis* branches as a sister group with the *U. parafilificum*–*U. filificum* clade. The above analyses support the contention that the genus *Uronemella* is monophyletic.

In conclusion, the morphological and molecular data consistently support the validity of the species *Uronemella sinensis* spec. nov.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of China (project nos 41176119 and 31272285), a Marie Curie International Incoming Fellowship within the 7th European Community Framework Programme (return phase; 909381), and a Research Group Project from King Saud University Deanship of Scientific Research, Saudi Arabia (no. RGP-VPP-083). We gratefully acknowledge Professor Weibo Song for his helpful suggestions on this paper.

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