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Description of a New Brackish Water Ciliate, *Uronychia xinjiangensis* n. sp. (Ciliophora, Euplotida) Based on Morphology, Morphogenesis and Molecular Phylogeny

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Abstract. A brackish water euplotid ciliate, *Uronychia xinjiangensis* n. sp., was discovered in a ditch in Yuli County, Xinjiang, China. Its morphology, ciliature and morphogenesis were investigated based on specimens examined *in vivo* and following protargol staining. The new species is characterized by the posterior part of the adoral zone composed of three membranelles, which has never been seen in all other known congeners. Other morphologic features include: (i) body oval-shaped, with conspicuous right anterior spur-like protrusion; (ii)size *in vivo* 60–90 × 40–68 μm; (iii) two macronuclear nodules; (iv) four frontal, two ventral, five transverse, three left marginal and three caudal cirri. Its morphogenesis proceeds in a usual way, except that the oral primordium forms only three proximal membranelles rather than four proximal membranelles within congeners. The small subunit rRNA gene of the new species (GenBank accession number: KX147287) comprises 1723 bp and 44.63% GC content, and differs by 0.12–1.81% from those of congeners. Phylogenetic analyses based on SSU rRNA gene sequence data reveal that *Uronychia xinjiangensis* n. sp. clusters with other *Uronychia* species with full support, which supports the monophyly of the genus *Uronychia* Stein, 1859.

Key words: euplotids, *Uronychia*, ciliature, ontogeny, SSU rDNA

INTRODUCTION

The euplotid genus *Uronychia* Stein, 1859 is commonly found in marine and saltwater biotopes, thus has a very long research history (e.g. Müller 1786; Stein 1859; Wallengren 1900; Young 1922; Mansfeld 1923; Kahl 1932; Wang and Nie 1932; Kirby 1934; Bullington 1940; Kattar 1970; Agamaliev 1971; Borror 1972; Wilbert and Kahan 1981; Valbonesi and Luporini 1990; Carey 1992; Song 1997; Kim and Min 2011). Based on the revision by Curds and Wu (1983) and the redefinition by Song (1999), four morphospecies were considered as valid species in the genus, i.e. *U. transfuga* (Müller 1786) Stein, 1859 (type species), *U. setigera* Calkins, 1902, *U. binucleata* Young, 1922, and *U. multicirrus* Song, 1997.

Although Song and Wilbert (1997) significantly enhanced our knowledge about *Uronychia* populations based on morphometric analyses, some morphological

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features at the species level are weak and easily overlooked. For example, the separation of *U. setigera* and *U. binucleata* is based almost exclusively on cell size and on the difference in the number of basal body pairs in the leftmost dorsal kinety, the latter character only being discernible in high quality protargol preparations.

Morphogenetic studies revealed that *Uronychia* species have an extremely similar developmental pattern of cortical structures and nuclear apparatus (Calkins 1911; Taylor 1928; Wilbert and Kahan 1981; Hill 1990; Wilbert 1995; Song 1996; Shi and Song 1999; Song *et al.* 2004), which do not contribute too much to species separation within the genus.

Recently, molecular biology techniques improve our understanding of high genetic diversity among ciliates (Chen and Song 2001; Chen *et al.* 2003; Shen *et al*. 2009; Yi *et al.* 2009; Huang *et al*. 2012, 2014; Gao *et al.* 2017).

During a survey on fauna in Xinjiang, western China, a species of *Uronychia* was isolated and investigated. Based on morphological and SSU rDNA sequence comparison, we believe it represents a new one, *Uronychia xinjiangensis* n. sp.

MATERIALS AND METHODS

Sampling and cultivation

Water samples were collected from temporary ditches near the 891 km spot on the national road 218 (40°25′47″N, 88°1′27″E), Yuli County, Xinjiang, China, where the altitude is 880 m, on July 23, 2007. The pH and salinity of water are 8.26 and 2–4‰, respectively. In the sample, only one kind of ciliate was found. After isolation, pure cultures were kept in the laboratory. For molecular work, clonal cultures were established and maintained in sterilized artificial brackish water at room temperature (*ca* 20°C). Squeezed wheat grains were added to enrich bacterial food.

Morphological and morphogenetic methods

Specimens were examined *in vivo* using bright-field and differential interference contrast microscopy. The ciliature and nuclear apparatus of cells at non-dividing and dividing stages was revealed using an improved protargol impregnation method according to Shi (2006). The protargol product from Merck was used for staining. Photomicrographs were obtained using a Leica DM6000B Microsystems (Leica Microsystems GmbH, Wetzlar, Germany). Schematic drawings of specimens *in vivo* and of different stages of morphogenesis were hand-drawn on parchment paper, based on photomicrographs or using a camera lucida. To illustrate the changes occurring during morphogenetic stages, the parental cirri were drawn only by outline in diagrams, whereas the new ones are shaded black.

Terminology and systematics are according to Shen *et al.* (2009) and Lynn (2008), respectively.

ZooBank registration

ZooBank registration of present work (see Recommendation 8A of ICZN2012): urn:lsid:zoobank.org:pub:147B2320-4AD2-46CA--829E-D698F95E82BE

DNA extraction, PCR amplification and sequencing

DNA was extracted according to Chen and Song (2001). In brief, cells were starved in artificial, sterilized brackish water (salinity 4‰) at room temperature overnight to minimize the contaminants from food and other sources. They were harvested by centrifugation at 640 g for 3 min and treated with lysis buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.6% Tween 20, 0.6% Nonidet P40, 60 mg/ml Proteinase K) at 56°C for 2–3 h, followed by phenol-chloroform purification. Universal primers (18sF 5'-AACCTGGTTGATCCTGCCAGT-3'; 18sR 5'-TGATCCTTCTGCAGGTTCACCTAC-3') (Medlin *et al*. 1988) were used to amplify the SSU rRNA gene. The amplification profile consisted of 35 cycles of 30 s at 98°C, 30 s at 67°C and 1 min at 72°C, followed by 5 min at 72°C for final extension. In order to minimize sequence errors, Q5® Hot Start High-Fidelity DNA Polymerase (Cat. #M0493L, New England Biolabs Inc. USA) was used for PCR amplification. PCR product was sequenced at the Tsingke biological technology Co., Ltd in Qingdao, China. The contig was assembled by SeqMan (DNASTAR).

Phylogenetic analyses

Phylogenetic analyses included the SSU rRNA gene sequence of *Uronychia xinjiangensis* n. sp. and 44 other species, which were downloaded from NCBI GenBank database (accession numbers as shown in Fig. 7). *Uronychia sinica* (FJ876982) was an unpublished name (personal communication) and it is treated as *Uronychia* sp. in the present study. All sequences were aligned by a website of The GUIDANCE2 Server (Sela *et al*. 2015) with default settings and further manually modified using the program BioEdit 7.0.1 (Hall 1999), resulting in a matrix of 45 sequences and 1729 characters. Maximum-likelihood (ML) analysis was performed in CIPRES Science Gateway using RAxML-HPC2 on XSEDE 8.2.6 (Stamatakis *et al*. 2008) with the model GTR+G. The reliability of internal branches was assessed using a nonparametric bootstrap method with 1000 replicates. Bayesian inference (BI) analysis was carried out using MrBayes on XSEDE 3.2.6 with the model GTR+I+G (selected by MrModeltest v.2.0; Nylander, 2004) in CIPRES Science Gateway. Markov chain Monte Carlo simulations were run with two sets of four chains for 6,000,000 generations with a frequency of 100 generations, and the first 25% of sampled trees were discarded as burn-in. MEGA 6.06 (Tamura *et al.* 2013) was used to visualize tree topologies.

RESULTS

Class Spiritrichea Bütschli, 1889
Order Euplotida Small and Lynn, 1985
Family Uronychiidae Jankowski, 1975
Genus *Uronychia* Stein, 1859
***Uronychia xinjiangensis* n. sp. (Figs 1–7, Tables 1 and 2)**

Diagnosis

Body oval-shaped, size about 60–90 × 40–68 μm *in vivo*. Two macronuclear nodules; the posterior part of adoral zone composted of three membranelles; always two fine ventral cirri and one small buccal cirrus; the dorsal kinety 1 consisting of about 36 basal body pairs. Brackish water habitat.

Deposition of types

The protargol slide with the holotype specimen (Fig. 1B, C) and paratypes was deposited in the Hangzhou Key Laboratory for Animal Adaptation and Evolution (registration number: 070723112), Hangzhou Normal University, Hangzhou, China.

Type locality

Brackish water (pH 8.26, salinity 2~4‰) from ditches in Yuli County (40°25′47″N, 88°1′27″E), Xinjiang, China (for details, see Materials and Methods).

Etymology

The species-group name "*xinjiangensis*" refers to Xinjiang Province where the new species was discovered.

Morphology

Cell approximately oval-shaped, *in vivo* about 60–90 × 40–68 μm (n = 13), mostly 80 × 55 μm (Figs 1A, 4A–D). When viewed ventrally, the right side is slightly even and the left side is arc-shaped (Figs 1A, 4A, B). Dorsoventrally ca. 1 : 2 flattened. A distinct spur-like protrusion on right anterior portion of body, while other protrusion inconspicuous (Figs 4B, F). Inconsistently two concaves present in posterior portion of cell on ventral side, which are the location of transverse and left marginal cirri, respectively. On dorsal side a large depression corresponding position of caudal cirri (CC) (Figs 1A, 4A–C, I).

Table 1. Morphometric characterization of *Uronychia xinjiangenesis* n. sp.

Character	Min	Max	Mean	M	SD	CV	n
Length of body * (μm)	60	90	77.1	76	3.64	4.7	13
Length of body (μm)	58	87	73.4	73	8.47	11.5	13
Width of body * (μm)	40	68	55.8	58	8.89	15.9	13
Width of body (μm)	39	66	53.8	56	8.47	15.7	13
Length of adoral zone (μm)	38	53	44.5	43	4.77	10.7	13
Number of membranelles in AZM1	11	11	11	11	0	0	15
Number of membranelles in AZM2	3	3	3	3	0	0	15
Number of buccal cirrus	1	1	1	1	0	0	15
Number of frontal cirri	4	4	4	4	0	0	15
Number of ventral cirri	2	2	2	2	0	0	15
Number of left marginal cirri	3	3	3	3	0	0	15
Number of caudal cirri	3		3	3	3	0	0
Number of transverse cirri		5	5	5	0	0	0
Number of dorsal kineties	6	6	6	6	0	0	15
Number of macronuclear nodules	2	2	2	2	0	0	15
Number of basal body pairs in DK1	34	38	36.3	34	1.41	3.9	9
Length of anterior MN (μm)	45	48	–	–	–	–	4
Width of anterior MN (μm)	12	16	–	–	–	–	4
Length of posterior MN (μm)	24	28	–	–	–	–	4
Width of posterior MN (μm)	17	19	–	–	–	–	4

Data based on protargol-impregnated specimens. * from live cells. Abbreviations: AZM1, 2, the anterior and posterior part of adoral zone of membranelles; CV, coefficient of variation in %; DK 1, dorsal kinety 1; Max, maximum; Mean, arithmetic mean; M, median value; Min, minimum; MN, macronuclear nodule; n, number of specimens observed; SD, standard deviation.

Table 2. Sequences divergence of SSU rRNA gene between *Uronychia xinjiangensis* n. sp. and congeners

	sequence variation (bp)	Sequence similarity
Uronychia transfuga AF260120	28	98.25%
Uronychia binucleata EF198667	26	98.38%
Uronychia binucleata HQ380024	25	98.44%
Uronychia multicirrus EU267929	25	98.44%
Uronychia multicirrus HQ380025	25	98.44%
Uronychia setigera EF198669	27	98.31%
Uronychia setigera HQ380023	26	98.38%
Uronychia setigera HQ380022	28	98.25%
Uronychia setigera HQ380021	29	98.19%
Uronychia setigera JF694042	21	98.69%
Uronychia sp. FJ876982	2	99.88%

Endoplasm colorless, comprising many to numerous granules (*ca.* 1–5 μm in diameter), which renders cell grayish to dark gray at low magnification (Figs 1A, 4A). Food vacuoles (FV) recognizable, low in number, containing green algae and unidentified inclusions (Fig. 4B–D). Two macronuclear nodules connected by funiculus, left of cell midline; individual nodule oval to elongate ellipsoidal, up to 50 μm long (Fig. 1C); micronuclei not observed *in vivo* or after protargol staining. Sometimes a small contractile vacuole located at the lower right-hand corner in buccal field (Fig. 4A, I).

The movement and behavior of the new species characteristic: they spend most of the time creeping and grazing on the substrate; they rapidly jump to elude backwards when stimulated, and swim very fast in the water, rotating around their longitudinal axes; the adoral membranelles and cirri stiffly spread during pauses.

Buccal field enormous, extending to 61% of body length on average in protargol preparations (Figs 1A, 4D, J). Constantly 11 adoral membranelles in anterior part (AZM1), mostly extending onto dorsal side (Fig. 5A, B, D); cilia of membranelles 15–25 μm long. Posterior part of adoral zone (AZM2) composed of three membranelles (Table 1), in which basal bodies are arranged in four rows and cilia are 12–16 μm long (Figs 4G, 5G). Single buccal cirrus (BC) 12–15 μm long, apart from AZM2 (Fig. 4G). Paroral membrane (PM) genus-specific, that is, highly developed and forming almost a circle with BC and AZM2; cilia of PM about 30 μm long (Figs 1A, B, 5A).

Somatic ciliature distributed on ventral and dorsal side: constantly four fine frontal cirri (FC; Table 1) resembling short membranelles in anterior right area of body (Figs 4H, 5E). Two fine ventral cirri (VC) near right posterior border of cell (Figs 4J, arrows; 5F, arrows). Cilia of both frontal and ventral cirri about 15 μm long. Always five transverse cirri (TC), four left ones considerably enlarged and about 40–45 μm long each and rightmost cirrus small and inconspicuous (Figs 1A, 4G, I). Three left marginal cirri (LMC; Table 1), curving to right at their distal ends, the uppermost one is extraordinary small while the middle one is considerably enlarged, and its cilia measures 45 μm in length (Figs 1A, B, 4A, G, J, 5A, H). Three mighty, hook-like caudal cirri (CC) about 40–60 μm long (Figs 4A, L, 5C, I). Invariably six dorsal kineties (DK), each situated in narrow longitudinal pellicular groove (Fig. 4L, arrows); kineties 1 and 2 near left margin of ventral side, and both terminated posteriorly in a short row consisting of tightly spaced kinetosomes (Figs 1B, 5A; kineties 3–6 positioned on dorsal side, of which kinety 3 extends over the entire length of body, while the other three extend only to the caudal cirri (Figs 1C, 5C); dorsal bristles about 3 μm long (Fig. 4K, arrows).

Stomatogenesis

Stomatogenesis begins with the appearance of the opisthe's oral primordium (OP), which appears as a small patch of kinetosomes within a subsurface pouch between cytostome and left marginal cirri (Figs 1D, 6A). Slightly later, the proter's oral primordium (POP) appears subcortically anterior to the parental AZM_2 (Figs 1E, 6D; at the same time the OP begins to differentiate into membranelles. Both the POP and OP develop then by rapid proliferation of kinetosomes (Fig. 2A), and soon a small streak-like anlage for paroral membrane (PMA, anlage I) appears within the subcortical pouch, and is located opposite to the posterior end of the POP and OP, respectively (Fig. 2C, D), meanwhile the parental paroral membrane begins to disintegrate. Subsequently, two groups in the OP, each with 11 and 4 membranelles, and two groups in the POP, each with 5 and 4 membranelles, are differentiated and finally migrate onto the surface of cell (Figs 2E, 4H). Meanwhile, the PMA begins to lengthen along the right edge of each opening pouch; the parental AZM_2 and BC become invisible. In the late stages, two groups of membranelles in two oral primordia are separated, and 11membranelles in the OP and 5 membranlles in the POP migrate anteriorly (Fig. 3A). As in its congeners, the newly built

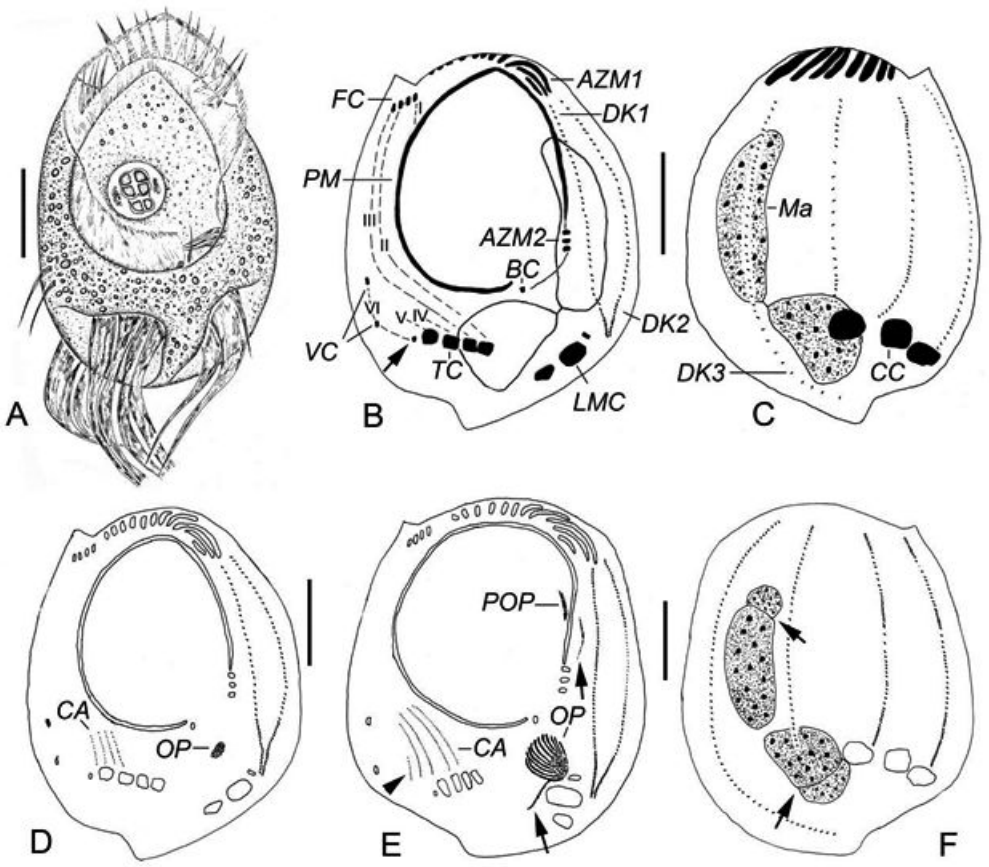

Fig. 1. Uronychia xinjiangensis n. sp. *in vivo* (A) and after protargol staining (B–F). **(A)** Ventral view of a representative individual. **(B, C)** Ventral and dorsal view of the holotype specimen, showing the ciliature and nuclear apparatus. Frontal-midventral-transverse cirri which originate from the same anlage are connected by a broken line. **(D)** Ventral view of an early divider, showing the formation of cirral anlagen and OP. **(E, F)** Ventral and dorsal view of the same early divider, showing the development of cirral anlagen and OP as well the formation of proter's oral primodirum, left marginal anlagen (arrows in E) and replication bands (arrow in F) and dorsal kinety anlagen in the two rightmost old structures; arrowhead indicates short cirral anlage. AZM1,2, anterior and proximal part of adoral zone of membranelles; BC, buccal cirrus; CA, cirral anlagen; CC, caudal cirri; DK1-3, dorsal kineties 1–3; FC, frontal cirri; LMC, left marginal cirri; Ma, macronuclear nodule; OP, opisther's oral primodium; PM, paroral membrane; POP, proter's oral primordium; TC, transverse cirri; VC, ventral cirri. Scale bars: 20 μm.

five membranelles will replace the posterior five membranelles of parental AZM_1 in the proter, while the anterior (parental) six membranelles are retained to reform the new AZM_1 of the proter; the first part in the opisthe will be the new AZM_1 of the opisthe (Fig. 3A). In the second group of AZM in both proter and opisthe, the posterior-most membranelle moves apart from the other three, and then migrates to the final position to differentiate into the new buccal cirrus (Fig. 3C, E).

Development of somatic ciliature

At about the same time as the OP is formed, four thread-like, frontal-ventral transverse cirral anlagen (CA), develop on the cell surface anterior to the transverse cirri (Figs 1D, E, 4A), and then lengthen. Soon the fifth anlage is generated right of them with kinetosomal proliferation (Fig. 1E), presenting as five streaks, they are appointed as CA II–VI from left to right. Apparently no parental cirri participate in the formation of these anlagen. Then the five anlagen proliferate and extend to its maximum length before they divide into two sets as the CA of both proter and opisthe (Fig. 2A). Subsequently, cirral anlagen begin to broaden at the posterior end, break apart and then migrate developing as distinct cirri (Fig. 2C, D). At this time, one short anlage (for the leftmost frontal cirrus) occurs near mid-portion of the anlage I in both proter and opisthe (Fig. 2E). Simultaneously, the cirral anlagen II–VI break apart into 3, 3, 2, 2, and

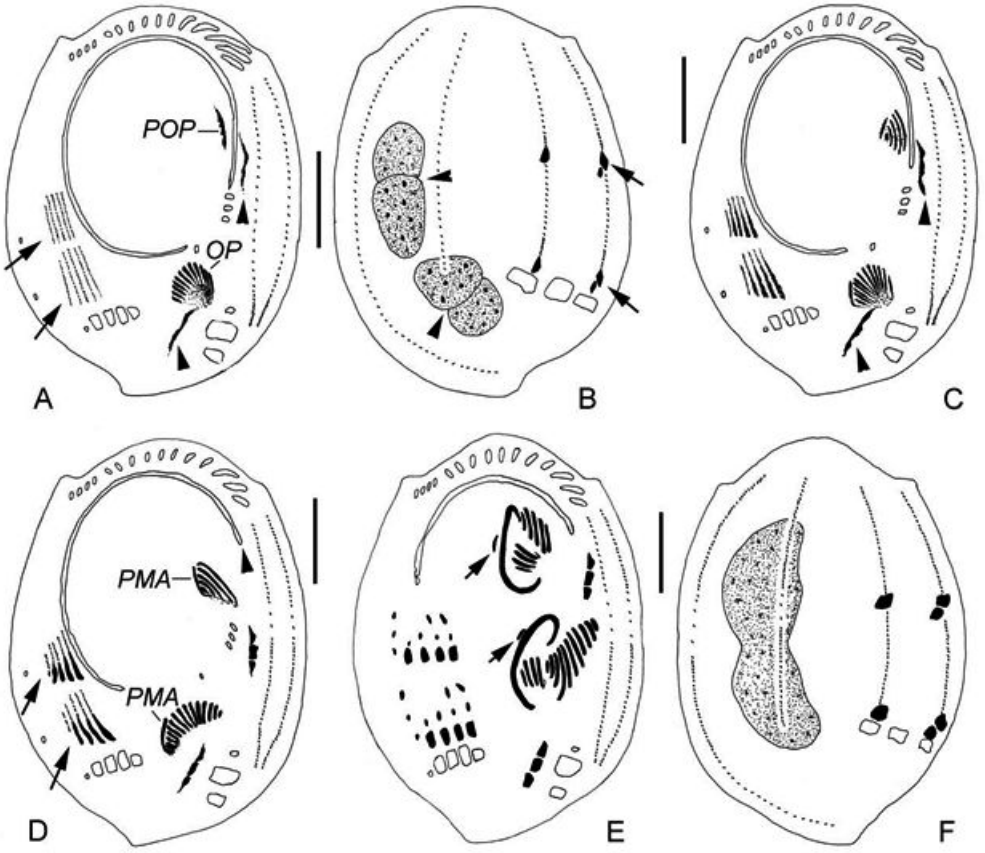

Fig. 2. Morphogenesis of *Uronychia xinjiangensis* n. sp. after protargol staining (A–F). **(A, B)** Ventral and dorsal view of the same specimen, arrows in A and B show cirral anlagen and two caudal cirri in the rightmost dorsal kinety anlagen of both proter and opisthe, respectively, arrowheads in A and B mark left marginal anlagen and replication bands, respectively. **(C)** Ventral view of a middle divider, arrowheads mark the development of left marginal anlagen. **(D)** Ventral view of a middle divider, showing the formation of paroral membrane anlagen and the segmentation of cirral anlagen (arrows), dedifferentiation of the old paroral membrane. **(E, F)** Ventral and dorsal view of the same late divider, showing the formation of new membranelles and cirri and other dorsal kineties anlagen, arrows show de novo formation of a frontal cirrus beside new paroral membrane OP, opisthe's oral primordium; PMA, paroral membrane anlage; POP, proter's oral primordium. Scale bars: 20 μm.

3 fragments (counted from left to right; Fig. 2E); these fragments finally develop into nine cirri, that is, 3 fronal, 2 ventral and 5 transverse cirri. The definite differentiation is: the anterior and middle segments in anlage II and the anterior one in anlage III will become frontal cirri; the middle segment from anlage III and the anterior one from anlagen IV and V will be resorbed before or after cell division is finished; the anterior and middle segments from anlage VI develop into ventral cirri, the remaining will become transverse cirri (Fig. 5A, C).

The anlagen of left marginal cirri appear on the cell surface anterior to the AZM2 and left of buccal cavity in the proter and behind the OP and right of the old LMC in the opisthe (Fig. 1E, arrows), when the POP is formed. These anlagen then enlarge by proliferation of kinetosomes and two depressions are formed (Fig. 2A). At last, the anlagen segment into three parts as the cirral anlagen break apart (Figs 2C, D, E; 3A, C) and ultimately form the marginal cirri for the daughter cells (Fig. 3E).

On dorsal side, the development of dorsal kineties anlagen occurs at two levels within each old kinety and seems to follow a gradient from right to left (Fig. 1F). Soon, caudal cirri are generated as follows: two caudal cirri are formed at the posterior end of the rightmost anlage in both proter and opisthe, and one caudal cirrus is formed at the posterior end of the penultimate anlage from right (Figs 1F, 2B, F). During the formation of new caudal cirri, morphogenesis of the other four old dorsal kineties is undergoing. The proliferation of new basal bodies occurs within each old kinety (Fig. 2E, F). These anlagen gradually lengthen towards both ends of the body to replace the old kineties (Fig. 3A–D).

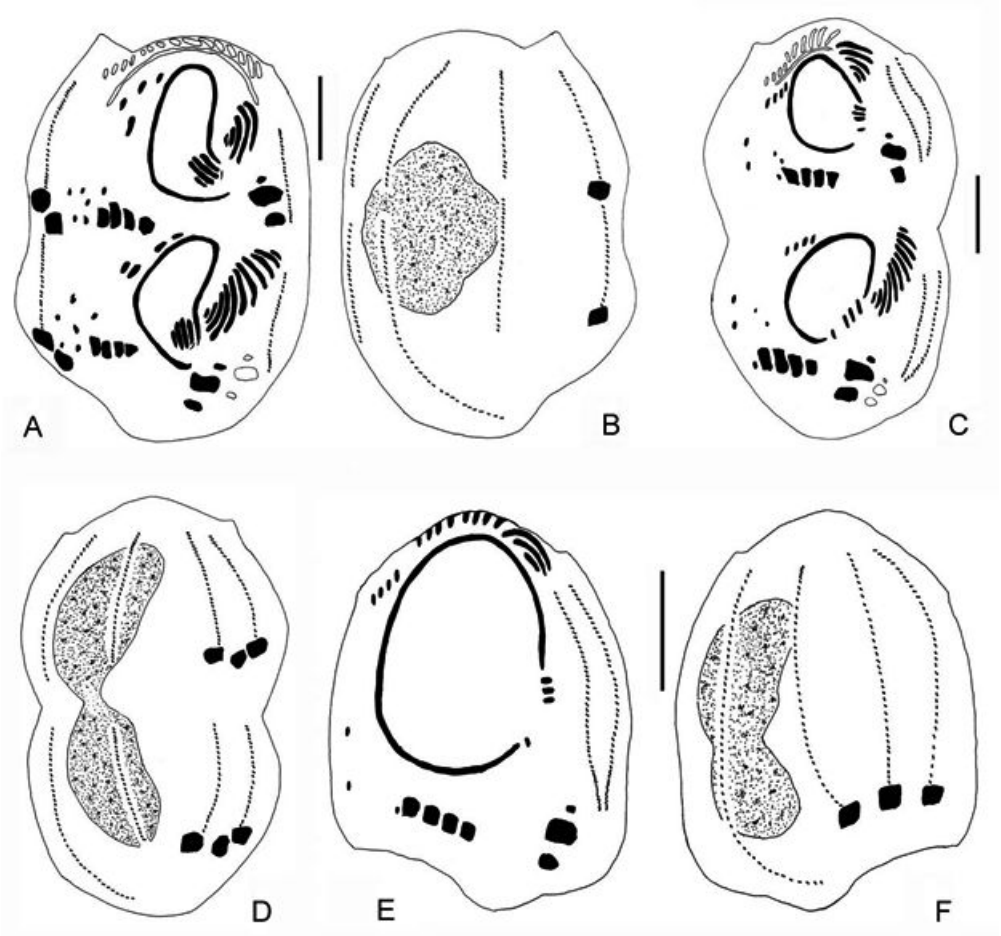

Fig. 3. Morphogenesis of *Uronychia xinjiangensis* n. sp. after protargol staining (A–F). **(A, B)** Ventral and dorsal view of the same late divider, showing migration of new structures and the formation of spherical fused macronucleus. **(C, D)** Ventral and dorsal view of the same late divider, five new membranelles combine with six retained membranelles to form anterior part of adoral zone of membranelles in the proter, the macronucleus dividing once. **(E, F)** Ventral and dorsal view of the same daughter cell just after fusion, showing infraciliature and nuclear apparatus. Scale bars: 20 μm.

Nuclear apparatus

In the early stage of morphogenesis, one replication band is recognized at far end of each macronuclear nodule (Fig. 1F) and then migrates towards the center of cell (Fig. 2B). At about the same time as the OP separate into two groups, DNA replication is completed, and two nodules begin to fuse (Fig. 2F). When the fusion is completed a globular mass is formed (Fig. 3B). In the late stage, it divides into two parts and gives one part to each daughter cell as the cell division is undergoing (Fig. 3D). At last, daughter cell acquire two macronuclear nodules as result of one dividing of macronucleus (Fig. 3F). Unfortunately, the development of the micronucleus was not observed.

SSU rDNA sequence and phylogenetic analyses

The SSU rRNA gene sequence of *Uronychia xinjiangensis* n. sp. was deposited in GenBank with accession number of KX147287. The length and GC content of the SSU rRNA gene are 1723 bp and 44.63%, respectively. Phylogenetic trees based on the SSU rRNA gene sequences using ML and BI algorithms are basically congruent, therefore, only ML tree is shown here with support values of both algorithms indicated on branches (Fig. 7). In both ML and BI trees, the family Uronychiidae Jankowski, 1975 is divided into two parts: one consists of the *Uronychia*, *Paradiophrys* and *Apodiophrys* (91 ML, 1.00 BI); and the other is formed with *Diophrys*, *Diophryopsis*, *Heterodiophrys*

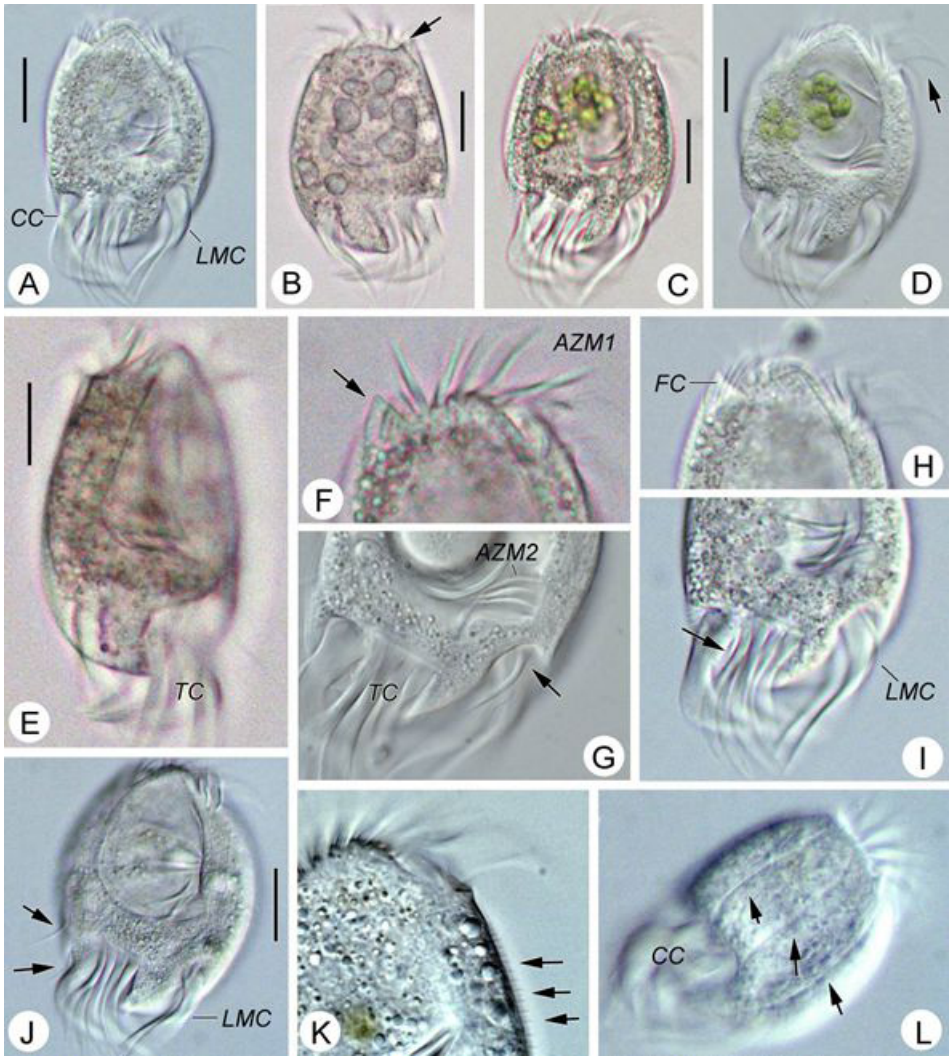

Fig. 4. Microphotographs of *Uronychia xinjiangensis* n. sp. from life (A–L). **(A, C, D)** Ventral views of different cells, showing variation of body shape, arrow shows cilia in AZM1. **(B)** Dorsal view showing inclusions, a small spur-like protrusion (arrow in B), and anterior membranelles (arrow in D). **(E)** Partially lateral view. **(F)** Anterior portion showing spur-like bulge (arrow) at anterior margin of body and AZM1. **(G)** Ventral view of posterior part showing AZM2, and small left marginal cirrus (arrow). **(H)** Ventral view of anterior part, showing frontal cirri. **(I)** Ventral view of posterior part, showing the fine rightmost transverse cirrus (arrow) and left marginal cirri. **(J)** Ventral view, showing two fine ventral cirri (arrows). **(K)** Depicting dorsal bristles. **(L)** Dorsal view, to show caudal cirri located at concave area and dorsal grooves (arrows). AZM1,2, anterior and proximal part of adoral zone of membranelles; CC, caudal cirri; FC, frontal cirri; LMC, left marginal cirri; TC, transverse cirri. Scale bars: 20 μm.

and *Pseudodiophrys* (81 ML, 1.00 BI). Up to now, there are 12 SSU rRNA gene sequences of *Uronychia* belonging to four morphospecies and an unidentified form in NCBI database and all of them cluster together to form a monophyletic clade with full support value (as shown in Fig. 7). Pairwise sequence similarities between *U. xinjiangensis* n. sp. and its congeners range from 99.88% to 98.19% (i.e. the divergence range from 0.12 to 1.81) (Table 2).

DISCUSSION

Morphological comparison with its congeners

As mentioned above, so far only four morphospecies are included in the genus *Uronychia*. In terms of body shape and size, number of macronuclear nodules and structure of oral apparatus and cirral arrangement, our form resembles *U. setigera* and *U. binucleata* very

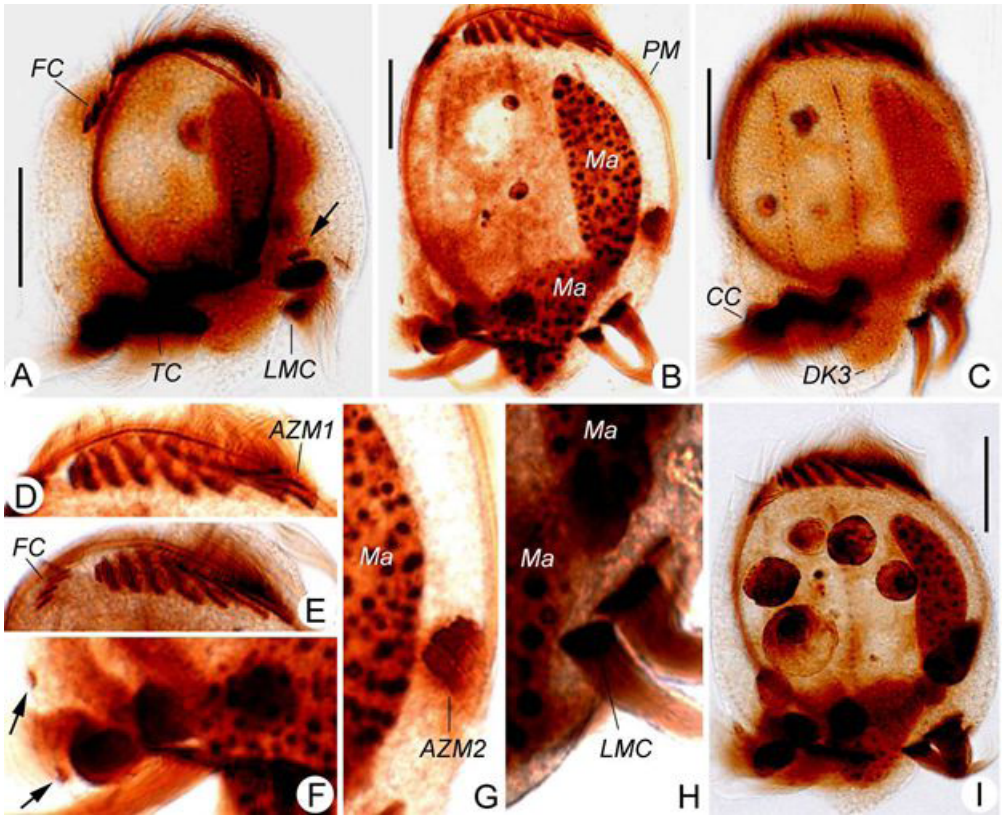

Fig. 5. Photomicrographs of *Uronychia xinjiangensis* n. sp. after protargol staining (A–I). **(A–C, I)** Ventral and dorsal views of specimens at interphase, showing ciliature and macronuclear nodules, arrow showing the small left marginal cirrus. **(D, E)** Anterior view, to show AZM1 and frontal cirri. **(F)** Arrows show two fine ventral cirri. **(G, H)** Depicting AZM2 and left marginal cirri. AZM1,2, anterior and proximal part of adoral zone of membranelles; CC, caudal cirri; DK3, dorsal kinety 3; FC, frontal cirri; LMC, left marginal cirri; Ma, macronuclear nodule; PM, paroral membrane; TC, transverse cirri. Scale bars: 20 μm.

well, however, differs in having three (vs. four) membranelles in the posterior part of adoral zone of membranelles, more basal body pairs in the leftmost dorsal kinety (ca. 36 pairs vs. ca. 15 or 30) (Song and Wilbert 1997; Song *et al.* 2004; Shi and Song 1999). Both *Uronychia transfuga* and *U. multicirrus* are larger forms (150–280 or 120–200 μm) and possess beaded macronuclear nodules (Curds and Wu 1983; Song 1997, 1999; Shi and Song 1999). Furthermore, the latter has long row of ventral cirri (6–8 vs. 2 in the new species). Thus these two species cannot be confused with *U. xinjiangensis* n. sp.

Morphogenetic features in *Uronychia*

All known species of *Uronychia* have been morphogenetically investigated using silver staining methods (e.g. Hill 1990; Wilbert 1995; Shi and Song 1999; Song *et al.* 2004; Shen *et al.* 2009). The present work further confirms that *Uronychia* species exhibit very similar developmental mode of cortical ciliature and nuclear apparatus. These common features are as follows: (1) The oral primordium (OP) in both proter and opisthe are de novo formed in a subcortical pouch; (2) the parental AZM_1 will be partly retained by the proter, i.e. the newly built membranelles formed in the OP will replace the AZM_2 and the leftmost 5 parental ones in the parental AZM_1; (3) five frontal ventral transverse cirral anlagen in both proter and opisthe are derived from the dividing of primary primordium; (4) the anlagen of the leftmost frontal cirrus and left marginal cirri develop de novo; (5) dorsal morphogenesis proceeds within the old kineties and three caudal cirri are formed at the posterior ends of the two rightmost dorsal kineties. However, *Uronychia xinjiangensis* n. sp. differs in forming 3 (vs. 4 in all congeners) membranelles in the AZM_2, 2 (vs. 5–8 in *U. multicirrus* and several to more

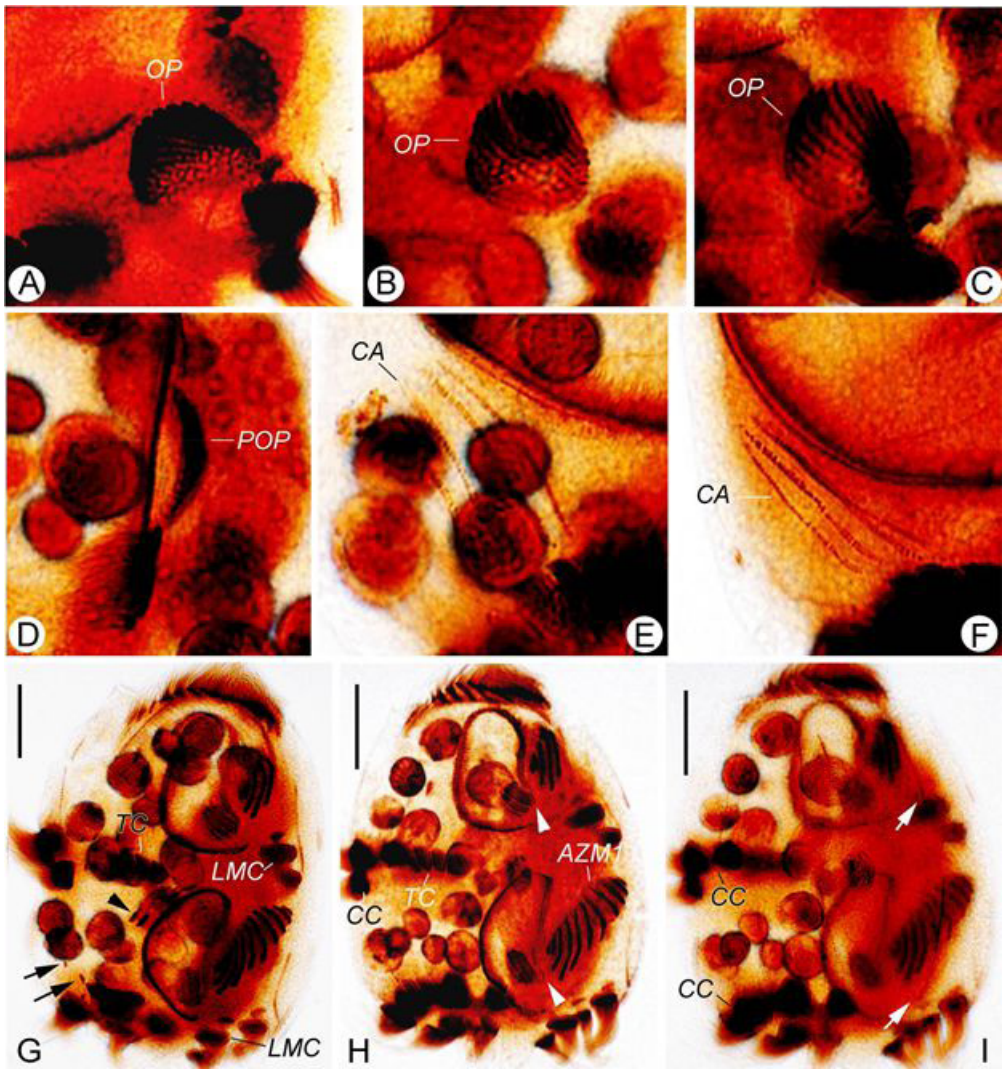

Fig. 6. Photomicrographs of *Uronychia xinjiangensis* n. sp. after protargol staining (A–I). **(A–C)** Opisthe's oral primordium at early dividers. **(D)** Proter's oral primordium. **(E, F)** Fontal-ventral-transverse cirral anlagen of early dividers. **(G)** A later divider showing the completion of development of oral primordium and cirral anlagen, arrows and arrow show newly formed ventral and frontal cirri respectively in the opisthe. **(H, I)** The same late divider showing the posterior part of adoral zone of membranelles (arrowheads) and the longest dorsal kinety 3 (arrows). AZM1, the anterior part of adoral zone of membranelles; CA, cirral anlagen; CC, caudal cirri; LMC, left marginal cirri; OP, opisthe's oral primordium; POP, proter's oral primordium; TC, transverse cirri. Scale bars: 20 μm.

than 10 in *U. transfuga*) macronuclear nodules during binary fission, which supports the separation of these five species.

Like several related euplotids (e.g. *Diophrys*, *Pseudodiophrys*, *Euplotes*), some caudal cirri are derived from the posterior end of the rightmost dorsal kinety in *Uronychia* (Hu 2008; Song *et al.* 2009; Shao *et al.* 2010; Fan *et al.* 2013), which is in accordance with the rightmost caudal cirrus often originated from the posterior end of the 4th dorsal kinety in some hypotrichs (Berger 1999; Kumar *et al.* 2014; Li *et al.* 2016; Lu *et al.* 2017; Luo *et al.* 2017), and implies this feature is an ancestral one. However, compared with other euplotids, *Uronychia* species demonstrate several unusual characters: (1) undulating membrane anlage forming only paroral membrane just like *Euplotes* and *Certesia* rather than paroral and endoral membranes as in *Diophrys* and most diverse hypotrichs, which seems to suggest it is a plesiomorphic character; nevertheless, it cannot be excluded that the highly differentiated paroral membrane

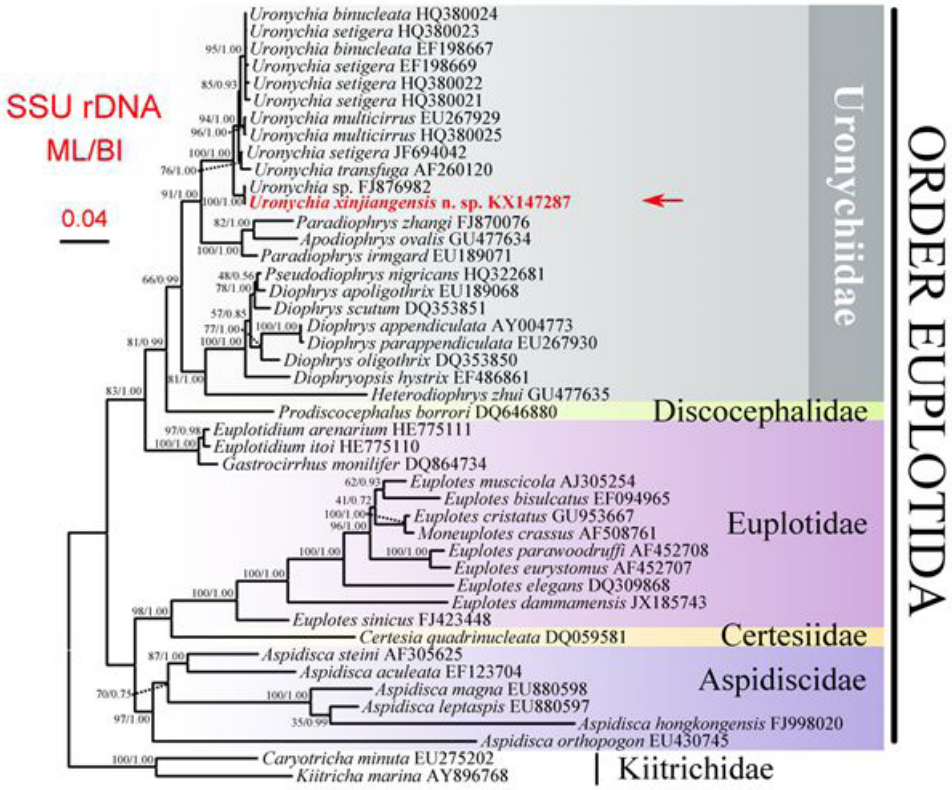

Fig. 7. Phylogenetic tree inferred by ML and BI of SSU rRNA gene sequences. Numbers near branches denote ML bootstraps value/BI posterior probability value. '*' indicates topology that differ between ML and BI phylogenies. All branches are drawn to scale. The scale bar corresponds to 5 substitutions per 100 nucleotide positions. GenBank accession numbers are given for each species. Classification is mainly according to Lynn (2008).

in *Uronychia* is a divergent feature as the basal bodies in it are arranged in short rows, which is distinct from anarchic distribution in *Euplotes*; (2) de novo formation of OP in the proter (vs. no OP in other euplotids); and (3) the piece-together-mode of the AZM_1 in the proter (vs. complete retaining of parental AZM or partly renewal of membranelles at proximal portion).

Molecular comparison with congeners

The phylogenetic analysis is consistent with the morphological result. *U. xinjiangensis* n. sp. clusters with the congeners to form the monophyletic clade with full value, which supports the monophyly of *Uronychia*. The divergence of SSU rRNA gene sequences between *U. xinjiangensis* and four valid *Uronychia* species is over 20 base pairs. Such differences, combined with its unique morphological and morphogenetic features, corroborate the establishment of this species as a distinctive member of *Uronychia*. The SSU rRNA gene sequence variation between *U. xinjiangensis* and *Uronychia* sp. is 2 base pairs and two forms cluster together to form a sister branch with full support value in phylogenetic trees (Fig. 7). From the molecular analyses, these two forms are very likely conspecific, however, the lack of morphologic description makes it unsolved.

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