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ORIGINAL ARTICLE

Morphology, Cell Division, and Phylogeny of *Uroleptus longicaudatus* (Ciliophora, Hypotricha), a Species of the *Uroleptus limnetis* Complex

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ABSTRACT

A Chinese population of the little-known freshwater hypotrich *Uroleptus longicaudatus* was investigated with emphasis on its living morphology and infraciliature. The characteristic, tripartite body consists of a narrowed (cephalized) anterior portion, a slender trunk, and a long, slender, and strongly contractile tail occupying up to 30% of body length. Contracted specimens with a tail length of about 12% closely resemble *Uroleptus limnetis* which has, like *U. longicaudatus*, its type locality on the East Coast of the United States so that it cannot be excluded that these two species are synonymous. Thus, we propose to subsume these and few other little-known species, which are not clearly distinguishable at the present state of knowledge, as *U. limnetis* complex. The morphogenesis of *U. longicaudatus* proceeds as in most congeners. The phylogenetic analyses reveal that *Uroleptus* is a monophyletic group, but due to the lack of detailed morphological data of the populations sequenced so far, the relationships within this taxon remain obscure. For the objective determination of the tail length of hypotrichs, we propose the "1/3-method", which says that the tail commences at that body width which corresponds one-third of the maximum width. *Paruroleptus ophryoglena* Gelei, 1954 is transferred to *Uroleptus*: *Uroleptus ophryoglena* (Gelei, 1954) comb. nov.

UROLEPTUS Ehrenberg, 1831 is a moderately large genus of hypotrichous ciliates which have, inter alia, a more or less distinct tail, three frontal cirri, a midventral complex composed of cirral pairs only, few to many transverse cirri, usually three bipolar dorsal kineties each bearing a caudal cirrus, and two or more dorsomarginal kineties (Berger 2001; He et al. 2011). The species occur throughout the world in limnetic and terrestrial habitats, but usually their abundance is low (for reviews, see Foissner 1998; Foissner et al. 1991). Previously, *Uroleptus* was classified, inter alia, in the Urostylidae Bütschli, 1889 (e.g. Small and Lynn 1985) or the Holostichidae Fauré-Fremiet, 1961 (e.g. Corliss 1979) because all these taxa have zigzagging midventral cirri. However, the presence of dorsomarginal kineties shows that *Uroleptus* is more closely related to the oxytrichids (with dorsomarginal kineties and kinety fragmentation) than to the urostyloids, which lack this part of the dorsal ciliature (Berger 1999, 2006; Foissner et al. 2004; He et al. 2011). The separation of *Uroleptus* from the Urostylidae and Holostichidae is not only evident from the dorsal kinety pattern but also from gene sequence analyses (e.g. Bharti et al. 2014; Chen et al. 2013; Fan et al. 2014; Foissner et al. 2014; Heber et al. 2014; Hewitt et al. 2003; Kumar et al. 2014, 2015; Li et al. 2014; Lv et al. 2015; Singh and Kamra 2014; Sonntag et al. 2008). Mainly for the latter reason, Foissner and Stoeck (2008) established the Uroleptidae, which are—due to the presence of dorsomarginal kineties—a subgroup of the Dorsomarginalia Berger 2006 according to Berger (2008).

Although some species are known for more than 240 yr, for example *Trichoda musculus* Müller, 1773, *Uroleptus* is still a difficult genus whose complex history and systematics, including its intricate type species problem, were briefly analyzed by He et al. (2011). Major issues are the lack of relevant morphological details (e.g. body size, presence/absence of transverse cirri) in some important early studies (e.g. Ehrenberg 1838; Müller 1786; Stein 1859); the overlap of some species in essential diagnostic features, for example, body size or length of tail (e.g. Kahl 1932; Stokes 1888); the difficulty to recognize the cirral and dorsal kinety pattern of the usually slender tail region correctly, even in protargol preparations; and the lack of detailed morphological studies and gene sequence analyses in most species.

In the present paper, we describe and analyze the morphology, the cell division, and the SSU rDNA of a distinctly tailed species from a limnetic habitat in China. We suppose that it is identical with *Uroleptus longicaudatus*, a little-known species discovered by Stokes (1886) on the East Coast of the United States. Our study is a further, small step to unravel the complex taxonomy of this group of spirotrichous ciliates.

MATERIALS AND METHODS

Sample site and cultivation

On 15 May 2012, a sample of surface water (0–10 cm) containing the present species was collected from a pond (water temperature about 19 °C, pH 8.0) near the Tengfei Tower in the center of the Xi'an Jiaotong University Campus (34°14′54″N, 108°59′01″E), Xi'an, China (Fig. S1). Subsamples were transferred to Petri dishes (10 cm across) and maintained as uniprotistan cultures in the laboratory at about 24 °C. Few wheat grains were added to support the growth of bacteria, besides diatoms an important food of our *Uroleptus* population (Fig. 1B,C, 2E).

Unfortunately, we could not establish a clone and therefore we cannot be 100% sure that the specimens used for the morphological studies and molecular analyses belong to the same species. However, as no other *Uroleptus* morphotypes have been present in the protargol preparations, the probability is extremely high that our morphological and molecular studies deal with the same species.

Morphology, morphogenesis, and voucher material

Ciliates were examined in vivo using bright field and differential interference contrast microscopy. The protargol method according to Wilbert (1975) was used to reveal the infraciliature and nuclear apparatus. Measurements and counts on protargol-prepared specimens were performed at a magnification of 1,250X and drawings were made with the help of a camera lucida. To illustrate the changes occurring during cell division, old (parental) structures are depicted by contour, whereas new structures are shaded black. Five voucher slides are deposited in the Laboratory of Protozoology, OUC, China, with the registration numbers CLY12051501/A to CLY12051501/E.

Uroleptus species have a more or less distinctly tailed body (Kahl 1932). Unfortunately, there is no useful structure at the base of the tail to which one could refer to describe the length of this body portion exactly. Thus, we propose the following procedure to describe the length of the tail objectively: (i) Measure the largest body width in ventral or dorsal view (for terminology, see Berger 2008, p. 2). (ii) Measure the distance from the posterior end of the cell (= tip of tail) to that body width which corresponds one-third of the largest body width; this is the tail length; when the tail is curved, measure the arc and not the chord. (iii) Divide the tail length by the total body length (that is, including tail) to get the relative length of the tail; to get the relative length in percent, multiply with 100. We recommend to refer to this procedure as the "1/3-method". Vdačný and Foissner (2012, p. 5) circumscribed the length of the tail in their revision on dileptids as follows: short (tail < 20% of body length); distinct (20%; likely they meant 20 to < 33%); long (33–50%). We omit such circumscriptions and just mention the percentage. The tail of *Uroleptus* species is more or less distinctly contractile, and therefore it has to be measured when it is fully extended. It is recommended to make micrographs or video sequences of undisturbed, freely motile specimens to estimate the tail length (e.g. Fig. 1A). Undoubtedly, it is also important to know the contractility of the tail to describe the morphology of a *Uroleptus* species in detail. Of course, the objectification of the length measurement is just one step in the refinement of *Uroleptus* taxonomy. Another, not yet solved problem is the separation of species on the basis of the tail length, the main feature applied by Stokes (1885, 1886, 1888) when separating *U. limnetis* (tail occupying about 20%) and *U. longicaudatus* (about 33%).

Terminology and systematics

General terminology is mainly according to Lynn (2008); for terms specific for hypotrichs (e.g. DE-value, midventral complex, pseudorow, mixed row), see Berger (1999, 2006, 2008, 2011) and Foissner and Al-Rasheid (2006). Systematics is according to Berger (2008), Foissner and Stoeck (2008), and Adl et al. (2012). As this is a mainly taxonomic paper, "nomenclatural" references are also listed in the reference section.

DNA extraction, PCR amplification, and sequencing

Three randomly selected cells of the *U. longicaudatus* morphotype were isolated and repeatedly washed using sterile water. Then they were transferred to a 2-ml microfuge tube with the minimum volume of water. Genomic DNA was extracted using REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO) following the manufacturer's instructions. The gene coding for the ribosomal small subunit (SSU rDNA) was amplified with the eukaryotic universal SSU rRNA primers pr Forward (5′-AAC CTG GTT GAT

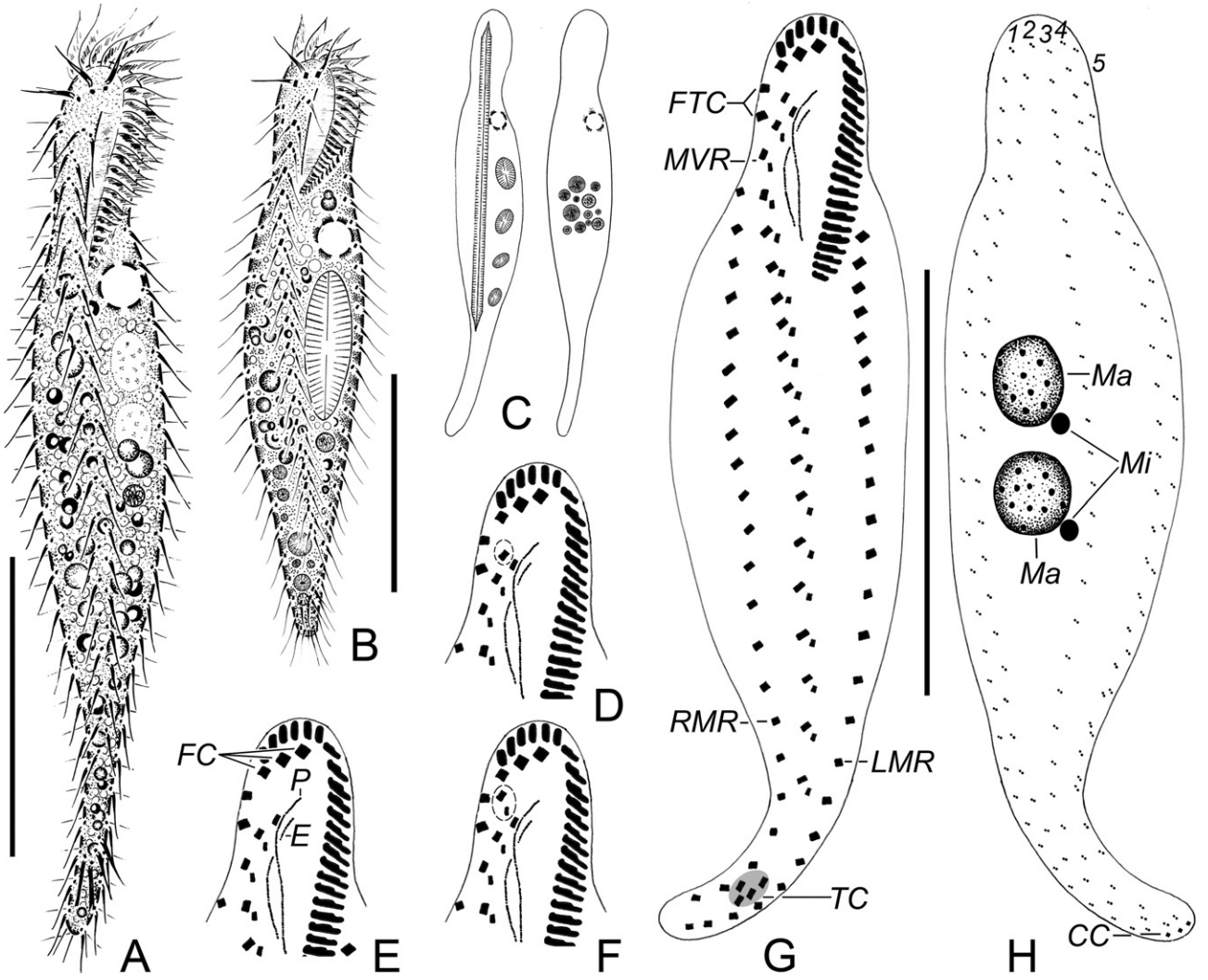

Figure 1 **A–H.** Morphology of Chinese population of *Uroleptus longicaudatus* from life (A–C) and after protargol preparation (D–H). **A.** Ventral view of a representative, completely(?) extended individual showing, inter alia, cirral pattern, nuclear apparatus, and position of contractile vacuole. **B.** Ventral view of cell with retracted tail and ingested diatom. **C.** Ventral views of fully(?) extended specimens having ingested short and long diatoms and green algae. **D–F.** Diagrammatic drawings of specimens which have one (D), zero (E), or two (F) parabuccal cirri (dashed circles). **G, H.** Infraciliature of ventral and dorsal side and nuclear apparatus of same specimen. CC = caudal cirri; E = endoral; FC = frontal cirri; FTC = frontoterminal cirri; LMR = left marginal row; Ma = macronuclear nodules; Mi = micronuclei; MVR = second pair of midventral complex; P = paroral; RMR = right marginal row; TC = transverse cirri; 1–5 = dorsal kineties (4 and 5 are dorsomarginal rows). Scale bars: 65 μm.

CCT GCC AGT-3′) and Reverse (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin et al. 1988; Yi and Song 2011). High-fidelity *Taq* polymerase (Takara Ex *Taq*; Takara Biomedicals) was used to minimize the possibility of amplification errors. The amplification cycles were as follows: 5 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min 50 s; the final extension was 7 min at 72 °C (Huang et al. 2010). The PCR product was purified using San-Prep DNA Gel Extraction Kit (Sangon Bio. Co., Shanghai, China) and then inserted into the pMD™ 19-T vector (Takara Biotechnology, Dalian Co., Ltd.). Sequencing was performed bidirectionally on an ABI 3700 sequencer (Invitrogen sequencing facility, Shanghai, China) using primers M13-47 (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′), M13-48 (5′-AGC GGA TAA CAA TTT CAC ACA GGA-3′), and three internal primers 900 F (5′-CGA TCA GAT ACC GTC CTA GT-3′), 900R (5′-ACT AGG ACG GTA TCT GAT CG-3′) and Pro B (5′-GGT TAA AAA GCT CGT AGT-3′).

Phylogenetic analyses

The SSU rDNA of *U. longicaudatus* was aligned to the sequences of 43 other spirotrichous ciliates from GenBank database using the online program Muscle 3.7 (Edgar 2004). The accession numbers are shown after the species names in the phylogenetic tree (Fig. 6). Subsequently, these sequences were aligned using Clustal

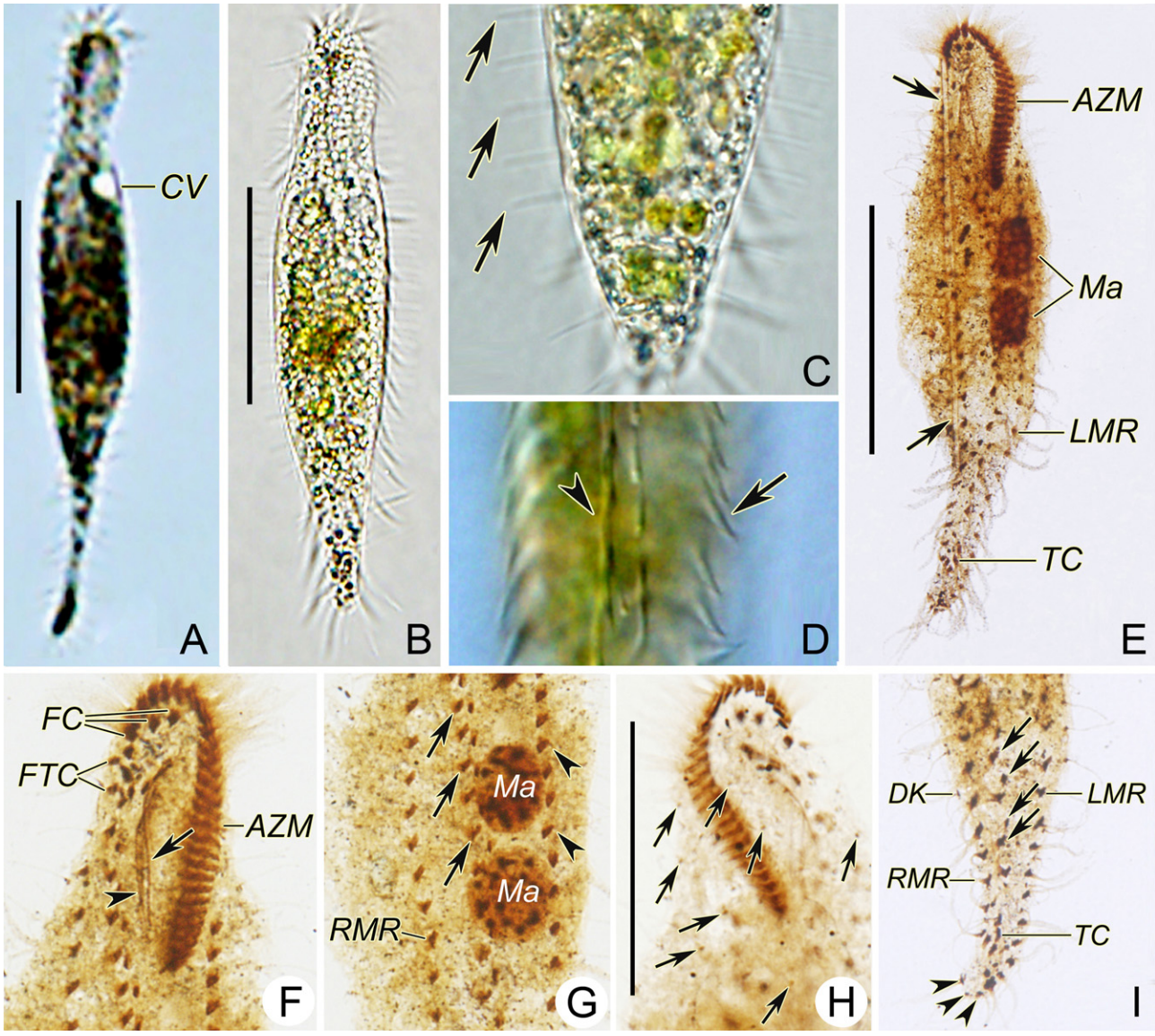

Figure 2 **A–I.** Chinese population of *Uroleptus longicaudatus* from life (A–D) and after protargol preparation (E–I). **A.** Ventral view of a representative specimen with fully extended tail. **B.** Ventral view of a cell slightly compressed under the cover glass and retracted tail. **C.** Posterior body portion (tail retracted) in ventral view showing dorsal cilia (arrows) of outermost kineties. **D.** Posterior body portion of contracted specimen showing left marginal row (arrow) and midventral cirri (arrowhead). **E.** Ventral view of infraciliature, arrows mark a long diatom (*Synedra* sp.). **F.** Infraciliature of anterior body portion showing, inter alia, the endoral (arrowhead) and paroral (arrow). **G.** Infraciliature of central body portion in ventral view and macronuclear nodules. Arrows mark right cirri of midventral pairs, arrowheads mark left marginal cirri. **H.** Dorsal view of anterior body portion showing some dikinetids of dorsal kineties (arrows) and adoral zone of membranelles. **I.** Infraciliature of tail region in ventral view showing rearmost cirri of midventral complex (arrows), transverse cirri, and caudal cirri (arrowheads). AZM = adoral zone of membranelles; CV = contractile vacuole; DK = rear end of dorsal kinety 4; FC = frontal cirri; FTC = frontoterminal cirri; LMR = left marginal row; Ma = macronuclear nodules; RMR = right marginal row; TC = transverse cirri. Scale bars: 60 μm (A, B); 55 μm (E); 40 μm (H).

W implemented in Bioedit 7.0.9 with default parameters (Hall 1999). Regions that could not be aligned unambiguously were removed and ends were trimmed manually, resulting in a matrix of 1,722 characters. The program MrModeltest v.2.0 (Nylander 2004) selected the GTR + *I* (=0.5232) + *G* (=0.4967) as the best model with Akaike information criterion, which was then used for Bayesian inference (BI). BI analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Maximum likelihood analysis was constructed with the RaxML online program (Stamatakis et al. 2008). TreeView v1.6.6 (Page 1996) and MEGA 4.0 (Tamura et al. 2007) were used to visualize tree topologies. *Tintinnopsis tubulosoides*, *T. dadayi*, *Parastrombidinopsis minima* and *Strombidinopsis acuminata* were selected as the outgroup taxa.

Table 1. Morphological characterization of *Uroleptus longicaudatus*[a]

Character	Min	Max	Mean	M	SD	CV	*n*
Body, length	121.0	182.0	149.4	150	17.9	12.0	18
Body, width	31.0	43.0	35.3	35	4.4	12.3	18
Tail, relative length (%)	30	35	31.8	31	1.7	5.2	11
Adoral zone of membranelles, length	23.0	46.0	35.8	35	5.5	15.3	18
AE to anterior end of RMR, distance	20	25	23.6	24	1.7	7.3	18
AE to anterior end of LMR, distance	25	30	27.1	26	2.0	7.6	18
AE to buccal cirrus, distance	10	12	11.1	11	0.6	5.2	18
AE to anteriormost midventral pair, distance	14	21	16.5	16	1.8	11.0	18
AE to anterior macronuclear nodule, distance	40	55	45.5	45	4.8	10.5	18
AE to posterior end of dorsal kinety 5, distance	100	120	113.6	115	6.8	6.0	10
AE to posterior end of kinety 4, distance	110	135	122.0	120	7.9	6.5	10
PE to rearmost transverse cirrus, distance	13	20	14.7	14	2.1	14.0	10
PE to rear end of midventral complex, distance	15	25	20.7	20	2.5	12.2	18
Anterior macronuclear nodule, length	14	21	15.8	15	1.7	10.8	18
Anterior macronuclear nodule, width	6	15	7.2	6	2.4	33.1	18
Posterior macronuclear nodule, length	10	20	14.4	14	2.2	15.2	18
Posterior macronuclear nodule, width	5	14	7.4	7	2.2	29.9	18
Macronuclear nodules, distance in between	1	5	4.3	5	1.3	29.6	18
Adoral membranelles, number	21.0	32.0	26.2	26	2.1	8.1	18
Frontal cirri, number	3.0	3.0	3.0	3	0.0	0.0	18
Buccal cirri, number	1.0	1.0	1.0	1	0.0	0.0	18
Parabuccal cirri, number	0	2.0	1.0	1	0.4	41.5	20
Frontoterminal cirri, number	2.0	3.0	2.2	2	0.4	17.7	18
Midventral complex, number of pairs	15.0	23.0	18.2	18	2.3	12.4	18
Transverse cirri, number	3.0	4.0	3.8	4	0.4	11.3	18
Left marginal cirri, number	16.0	33.0	25.2	24	4.1	16.2	18
Right marginal cirri, number	16.0	28.0	21.4	22	2.6	12.3	18
Dorsal kineties, number	5.0	5.0	5.0	5	0.0	0.0	15
Dorsal kinety 1, number of dikinetids	27	35	31.4	32	2.5	8.1	10
Dorsal kinety 2, number of dikinetids	26	31	29.0	30	1.9	6.5	10
Dorsal kinety 3, number of dikinetids	24	30	26.8	28	2.1	7.8	10
Dorsal kinety 4, number of dikinetids	23	29	27.2	28	1.6	6.0	10
Dorsal kinety 5, number of dikinetids	17	23	20.0	20	2.0	9.9	10
Dorsal dikinetids, total number	118	143	134.7	138	8.8	6.6	10
Caudal cirri, number	3.0	3.0	3.0	3	0.0	0.0	11
Macronuclear nodules, number	2.0	2.0	2.0	2	0.0	0.0	18
Micronuclei, number	1	2	1.9	2	0.3	17.1	18

[a]All data are based on protargol-prepared specimens. All measurements are in μm.
AE = anterior end of cell; CV = coefficient of variation in %; LMR = left marginal row; M = median; Max = maximum; Mean = arithmetic mean; Min = minimum; n = sample size; PE = posterior end of cell; RMR = right marginal row; SD = standard deviation.

RESULTS

Morphology of Chinese population of *U. longicaudatus*

Body size in vivo about 120–200 × 30–45 μm, usually around 150 × 35 μm; ratio of length to width of extended specimens about 6–7:1 (Fig. 1A, 2A). Body outline of stretched specimens in ventral view distinctly tripartite: (i) anterior portion "cephalized", that is, somewhat narrowed with smallest width at about 20% of body length; (ii) body proper (trunk) elongate elliptical, that is, body margins slightly convex; and (iii) more or less parallel-sided tail slightly curved rightwards and occupying up to 30% (1/3-method) of body length (Fig. 2A). Contracted specimens (e.g. under cover glass pressure; Fig. 1B, 2B) fusiform, that is, anterior portion only indistinctly cephalized and tail strongly retracted to about 12% of body length in specimen shown in Fig. 2B. Body distinctly flattened dorsoventrally; ventral side plane, dorsal side of body proper vaulted. Invariably two macronuclear nodules closely spaced left of cell midline in central body portion; individual nodules ellipsoidal, average length:width ratio 2.2:1 and 1.9:1 in anterior and posterior nodule respectively, with small nucleoli (Table 1; Fig. 1A,H, 2E,G). Contractile vacuole in ordinary position, that is, near left cell margin at about 25% of body length in extended specimens (Fig. 2A). No collecting canals seen. Cortical granules lacking. Cytoplasm colorless, typically with food vacuoles containing green algae on average 5 μm across, roundish and

longish (up to 90 μm) diatoms (*Synedra* sp.), and bacteria (Fig. 1A–C, 2A–E); thus, central body portion often dark at low magnification (Fig. 2A). Movement without peculiarities, that is, slowly to moderately fast crawling on substrate; sometimes motionless.

Adoral zone occupies 20–30% of body length in vivo and 24% on average in protargol preparations, composed of an average of 26 membranelles, formed like a question mark, extends only slightly onto right body margin, that is, DE value 0.18–0.20. Membranelles of ordinary fine structure, that is, composed of two long and one moderately and one very strongly shortened row of basal bodies; bases of largest membranelles about 6-μm wide and cilia up to 20-μm long in vivo. Both undulating membranes distinctly curved, intersect optically somewhat behind level of buccal cirrus, paroral slightly longer than endoral (Table 1; Fig. 1A,G, 2E,F,H).

Cirral pattern typically uroleptid, rather constant; number of cirri of usual variability, i.e. coefficient of variation about 15% or less, except for number of parabuccal cirri (see below; Table 1; Fig. 1D–H, 2E–I). Three slightly enlarged frontal cirri arranged in somewhat oblique pseudorow with right cirrus immediately behind distal end of adoral zone; cirri in vivo about 20-μm long. Buccal cirrus somewhat behind level of anterior end of paroral. Parabuccal cirrus (=III/2) about at level of frontoterminal cirri and, as usual, smaller than right frontal cirrus; rarely (about 10% of individuals analyzed) no cirrus or two cirri present (Fig. 1D–G). Usually two, sometimes three frontoterminal cirri between distal end of adoral zone and anterior end of midventral complex. Midventral complex typically uroleptid, that is, composed of pairs forming characteristic zigzag pattern; right (=anterior) cirrus of each pair larger and differently aligned than left (=posterior) cirrus; complex commences slightly behind parabuccal cirrus, extends into proximal portion of tail, composed of 18 pairs on average, distance between individual pairs somewhat wider in posterior half than in anterior (Table 1; Fig. 1A,B,D–H, 2E–G,I). Pretransverse ventral cirri obviously lacking in interphasic specimens. Usually four, sometimes only three transverse cirri arranged in hook-shaped pseudorow in between rearmost portion of marginal rows; transverse cirri of about same size and length as marginal cirri, not projecting beyond posterior end of tail and only inconspicuously protruding left laterally and thus very difficult to recognize in life (Fig. 1A,G, 2B,E,I). Right marginal row commences behind frontoterminal cirri, extends—like left row—to end of tail leaving blank a small gap optically occupied by the dorsally inserted caudal cirri; left marginal row begins left of proximal portion of adoral zone and terminates at tip of tail (Fig. 1G, 2E,I).

Dorsal bristles about 5- to 8-μm long, conspicuous because orthogonally protruding from cell margin; constantly arranged in five kineties (Table 1; Fig. 1A,H, 2C,H): dorsal kineties 1, 2, and 3 bipolar, that is, extending from anterior to posterior end of cell; dorsal kineties 4 and 5 (=dorsomarginal kineties) terminating roughly at base of tail (Fig. 1H). Kinety 1 composed of 31 dikinetids on average, kinety 2 of 29, kinety 3 of 27, kinety 4 of 27, and kinety 5 of 20 (Table 1), that is, in total 134 kinetids on average. Caudal cirri inconspicuous because of about same length (ca. 8 μm) as marginal cirri; each one cirrus at rear end of kineties 1, 2, and 3 (Fig. 1H, 2I).

Cell division

We found several dividers in various stages and therefore can provide a brief characterization of this part of the life cycle. Unfortunately, some preparations are not perfect so that some details remain obscure.

Oral apparatus and frontal, midventral, and transverse cirri

Stomatogenesis commences with the epiapokinetal formation of the oral primordium left of the middle portion of the midventral complex (Fig. 3A, 5A). Somewhat later, the right anterior portion of the oral primordium is frayed; obviously, no parental cirri are involved in anlagen formation at this stage (Fig. 3B, 5B). In the next stage observed, the anterior portion of the oral primordium is modified into membranelles (Fig. 3C,D, 5C). In the same specimen, two longitudinal fields of frontal–midventral–transverse cirri anlagen are present. The major part of the anterior field, which will form the cirri of the proter, extends left of the anterior portion of the parental midventral complex; only the posterior quarter crosses the midventral complex and terminates next to the right marginal row. The posterior field, which will form the cirri of the opisthe, extends mainly right of the parental midventral complex and almost parallel to the oral primordium. It remains unclear whether the two fields are connected in the area where they cross the midventral complex in a somewhat earlier divider. In addition, we cannot ascertain beyond doubt whether cirri of the parental midventral complex have been involved in the formation of the anlagen fields. As in many other hypotrichous ciliates, the parental adoral zone of membranelles is retained without distinct changes during morphogenesis. Thus, the alterations of the oral structures of the proter are confined to the paroral and endoral (Fig. 3A–D,F,H, 4A,C, 5G). At early stages, the parental undulating membranes appear unchanged (Fig. 3A, B).

In the next stage which we found, all new membranelles of the opisthe are available (Fig. 3F). Twenty-two frontal–midventral–transverse cirri anlagen (including the undulating membrane anlage) are present in the proter while 23 anlagen have been formed in the opisthe. The differentiation of cirri is in progress. The undulating membrane anlage of both filial products is a single, almost straight row, likely composed of basal body pairs. As is usual for hypotrichs, the left frontal cirrus (=cirrus I/1) originates from this anlage (Fig. 3F).

In late and very late dividers, the anterior portion of the adoral zone of the opisthe turns to the right while the undulating membrane anlagen are modified into the paroral and endoral in both the proter and the opisthe (Fig. 3H, 4A,C, 5F). Simultaneously, the final number of frontal–midventral–transverse cirri is formed and the cirri begin to migrate to their final positions. The most conspic-

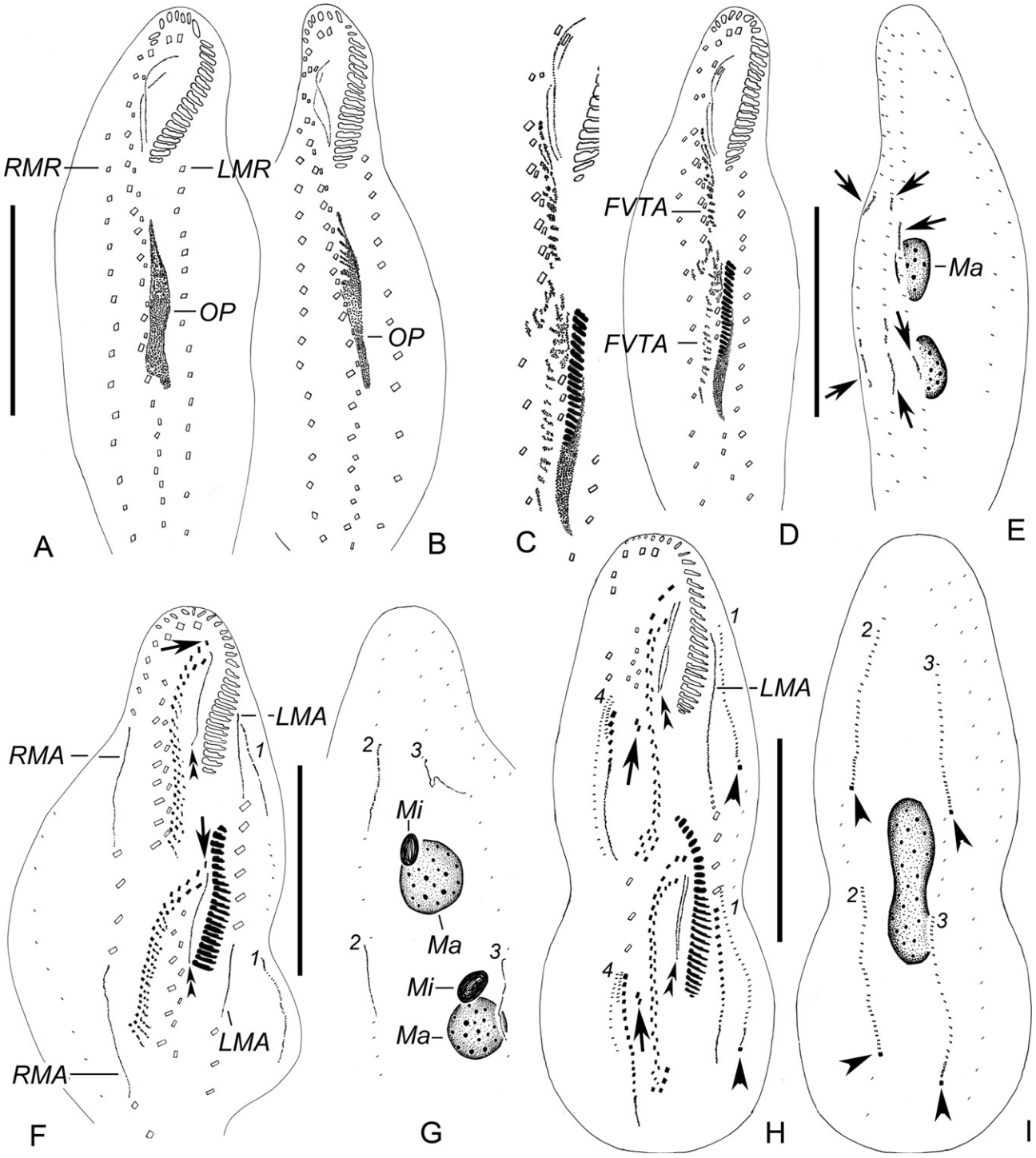

Figure 3 **A–I.** Morphogenesis of Chinese population of *Uroleptus longicaudatus* after protargol preparation. **A, B.** Infraciliature of ventral side of very early dividers. **C–E.** Infraciliature of ventral and dorsal side and macronuclear apparatus of an early divider; arrows mark the anlagen in dorsal kineties 1–3. **F, G.** Infraciliature of ventral and dorsal side and nuclear apparatus of a middle divider. The undulating membranes anlagen (double arrowheads) produce as usual the leftmost frontal cirrus (arrows) in both filial products. **H, I.** Infraciliature of ventral and dorsal side and fused macronucleus of a late divider showing, inter alia, the undulating membranes anlage generating the paroral and endoral in each filial product (double arrowheads), the anteriorly migrating frontoterminal cirri (arrows), and the caudal cirri (arrowheads) on dorsal kineties 1, 2, and 3. FVTA = frontal-ventral-transverse cirral anlagen; LMA = left marginal row anlagen; LMR = left marginal row; Ma = macronuclear nodules; Mi = micronucleus; OP = oral primordium; RMA = right marginal row anlagen; RMR = right marginal row; 1–4 = dorsal kineties. Scale bars: 40 μm.

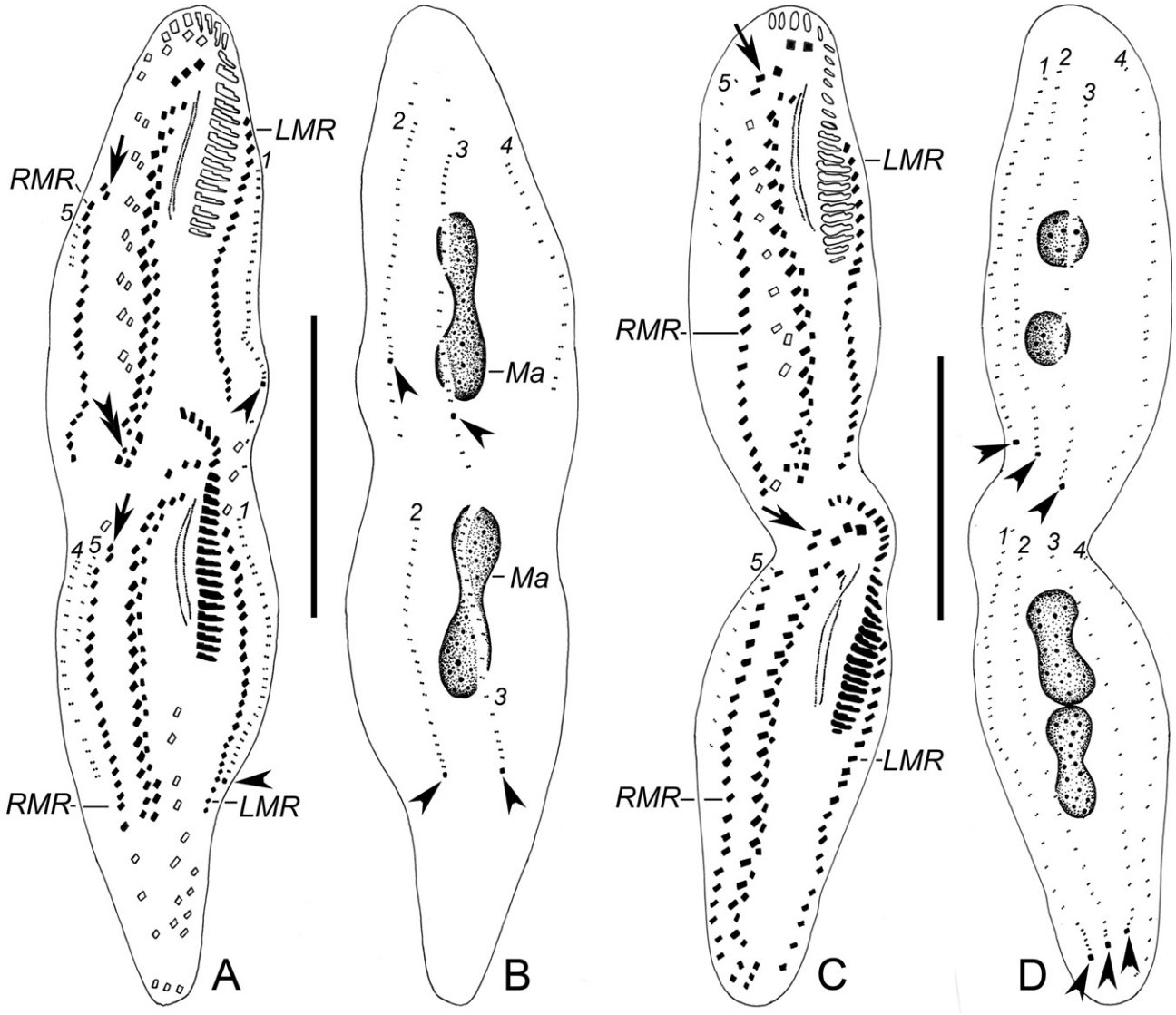

Figure 4 **A–D.** Morphogenesis of Chinese population of *Uroleptus longicaudatus* after protargol preparation. Infraciliature of ventral (**A**, **C**) and dorsal (**B**, **D**) side and macronuclear apparatus of late (A, B) and very late (C, D) divider showing, inter alia, the new frontoterminal (arrows), and caudal cirri (arrowheads). Double arrowhead in (A) marks pretransverse ventral cirrus of proter. LMR = left marginal row; Ma = macronuclear nodules; RMR = right marginal row; 1–3 = bipolar dorsal kineties; 4 , 5 = dorsomarginal kineties. Scale bars: 60 μm.

uous migration is done by the two anteriormost cirri of the rightmost anlage; they are displaced to near the frontal cirri to form the frontoterminal cirri. The majority of anlagen forms two cirri each, including anlagen II (middle frontal cirrus and buccal cirrus) and III (right frontal cirrus and parabuccal cirrus) (Fig. 3H, 4A,C). As described above, anlage I forms the left frontal cirrus only. Only the rearmost four (n−3 to n; sometimes only three, then n−2 to n) anlagen produce more than two cirri. Anlage n forms the rightmost transverse cirrus and the two (rarely three) frontoterminal cirri; anlage n−1 forms the next transverse cirrus, a pretransverse ventral cirrus (Fig. 3H, 4A,C), and the rearmost midventral pair; anlage n−2 and n−3 form each a transverse cirrus and a midventral pair. The pretransverse ventral cirrus formed from anlage n−1 (see above) is not recognizable in interphasic specimens, indicating that it is dissolved in postdividers (Fig. 1G, 4A,C).

Marginal rows

The new marginal rows originate as usual. One anlage each develops for the proter and the opisthe within each parental row in middle dividers (Fig. 3F, 5D). These anlagen then stretch into both directions and gradually replace the parental rows (Fig. 3H, 4A,C, 5D,G).

Dorsal ciliature

The dorsal ciliature develops according to the *Urosomoida*-pattern (Berger and Foissner 1997; Berger 1999; =type 2 in Foissner and Adam 1983). The new dorsal kineties 1–3 originate at two levels within the parental rows 1–3

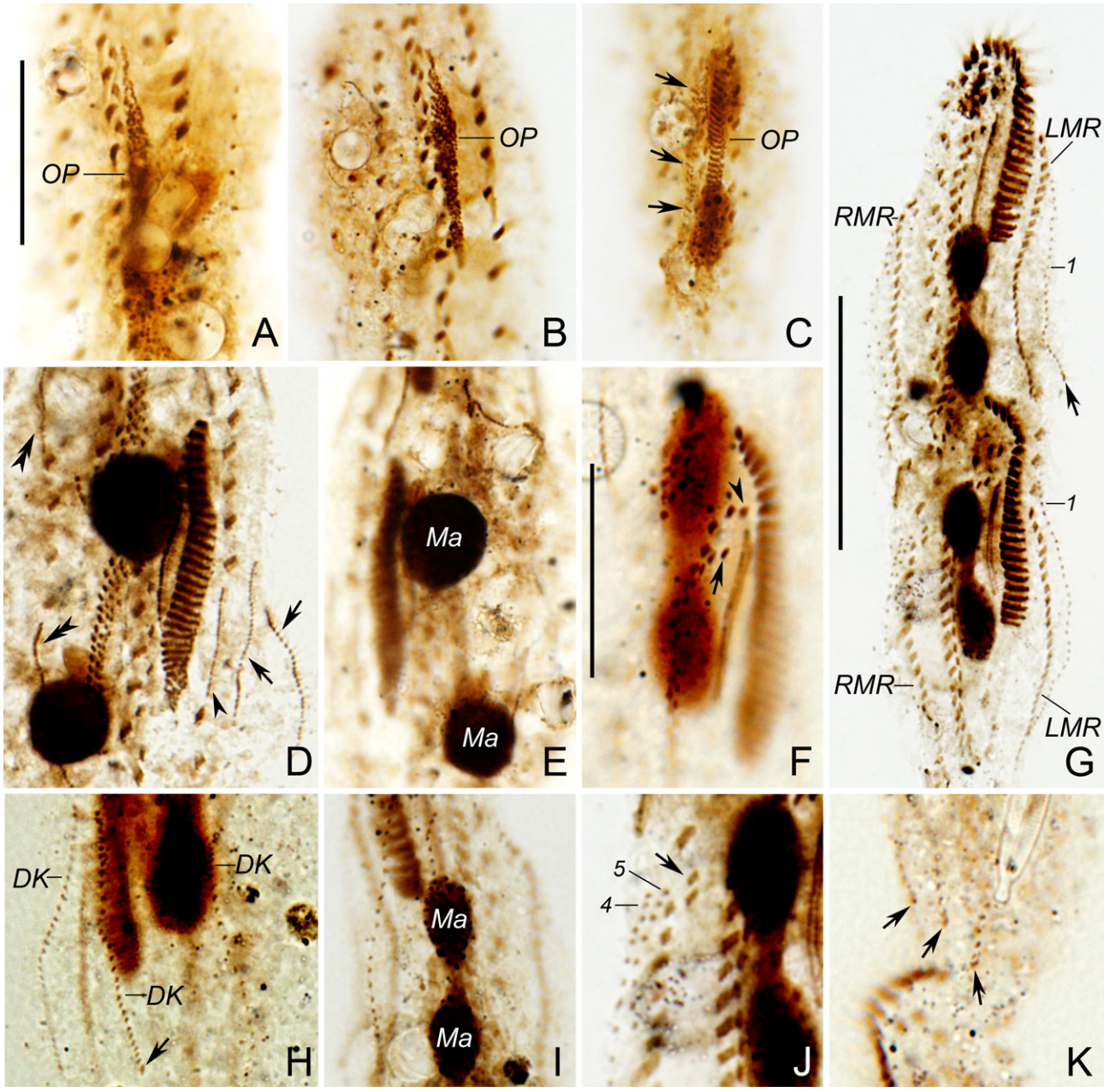

Figure 5 **A–K.** Photomicrographs of dividers of Chinese population of *Uroleptus longicaudatus* after protargol preparation. **A–C.** Ventral views of early dividers showing oral primordium and frontal-ventral-transverse cirral anlagen (arrows). **D.** Ventral view of middle divider. Arrowhead marks left marginal row anlage of opisthe, double arrowhead denotes right marginal row anlagen; arrows marks dorsal kinety anlagen 1 and 2 of opisthe. **E.** Same divider as shown in (D) with focus on macronuclear nodules of opisthe (seen from dorsal). **F.** Ventral view of middle to late divider showing, inter alia, the left frontal cirrus (I/1; arrowhead) and the buccal cirrus (II/2; arrow) of the opisthe and the fused macronucleus. **G, I, J.** Ventral (G, J) and dorsal (I) view of late divider showing, inter alia, new caudal cirrus at rear end of dorsal kinety 1 of proter (arrow in G), dividing macronucleus of proter (I), and anteriorly migrating frontoterminal cirri of opisthe (arrow in J). **H, K.** Dorsal view of late and very late divider showing new dorsal kineties 1–3 of opisthe (H) and proter (K); arrows mark new caudal cirri. DK = dorsal kineties; LMR = new left marginal row; Ma = macronuclear nodules; OP = oral primordium; RMR = new right marginal row; 1 = new dorsal kinety 1 of proter and opisthe; 4, 5 = anlagen of dorsomarginal kineties of opisthe. Scale bars: 40 μm.

(Fig. 3E). The anlagen subsequently elongate and the parental structures are incorporated or resorbed (Fig. 3G,I, 4B, 5D,G–I). By contrast, rows 4 and 5 are dorsomarginal kineties, that is, their anlagen originate close to (from?) the anterior end of the right marginal row anlage in each filial product, and subsequently they migrate onto the dorsolateral surface (Fig. 3H, 4A–D, 5J). One caudal cirrus each is formed at the posterior end of dorsal kineties 1, 2,

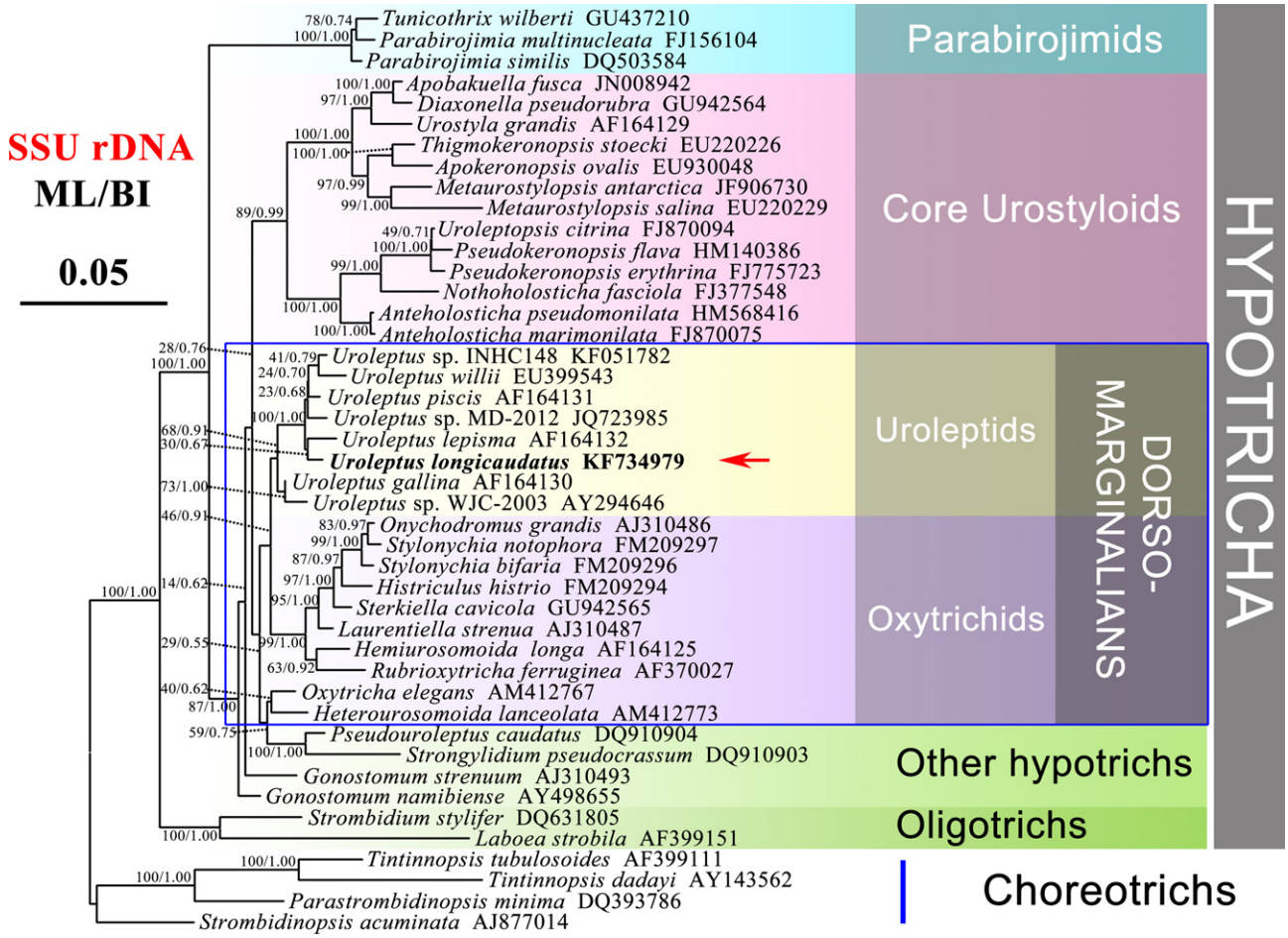

Figure 6 The maximum likelihood (ML) tree based on the SSU rDNA gene sequences showing the position of the Chinese population of *Uroleptus longicaudatus* (arrow). Numbers near nodes are nonparametric bootstrap values for maximum likelihood and posterior probability values for Bayesian inference (BI). The scale bar corresponds to 0.05 expected substitutions per site.

and 3 (Fig. 1H, 3H,I, 4A,B,D, 5G,H,K). No parental structures are retained after division and no dorsal kinety fragmentation occurs.

Nuclear apparatus

The division of the macronuclear apparatus proceeds as described for the ground pattern of the hypotrichs and hence needs no detailed description (Berger 2008). Briefly, the two macronuclear nodules fuse to a single mass which subsequently makes successive amitotic divisions to produce the species-specific number of nodules in each filial product (Fig. 3E,G,I, 4B,D, 5E,I,J). We could not recognize the micronuclei in most dividers and therefore cannot make a comment about their division.

SSU rRNA gene sequence analysis and phylogenetic analyses

The partial SSU rDNA (GenBank accession number KF734979) of our population of *U. longicaudatus* has a length of 1,594 bp and a G + C content of 46.42%. The topologies of the ML and BI trees are similar and therefore only the ML tree is shown (Fig. 6). According to the phylogenetic analyses of the 44-taxon alignment, *U. longicaudatus* is sister of *U. lepisma* (AF164132) in both trees, however, with low support (ML/BI, 30/0.67); the SSU rDNA sequence similarity is 98.3%. This clade clusters with strong support (100/1.00) with a weakly supported clade consisting of *U. piscis* (AF164131), *U. willii* (EU399543) and two unidentified *Uroleptus* species (JQ723985 and KF051782). *Uroleptus gallina* (AF164130) + *Uroleptus* sp. WJC-2003 (AY294646) are sister to this group. The entire *Uroleptus* clade is moderate weakly supported (68/0.91) and is sister (also with not high support 46/0.91) of a group containing the stylonychines. Both groups have dorsomarginal kineties and are major taxa of the Dorsomarginalia Berger 2006.

DISCUSSION

General remarks

As already briefly mentioned in the introduction, *Uroleptus* is a very difficult genus due to various serious problems.

Thus, we recommend reading relevant chapters of the discussion in He et al. (2011), where the complex situation is briefly reviewed, for example, the type species problem of *Uroleptus* and the invalidity of the subgenus *Holosticha (Paruroleptus)* Kahl, 1932.

Identification of Chinese population as *U. longicaudatus* and comparison with similar species

Morphologically speaking, two groups can be clearly distinguished in *Uroleptus*. One group comprises those species which have distinctly more than five, conspicuous transverse cirri, for example, *Uroleptus gallina* (Müller, 1786) Foissner et al., 1991 and *Uroleptus piscis* (Müller, 1773) Ehrenberg, 1831 (for reviews, see Foissner et al. 1991). The present population belongs to the second group whose species have five or less, inconspicuous transverse cirri (Fig. 1G, 2I, 4A). Wenzel (1953) established *Paruroleptus* for this group with *Holosticha caudata* Stokes, 1886 as type species (Fig. 7A). This species has five transverse cirri and is 508 × 63 μm large according to the original description and Stokes (1888), strongly indicating that our population (three or four transverse cirri, in vivo 120–200 × 30–45 μm, usually around 150 × 35 μm) does not belong to this species. Just recently, He et al. (2011) confirmed the existence of the very large *U. magnificus* (in vivo 400–500 μm long according to Kahl 1932; up to 400 μm in protargol preparations) so that it would be unwise to assume that the size provided by Stokes (1886) is a measuring error or the size of this species is extremely variable.

The specimens of our limnetic population (Fig. 1A,B, 2A,B, 7C,F) closely resemble *Uroleptus limnetis* Stokes, 1885 (Fig. 7B) and *U. longicaudatus* Stokes, 1886 (Fig. 7E), two little-known species discovered in fresh water from the East Coast of the United States. These two species differ almost exclusively in the length of the tail, namely about 17% in *U. limnetis* against about 36% in *U. longicaudatus* according to the 1/3-method. Strongly contracted specimens of the Chinese population have a tail length of about 12% (Fig. 2B, 7C) while in more or less fully(?) stretched cells, the tail occupies about 30% (Fig. 2A, 7F). The body sizes are also rather similar, namely 212 × 37 μm in *U. limnetis* (Stokes 1885; width calculated via length:width ratio of specimen illustrated), 210 × 26 μm in *U. longicaudatus* (Stokes 1886), and about 120–200 × 30–45 μm in the Chinese population. The macronuclear apparatus is composed of two nodules in all populations (Stokes 1885, 1886; Fig. 1A, H). However, Stokes (1885, 1886) neither described transverse nor caudal cirri in both species, but he wrote in both cases that the marginal cirri are longest, largest, and most abundantly developed on the caudal prolongation. This wording indicates that at least one or both cirral groups were present in both species, an assumption which is justified because it was and is almost impossible to recognize the infraciliature of the slender tail region correctly in life (Fig. 2A, B). Even with protargol preparations it is sometimes difficult to describe and interpret the pattern exactly

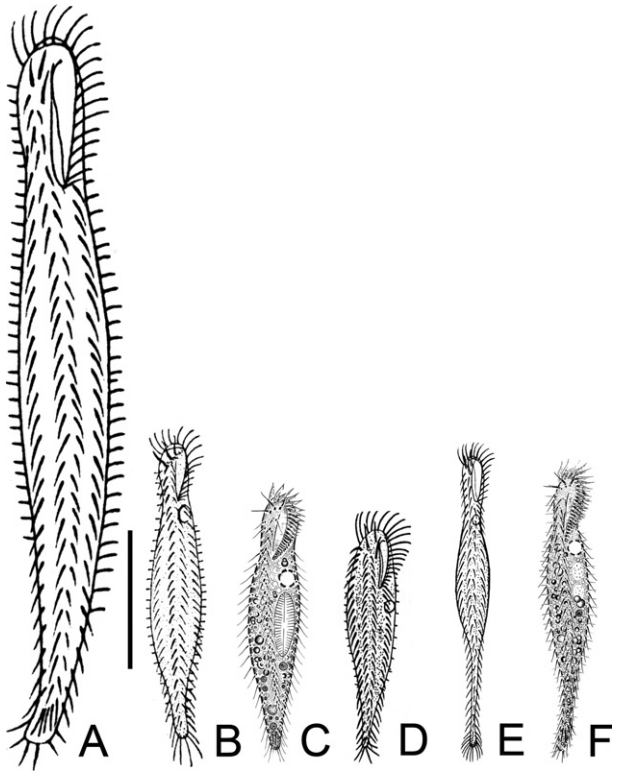

Figure 7 **A–F.** *Uroleptus caudatus* (A) and limnetic species of the *U. limnetis* complex (B–F). **A.** *Uroleptus caudatus* (from Stokes 1886). **B.** *Uroleptus limnetis* (from Stokes 1885). **C, F.** Chinese population of *U. longicaudatus* (originals). **D.** *Uroleptus dispar* (from Stokes 1886). **E.** *Uroleptus longicaudatus* (from Stokes 1886). Scale bar: 100 μm (drawn to scale).

when ontogenetic data are lacking. Dragesco (1966) redescribed *U. longicaudatus* from an alpine pond in France using protargol preparations. Accordingly, the marginal rows are strongly shortened posteriorly which is very unusual and transverse cirri are not described as in the original description. However, Dragesco (1966) illustrated two elongated midventral cirri in the tail region so that it cannot be excluded that he misinterpreted transverse cirri as midventral cirri. Thus, details of this redescription should not be over-interpreted.

Our data on live specimens (Fig. 7C, F) suggest that *U. limnetis* Stokes, 1885 is the contracted and *U. longicaudatus* the extended form of the same species (Fig. 7B, E). However, as none of Stokes' species is very well defined via a detailed redescription, molecular characterization, and neotypification of an East Coast population, we refrain from a final synonymy of these two species. The type localities of *U. limnetis* and *U. longicaudatus* are marsh waters with *Sphagnum* in the vicinage where A. C. Stokes lived and worked, that is, in Trenton, New Jersey, USA (Stokes 1886, 1891). It cannot even be excluded that Stokes found both species in the same pond, which would support the synonymy. The large distance between the site where we found our population (Xi'an, Shaanxi Province, China) and the original type localities (East Coast

of the USA) is the major reason why we do not use the Chinese population as neotype. According to the ICZN (International Commission on Zoological Nomenclature) (1999), Article 75.3.6), the neotype has to come as nearly as practicable from the original type locality. Note that "*Uroleptus longicaudatus*, Stokes" in Stokes (1885, p. 187) is a nomen nudum because not accompanied by a description or a reference to a description (ICZN (International Commission on Zoological Nomenclature) 1999, Article 12), that is, when the two species are synonymized, *U. limnetis* Stokes, 1885 is the correct name.

As a consequence of this intricate situation, we preliminary identify our population as *U. longicaudatus*. In addition, we propose to subsume these species, which are not clearly distinguishable at the present state of knowledge, as *Uroleptus limnetis* complex. Foissner et al. (1992) made the same proposal for some vorticellids. Furthermore, we suggest including *U. dispar* Stokes, 1886 into the complex. It is slightly smaller, but somewhat clumsier than *U. limnetis*. By contrast, *Uroleptus poianae* Lepsi, 1957 from an ombrogenic bog in the eastern Carpathians seems to be a junior synonym of *U. longicaudatus* because it has roughly the same size and shape (Lepsi 1957).

The little-known *U. lacteus* (Kahl, 1932) Borror, 1972 has a conspicuously milky cytoplasm and, like its synonym *U. pectinatus* Vuxanovici, 1963, 12 μm (12–14 μm in *U. pectinatus*) long dorsal bristles, that is, they are distinctly longer than those of the species of the *U. limnetis* complex (5–8 μm).

The molecular biological analyses show that the *Uroleptus* populations analyzed so far form a monophyletic group (Fig. 6). The Chinese population (GenBank KF734979) is closely related with *U. lepisma* (AF164132), *U. piscis* (AF164131), *U. willii* (EU399543), and unidentified *Uroleptus* populations (JQ723985, KF051782). The identifications of *U. lepisma* and *U. piscis* were done by nontaxonomists (Prescott and co-workers; see GenBank), likely not checked by silver preparations, and not supported by morphological data (for discussion, see Foissner et al. 2004). Thus, it makes no sense to discuss details, but the results suggest that the American workers studied populations which were very similar to our material. However, the discussion also demonstrates that detailed morphological data of the molecularly analyzed populations are urgently needed to improve the fine-systematics of *Uroleptus* species and ciliates in general.

Uroleptus willii Sonntag et al. 2008, a limnetic species with symbiotic green algae, has an inconspicuous tail which occupies only about 9% of body length according to the 1/3-method (length of tail estimated from fig. 1a, 2a in Sonntag et al. 2008). In addition, *U. willii* differs from our population in the relative length of the adoral zone (37% vs. 24%) and the number of adoral membranelles (on average 38 vs. 26), midventral pairs (36 vs. 18), marginal cirri (33 vs. 21 right; 33 vs. 25 left), and transverse cirri (constantly 5 [n only 3] vs. usually 4, sometimes 3).

Uroleptus lepisma Wenzel, 1953 is a mainly terrestrial species, which has, like the species of the *U. limnetis* complex, five or less transverse cirri. However, it is smaller (90–110 μm according to original description), has a rather short tail, and is not so distinctly cephalized like *U. longicaudatus* (Foissner 1998; Olmo 2000; Wenzel 1953). The specimens of other populations identified as *U. lepisma* (e.g. Berger and Foissner 1989; Shin et al. 1992) are larger and have a somewhat more distinct tail (about 22% in Berger and Foissner 1989 according to 1/3-method) and significantly more dorsal bristles (usually less than 90 vs. usually more than 130; Berger, unpubl. data). Perhaps, they are identical with the little-known *U. ophryoglena* (Gelei, 1954) comb. nov. (original combination: *Paruroleptus ophryoglena* Gelei, 1954) which was (obviously incorrectly) synonymized with the huge (more than 500-μm long according to original description; Fig. 7A) *U. caudatus* by Borror (1972). Whether *U. ophryoglena* is a distinct species or a junior synonym of a species of the *U. limnetis* complex cannot be estimated at the present state of knowledge. Thus, we recommend to include this species, which is mainly terrestrial and obviously less distinctly cephalised than the limnetic populations, into the *Uroleptus limnetis* complex.

Divisional morphogenesis

The ontogenetic stages of *U. longicaudatus* found (Fig. 3A–I, 4A–D, 5A–K) agree with the observations of previous studies (He et al. 2011; Martin et al. 1981; Olmo 2000) which were compared by He et al. (2011). As some early stages are lacking in our analysis, it remains obscure if the frontal-ventral-transverse cirri anlagen are formed via primary primordia (i.e. common anlagen for proter and opisthe) which are likely present for a short period only. So far, primary primordia have only been described for *U.* cf. *magnificus* (He et al. 2011). The formation of the frontoterminal cirri proceeds in the plesiomorphic way, that is, they are formed from the anterior portion of the rightmost anlage (Fig. 3F,H, 4A,C). Eigner (2001) reported a different formation in a *U. lepisma*-like population, namely, the anterior cirrus is formed by the penultimate anlage while the rear frontoterminal cirrus originates in the ordinary way (for comments on misidentification by Eigner 2001; see He et al. 2011). He et al. (2011) already stated that studies on further *U. lepisma* populations are needed to check whether Eigner's observations are correct or a misinterpretation.

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LITERATURE CITED

Adl, S. M., Simpson, A. G. B., Lane, C. E., Lukes, J., Bass, D., Bowser, S. S., Brown, M. W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., Gall, L. L., Lynn, D. H., McManus, M. H., Mitchell, E. A. D., Mozley-Stanridge, S. E., Parfrey, L. W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C. L., Smirnov, A. & Spiegel, F. W. 2012. The revised classification of eukaryotes. *J. Eukaryot. Microbiol.*, 59:429–493.

Berger, H. 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monogr. Biol.*, 78:i–xii, 1–1080.

Berger, H. 2001. Catalogue of Ciliate Names 1. Hypotrichs. Verlag Helmut Berger, Salzburg.

Berger, H. 2006. Monograph of the Urostyloidea (Ciliophora, Hypotricha). *Monogr. Biol.*, 85:i–xvi, 1–1304.

Berger, H. 2008. Monograph of the Amphisiellidae and Trachelostylidae (Ciliophora, Hypotricha). *Monogr. Biol.*, 88:i-xvi, 1–737.

Berger, H. 2011. Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha). *Monogr. Biol.*, 91:i–xiv, 1–741.

Berger, H. & Foissner, W. 1989. Morphology and biometry of some soil hypotrichs (Protozoa, Ciliophora) from Europe and Japan. *Bull. Br. Mus. Nat. Hist.*, 55:19–46.

Berger, H. & Foissner, W. 1997. Cladistic relationships and generic characterization of oxytrichid hypotrichs (Protozoa, Ciliophora). *Arch. Protistenkd.*, 148:125–155.

Bharti, D., Kumar, S. & La Terza, A. 2014. Morphology, morphogenesis and molecular phylogeny of a novel soil ciliate, *Pseudouroleptus plestiensis* n. sp. (Ciliophora, Oxytrichidae), from the uplands of Colfiorito, Italy. *Int. J. Syst. Evol. Microbiol.*, 64:2625–2636.

Borror, A. C. 1972. Revision of the order Hypotrichida (Ciliophora, Protozoa). *J. Protozool.*, 19:1–23.

Bütschli, O. 1889. Protozoa. III. Abtheilung: Infusoria und System der Radiolaria. *In*: Bronn, H. G. (ed.), Klassen und Ordnungen des Thier-Reichs, wissenschaftlich dargestellt in Wort und Bild, Vol. 1. Winter'sche Verlagshandlung, Leipzig. p. 1585–2035.

Chen, X., Miao, M., Ma, H., Shao, C. & Al-Rasheid, K. A. S. 2013. Morphology, morphogenesis and small-subunit rRNA gene sequence of the novel brackish-water ciliate *Strongylidium orientale* sp. nov. (Ciliophora, Hypotrichia). *Int. J. Syst. Evol. Microbiol.*, 63:1155–1164.

Corliss, J. O. 1979. The Ciliated Protozoa. Characterization, Classification and Guide to the Literature. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt. I–XVI, 455 p.

Dragesco, J. 1966. Ciliés libres de Thonon et ses environs. *Protistologica*, 2:59–95.

Edgar, R. C. 2004. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32:1792–1797.

Ehrenberg, C. G. 1831. Über die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abh. Preuss. Akad. Wiss., Phys. – Math. Kl.,* Year 1831:1–154.

Ehrenberg, C. G. 1838. Die Infusionsthierchen als vollkommene Organismen. Ein Blick in das tiefere organische Leben der Natur. L. Voss, Leipzig. 548 p.

Eigner, P. 2001. Divisional morphogenesis in *Uroleptus caudatus* (Stokes, 1886), and the relationship between the Urostylidae and the Parakahliellidae, Oxytrichidae, and Orthoamphisiellidae on the basis of morphogenetic processes (Ciliophora, Hypotrichida). *J. Eukaryot. Microbiol.*, 48:70–79.

Fan, Y., Chen, X., Hu, X., Shao, C., Al-Rasheid, K. A. S., Al-Farraj, S. A. & Lin, X. 2014. Morphology and morphogenesis of *Apoholosticha sinica* n. g., n. sp. (Ciliophora, Hypotrichia), with consideration of its systematic position among urostylids. *Eur. J. Protistol.*, 50:78–88.

Fauré-Fremiet, E. 1961. Remarques sur la morphologie comparée et la systématique des ciliata Hypotrichida. *C. R. Hebd. Séanc. Acad. Sci. Paris*, 252:3515–3519.

Foissner, W. 1998. An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. *Eur. J. Protistol.*, 34:195–235.

Foissner, W. & Adam, H. 1983. Morphologie und morphogenese des bodenciliaten *Oxytricha granulifera* sp. n. (Ciliophora, Oxytrichidae). *Zool. Scr.*, 12:1–11.

Foissner, W. & Al-Rasheid, K. 2006. A unified organization of the stichotrichine oral apparatus, including a description of the buccal seal (Ciliophora: Spirotrichea). *Acta Protozool.*, 45:1–16.

Foissner, W., Blatterer, H., Berger, H. & Kohmann, F. 1991. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. *Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft*, 1/91:1–478.

Foissner, W., Berger, H. & Kohmann, F. 1992. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band II: Peritrichia, Heterotrichida, Odontostomatida. *Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft*, 5/92:1–502.

Foissner, W., Filker, S. & Stoeck, T. 2014. *Schmidingerothrix salinarum* nov. spec. is the molecular sister of the large oxytrichid clade (Ciliophora, Hypotricha). *J. Eukaryot. Microbiol.*, 61:61–74.

Foissner, W., Moon-van der Staay, S. Y., van der Staay, G. W. M., Hackstein, J. H. P., Krautgartner, W. & Berger, H. 2004. Reconciling classical and molecular phylogenies in the stichotrichines (Ciliophora, Spirotrichea), including new sequences from some rare species. *Eur. J. Protistol.*, 40:265–281.

Foissner, W. & Stoeck, T. 2008. Morphology, ontogenesis and molecular phylogeny of *Neokeronopsis (Afrokeronopsis) aurea* nov. subgen., nov. spec. (Ciliophora: Hypotricha), a new African flagship ciliate confirms the CEUU hypothesis. *Acta Protozool.*, 47:1–33.

Gelei, J. 1954. Über die Lebensgemeinschaft einiger temporärer Tümpel auf einer Bergwiese im Börzsönygebirge (Oberungarn) III. Ciliaten. *Acta Biol. Hung.*, 5:259–343.

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

He, W., Shi, X., Shao, C., Chen, X. & Berger, H. 2011. Morphology and cell division of the little known freshwater ciliate *Uroleptus* cf. *magnificus* (Kahl, 1932) Olmo, 2000 (Hypotricha, Uroleptidae), and list of published names in *Uroleptus* Ehrenberg, 1831 and *Paruroleptus* Wenzel, 1953. *Acta Protozool.*, 50:175–203.

Heber, D., Stoeck, T. & Foissner, W. 2014. Morphology and ontogenesis of *Psilotrichides hawaiiensis* nov. gen., nov. spec. and molecular phylogeny of the Psilotrichidae (Ciliophora, Hypotrichia). *J. Eukaryot. Microbiol.*, 61:260–277.

Hewitt, E. A., Müller, K. M., Cannone, J., Hogan, D. J., Gutell, R. & Prescott, D. M. 2003. Phylogenetic relationships among 28 spirotrichous ciliates documented by rDNA. *Mol. Phylogenet. Evol.*, 29:258–267.

Huang, J., Yi, Z., Al-Farraj, S. A. & Song, W. 2010. Phylogenetic positions and taxonomic assignments of the systematically controversial genera, *Spirotrachelostyla*, *Uroleptopsis* and *Tunicothrix* (Protozoa, Ciliophora, Stichotrichia) based on small subunit rRNA gene sequences. *Syst. Biodiv.*, 8:409–416.

ICZN (International Commission on Zoological Nomenclature) 1999. International Code of Zoological Nomenclature. International Trust for Zoological Nomenclature, London, 306 p.
Kahl, A. 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Dtl.*, 25:399–650.
Kumar, S., Bharti, D., Marinsalti, S., Insom, E. & Terza, A. L. 2014. Morphology, morphogenesis, and molecular phylogeny of *Paraparentocirrus sibillinensis* n. gen., n. sp., a"Stylonychine Oxytrichidae" (Ciliophora, Hypotrichida) without transverse cirri. *J. Eukaryot. Microbiol.*, 61:247–259.
Kumar, S., Kamra, K., Bharti, D., Terza, A. L., Sehgal, N., Warren, A. & Sapra, G. R. 2015. Morphology, morphogenesis, and molecular phylogeny of *Sterkiella tetracirrata* n. sp. (Ciliophora, Oxytrichidae), from the Silent Valley National Park, India. *Eur. J. Protistol.*, 51:86–97.
Lepsi, I. 1957. Infuzori spirotrichi si peritrichi din tinoavele de la poiana stampei, raionul vatra dornei (Spirotriche und peritriche Infusorien aus den Hochmooren von Poiana Stampei, Bezirk Vatra Dornei). *Buletin sti. Acad. Repub. pop. rom., Sectia de biologie si stiinte agricole, Seria zoologie*, 9:231–240.
Li, F., Lv, Z., Yi, Z., Al-Farraj, S. A., Al-Rasheid, K. A. S. & Shao, C. 2014. Taxonomy and phylogeny of two species of the genus *Deviata* (Protista, Ciliophora) from China, with description of a new soil form, *Deviata parabacilliformis* sp. nov. *Int. J. Syst. Evol. Microbiol.*, 64:3775–3785.
Lv, Z., Shao, C., Yi, Z. & Warren, A. 2015. A molecular phylogenetic investigation of *Bakuella*, *Anteholosticha*, and *Caudiholosticha* (Protista, Ciliophora, Hypotrichia) based on three-gene sequences. *J. Eukaryot. Microbiol.*, 62:391–399.
Lynn, D. H. 2008. The Ciliated Protozoa: Characterization, Classification and Guide to the Literature, 3rd ed. Springer Press, Dordrecht.
Martin, J., Fedriani, C. & Nieto, J. 1981. Étude comparée des processus morphogénétiques d'*Uroleptus* sp. (Kahl, 1932) et de *Holosticha* (*Paruroleptus*) *musculus* (Kahl, 1932) (Ciliés Hypotriches). *Protistologica*, 17:215–224.
Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71:491–499.
Müller, O. F. 1773. Vermium Terrestrium et Fluviatilium, seu Animalium Infusoriorum, Helminthicorum et Testaceorum, non Marinorum, Succincta Historia. Heineck and Faber, Havniae and Lipsiae. 135 p.
Müller, O. F. 1786. Animalcula Infusoria Fluviatilia et Marina, quae Detexit, Sytematice Descripsit et ad Vivum Delineari Curavit. Mölleri, Hauniae. 367 p.
Nylander, J. A. A. 2004. MrModeltest, Version 2.2. Evolutionary Biology Centre, Uppsala University, Uppsala.
Olmo, J. 2000. Morphology and morphogenesis of *Uroleptus lepisma* (Wenzel, 1953) Foissner, 1998 (Ciliophora, Hypotrichida). *Eur. J. Protistol.*, 36:379–386.
Page, R. D. M. 1996. Tree view: an application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.*, 12:357–358.
Ronquist, F. & Huelsenbeck, J. P. 2003. MrBAYES 3: Bayesian Phylogenetic inference under mixed models. *Bioinformatics*, 19:1572–1574.
Shin, M. K., Kim, H. S. & Kim, W. 1992. New records of hypotrichs from Korea (Protozoa, Ciliophora, Hypotrichida). *Korean J. Syst. Zool. Special Issue*, 3:15–22.
Singh, J. & Kamra, K. 2014. Molecular phylogeny of an Indian population of *Kleinstyla dorsicirrata* (Foissner, 1982) Foissner et al., 2002. comb. nov. (Hypotrichia, Oxytrichidae): an oxytrichid with incomplete dorsal kinety fragmentation. *J. Eukaryot. Microbiol.*, 61:630–636.
Small, E. B. & Lynn, D. H. 1985. Phylum Ciliophora Doflein, 1901. *In*: Lee, J., Hutner, S. H. & Bovee, E. C. (ed.), An Illustrated Guide to the Protozoa. Society of Protozoologists, Lawrence, KS. p. 393–575.
Sonntag, B., Strüder-Kypke, M. & Summerer, M. 2008. *Uroleptus willii* nov. sp., a euplanktonic freshwater ciliate (Dorsomarginalia, Spirotrichea, Ciliophora) with algal symbionts: morphological description including phylogenetic data of the small subunit rRNA gene sequence and ecological notes. *Denisia*, 23:279–288.
Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol.*, 57:758–771.
Stein, F. 1859. Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. Engelmann, Leipzig. 206 p.
Stokes, A. C. 1885. Notices of new fresh-water infusoria – IV. *Am. Mon. Microsc. J.*, 6:183–190.
Stokes, A. C. 1886. Some new hypotrichous infusoria. *Proc. Am. Phil. Soc.*, 23:21–30.
Stokes, A. C. 1888. A preliminary contribution toward a history of the fresh-water infusoria of the United States. *J. Trenton Nat. Hist. Soc.*, 1:71–319.
Stokes, A. C. 1891. Notes of new infusoria from the fresh waters of the United States. *Jl R. Microsc. Soc.*, 1891:697–704.
Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24:1596–1599.
Vdačný, P. & Foissner, W. 2012. Monograph of the dileptids (Protista, Ciliophora, Rhynchostomatia). *Denisia*, 31:1–529.
Vuxanovici, A. 1963. Contributii la studiul speciilor din subordinul Hypotricha (Ciliata) (Nota I) (Contribution à l'étude des espèces appartenant au sous-ordre Hypotricha (Ciliata) (Note I)). *Studii Cerc. Biol. Seria Biol. Animala*, 15:199–222.
Wenzel, F. 1953. Die Ciliaten der Moosrasen trockner Standorte. *Arch. Protistenk.*, 99:70–141.
Wilbert, N. 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. *Mikrokosmos*, 64:171–179.
Yi, Z. & Song, W. 2011. Evolution of the order Urostylida (Protozoa, Ciliophora): new hypotheses based on multigene information and identification of localized incongruence. *PLoS ONE*, 6:e17471. doi:10.1371/journal.pone.0017471.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Site and surrounding scenery where the sample containing *Uroleptus longicaudatus* was collected. Arrow in lower inset marks Shaanxi Province and arrow in upper inset indicates the Tengfei Tower in the center of the Xi'an Jiaotong University Campus.