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Morphology, morphogenesis, and molecular phylogeny of *Uroleptus* (*Caudiholosticha*) *stueberi* (Foissner, 1987) comb. nov. (Ciliophora, Hypotricha), and reclassification of the remaining *Caudiholosticha* species

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Abstract

The morphology, morphogenesis, and SSU rDNA sequence of a *Caudiholosticha stueberi* population from Southwest China soil were analyzed. The studies confirm the assumption of previous workers that this species has dorsomarginal kineties and thus does not belong to the urostyloids, but to the non-oxytrichid dorsomarginalian genus *Uroleptus* whose members have, in contrast to *C. stueberi*, a distinct tail. On the basis of two morphological features we split *Uroleptus* into three subgenera: *U. (Uroleptus)* (tail present; more than five transverse cirri; habitat freshwater), *U. (Paruroleptus)* (present; five or less; freshwater or soil), and *U. (Caudiholosticha)* stat. nov. (lacking; five or less; soil). Since *Uroleptus (Caudiholosticha) stueberi* comb. nov. is the type of *Caudiholosticha*, the other 16 species so far assigned to *Caudiholosticha* have to be reclassified because they obviously lack dorsomarginal kineties. Based on published data, six new urostyloid genera are established: *Extraholosticha* gen. nov. (type: *Holosticha sylvatica*; monotypic); *Adumbratosticha* gen. nov. (type: *H. tetracirrata*; three species); *Acuholosticha* gen. nov. (type: *U. paranotabilis*; five species); *Limnoholosticha* gen. nov. (type: *H. (Holosticha) navicularum*; four species); *Multiholosticha* gen. nov. (type: *H. multicaudicirrus*; two species); and *Caudikeronopsis* gen. nov. (type: *Caudiholosticha marina*; monotypic). *Urosomoida sejongensis* is transferred to *Oxytrichella*: *O. sejongensis* comb. nov.

Keywords: China; Dorsomarginalia; SSU rDNA; Taxonomy; *Uroleptus*; Urostyloids

Introduction

Holosticha stueberi was discovered by Foissner (1987) in soil from the Austrian Alps. He classified it in the large urostyloid genus *Holosticha* Wrzesniowski, 1877 because of

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resemblance with *H. (Keronopsis) spectabilis* Kahl, 1932 (now type of the oxytrichid genus *Neokeronopsis* Warren et al., 2002) and *H. camerounensis* Dragesco, 1970 (now type of *Afrophrya* Foissner and Stoeck, 2006; perhaps a rigidotrichid). In 2003, Berger reviewed *Holosticha* (type species *Oxytricha kessleri* Wrzesniowski, 1877) and confined it to aquatic species without caudal cirri which have some remarkable apomorphies, for example, anterior end of left marginal row composed of narrowly spaced cirri and curved rightwards, and number of transverse cirri equal to or only slightly lower than number of midventral pairs. The other species have been transferred to *Anteholosticha* Berger, 2003 (type species *Holosticha monilata* Kahl, 1928; mainly characterized by lacking caudal cirri), *Biholosticha* Berger, 2003 (type species *Holosticha (Holosticha) discocephalus* Kahl, 1932; e.g., only two frontal cirri present, buccal cirrus lacking), and *Caudiholosticha* Berger, 2003 (caudal cirri present) with *H. stueberi* as type species (Berger 2003). Berger (2003, 2006) already mentioned that *Anteholosticha* and *Caudiholosticha* are non-monophyletic because defined only via plesiomorphies, an assumption later supported by molecular studies (e.g., Huang et al. 2014; Lv et al. 2015; Shao et al. 2011).

Berger (2006, p. 237) and Foissner and Stoeck (2006) hypothesized that *Caudiholosticha stueberi* is a *Uroleptus* because they supposed that dorsomarginal kineties are present. These kineties originate from/near the anterior end of the right marginal row anlagen, are usually more or less distinctly shortened posteriorly, and lack caudal cirri (Martin 1982). Both Berger (2006) and Foissner and Stoeck (2006) proposed to await further data for a restructuring of *Caudiholosticha*.

During a survey of ciliate communities of Southwest China soils, we isolated *Caudiholosticha stueberi*. The morphology, morphogenesis, and phylogeny based on the small subunit rDNA gene sequence of the Chinese population were analyzed with standard methods. The new data support the hypothesis of Berger (2006) and Foissner and Stoeck (2006) that *C. stueberi* is a *Uroleptus* species. All other 16 species at present assigned to *Caudiholosticha* are misclassified in this group; thus, we transfer them into six genera, basing on available morphological and molecular data.

Material and Methods

Sampling and cultivation

Samples of fine, brownish soil (0–10 cm) were collected from the floodplain of a small river near the village of Zhonghuashan (27°34.8′N, 109°15.7′E, altitude 394 m), Aojiazhai Township, Tongren City, Guizhou Province, China on 19 July, 2014 (Fig. 1). Ciliates were re-activated from air-dried samples using the non-flooded Petri dish method (Foissner et al. 2002). We established a non-clonal culture at room temperature in Petri dishes containing mineral water (Nongfu Spring) with squeezed rice grains to enrich the bacterial food. Specimens of this culture were used to study morphology and cell division and for molecular analyses. Since *C. stueberi* was the sole hypotrich in the permanent preparations, we are sure that all data deal with this species.

Morphological and morphogenetic analyses

Living cells were examined using bright-field and differential interference contrast microscopy. The protargol method was used to reveal the infraciliature and the nuclear apparatus (Wilbert 1975). Drawings of protargol-prepared specimens were performed at a magnification of 1000× with the aid of a camera lucida. Measurements were made with an ocular micrometer. To illustrate the changes occurring during divisional morphogenesis, the bases of the parental cirri and membranelles are depicted by contour whereas those of new ones are shaded black.

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

Voucher slides (reg. no. LFC2014080102A–O) of the Chinese population have been deposited in the Hydrobiological Laboratory of Hebei University, China.

DNA extraction, PCR amplification, and sequencing

Specimens of the present species were isolated and repeatedly washed using sterile distilled 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 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).

Phylogenetic analyses

The SSU rDNA gene sequence of *C. stueberi* was aligned to the sequences of 63 other spirotrichous ciliates from GenBank database using the online program Muscle 3.7 (Edgar

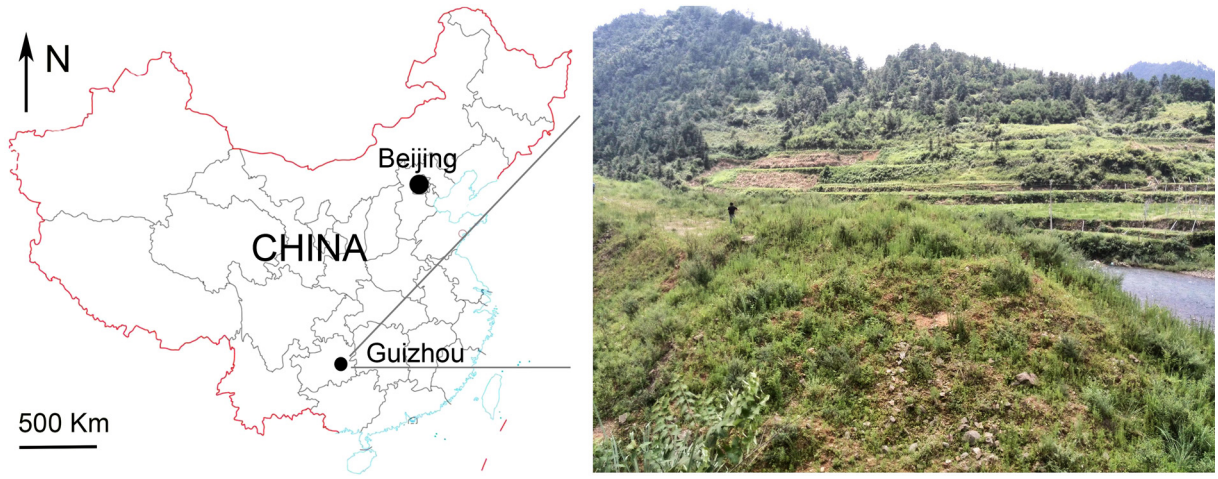

Fig. 1. Sampling site and surrounding areas.

2004). The accession numbers are mentioned after the species names in the phylogenetic tree (Fig. 5). Subsequently, these sequences were aligned using Clustal 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 1727 characters. The program MrModeltest v.2.0 (Nylander 2004) selected the GTR + *I* (= 0.5522) + *G* (= 0.4820) as the best model with Akaike Information Criterion (AIC), which was then used for Bayesian inference (BI) and maximum likelihood (ML) analyses. Bayesian inference analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Maximum-likelihood analysis was carried out online using RAxML-HPC2 on XSEDE (8.0.24) on the CIPRES Science Gateway (Stamatakis et al. 2008). TreeView v1.6.6 (Page 1996) and MEGA 4.0 (Tamura et al. 2007) were used to visualize tree topologies. *Parastrombidinopsis minima*, *Strombidinopsis acuminata*, and *Rimostrombidium lacustris* were selected as the outgroup taxa.

Results

Caudiholosticha stueberi (Figs 2A–K, 3A–J, 4A–L; Table 1)

Morphology of Chinese population (Figs 2A–E, H–K, 4A–E; Table 1)

Body size in vivo about 200–310 × 70–100 μm; on average 226 × 81 μm in protargol preparations, that is, length:width ratio 2.8:1 on average (Table 1). Body outline elongate elliptical, anterior end widely rounded; posterior portion usually slightly narrowed and moderately widely rounded, sometimes broadly rounded, that is, not tailed (Fig. 2A, B, H–K); dorsoventrally flattened about 2:1 (Fig. 2C). Body flexible and slightly contractile. Cells appear often darkish at low magnification due to various inclusions. Usually two, sometimes three (one of 15 specimens analyzed) or four (one of 15 specimens) macronuclear nodules in central body portion slightly left of midline; mostly three, sometimes up to six globular micronuclei usually closely attached to macronuclear nodules (Figs 2B, E, J, 4E). Contractile vacuole about at 40% of body length, that is, slightly behind proximal end of adoral zone near left body margin, during diastole with two inconspicuous collecting canals (Fig. 2A, B, J, K). Cortical granules lacking. Cytoplasm with crystals, undefined inclusions, and food vacuoles containing diatoms and ciliates; individual cells with up to 50 *Halteria grandinella* specimens. Movement without peculiarities, that is, crawling moderately fast on bottom of Petri dish or on debris with occasional pauses and then changing direction of movement; rotating about main body axis when swimming.

Adoral zone occupies about 38% of body length on average, composed of 39–52 membranelles (Table 1). Buccal field deep and large. Undulating membranes as in *Uroleptus* species, that is, both membranes long, curved, and optically intersecting; paroral commences and terminates slightly more anteriorly than endoral. Pharyngeal fibers extend almost longitudinally posteriorly (Figs 2A, B, D, H, K, 4A, C).

Cirral pattern uroleptid and of usual variability (Fig. 2D, Table 1). Three enlarged frontal cirri close behind distal portion of adoral zone. Buccal cirrus slightly behind level of parabuccal cirrus (III/2). Usually two, rarely three (three of 15 specimens analyzed) frontoterminal cirri between distal end of adoral zone and anterior end of pseudorow formed by right midventral cirri. Midventral complex composed of 15–21 cirral pairs, extends from about level of anterior end of right marginal row to near transverse cirri; right cirrus of each pair distinctly larger than left except for anteriormost pairs where both cirri of about same size. Rearmost pair and related pretransverse ventral cirrus usually longitudinally arranged, thus feigning short midventral row. Constantly three slightly enlarged transverse cirri arranged in oblique pseudorow near cell end, cirri thus distinctly projecting beyond rear end of cell. Right marginal row commences about at level of anteriormost midventral pair, ends, like left row, slightly

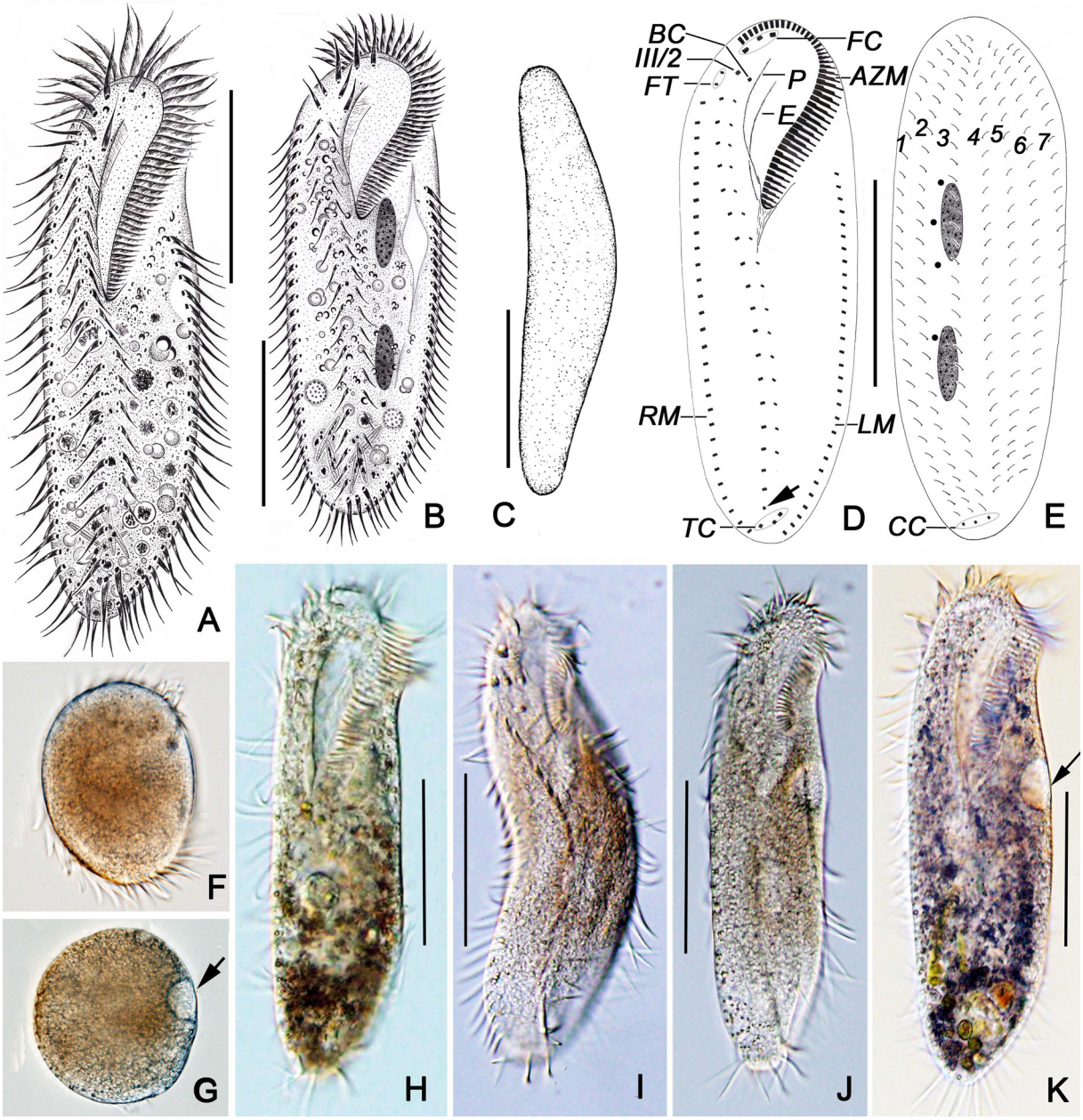

Fig. 2A–K. Morphology of Chinese population of *Uroleptus* (*Caudiholosticha*) *stueberi* comb. nov. from life (A–C, F–K) and after protargol preparation (D, E). **A, B:** Ventral view of a representative individual and a shape variant. **C:** Left lateral view. **D, E:** Infraciliature of ventral and dorsal side and nuclear apparatus of same specimen. Arrow in (D) marks pretransverse ventral cirrus. **F, G:** Encystment; arrow in (G) marks the contractile vacuole. **H–K:** Ventral views of typical, freely motile individuals showing, inter alia, flexibility (I) and contractile vacuole (K, arrow). AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; E, endoral; FC, frontal cirri; FT, frontoterminal cirri; LM, left marginal row; P, paroral; RM, right marginal row; TC, transverse cirri; III/2, cirrus III/2 (= parabuccal cirrus); 1–3, bipolar dorsal kineties; 4–7, dorsomarginal kineties. Bars, 90 μm.

subterminally, thus rows more or less distinctly separated posteriorly; left row commences left of proximal portion of adoral zone.

Three bipolar dorsal kineties left of cell midline, each with one caudal cirrus; four or five dorsomarginal kineties, right one usually terminating about in mid-body, others only slightly shortened posteriorly. Dorsal cilia about 3–5 μm long. Caudal cirri of about same length as marginal cirri, thus indistinguishable in vivo (Figs 2A, E, 4B, D).

Note on encystment of Chinese population (Fig. 2F, G)

Pre-encystment cells crawl very fast. When the encystment is almost finished, the locomotion becomes much slower and the number of food vacuoles decreases (Fig. 2F, G).

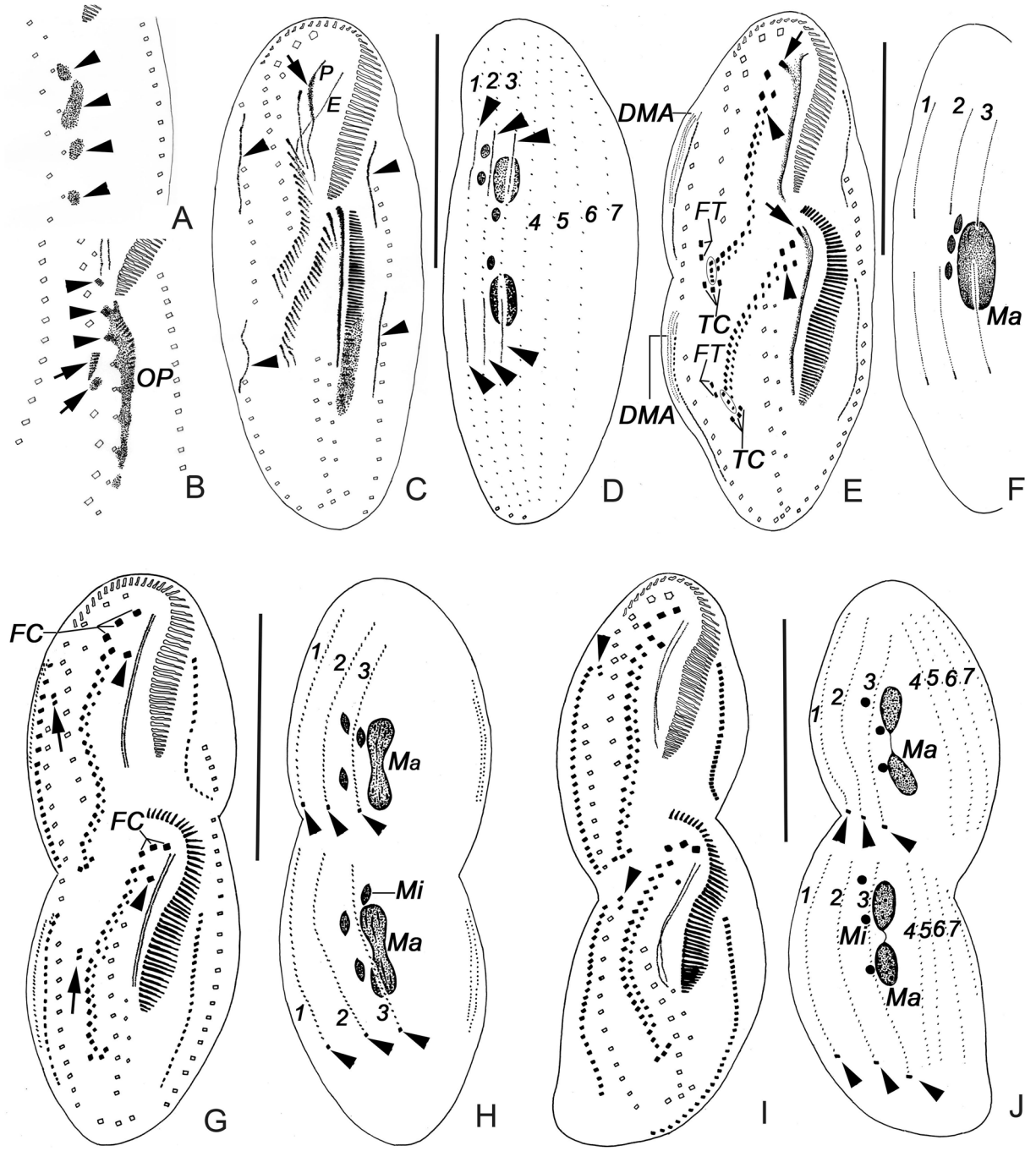

Fig. 3A–J. Morphogenesis of Chinese population of *Uroleptus (Caudiholosticha) stueberi* comb. nov. after protargol preparation. **A, B:** Postoral region of very early and early divider showing newly formed groups of basal bodies (arrowheads in A) and oral primordium for the opisthe (B). Arrows and arrowheads in (B) mark some midventral cirri modified to anlagen. **C, D:** Infraciliature of ventral and dorsal side and nuclear apparatus of an early to middle divider showing, inter alia, the oblique frontoventral-transverse cirri anlagen, the newly formed marginal anlagen (arrowheads in C), the dedifferentiation of the parental paroral (arrow in C), and the newly formed dorsal kineties anlagen (arrowheads in D). **E, F:** Infraciliature of ventral and dorsal side and nuclear apparatus of middle divider showing, inter alia, newly formed cirri III/2 (arrowheads), left frontal cirri (arrows) originating from anlage I, and short rows (ellipses) formed by anlage n–1 and producing rearmost midventral pair, pretransverse ventral cirrus, and penultimate transverse cirrus. **G–J:** Infraciliature of ventral and dorsal side and nuclear apparatus of late (G, H) and very late (I, J) divider showing new buccal cirri (arrowheads in G) and anteriorly migrating frontoterminal cirri (arrows in G, I). Each one caudal cirrus (arrowheads in H, J) is formed at rear end of dorsal kineties 1–3. DMA, dorsomarginal kineties anlagen; E, endoral; FC, frontal cirri; FT, frontoterminal cirri; Ma, macronuclear nodules; Mi, micronuclei; OP, oral primordium; P, paroral; TC, transverse cirri; 1–3, dorsal kineties; 4–7, dorsomarginal kineties. Bars, 100 μm.

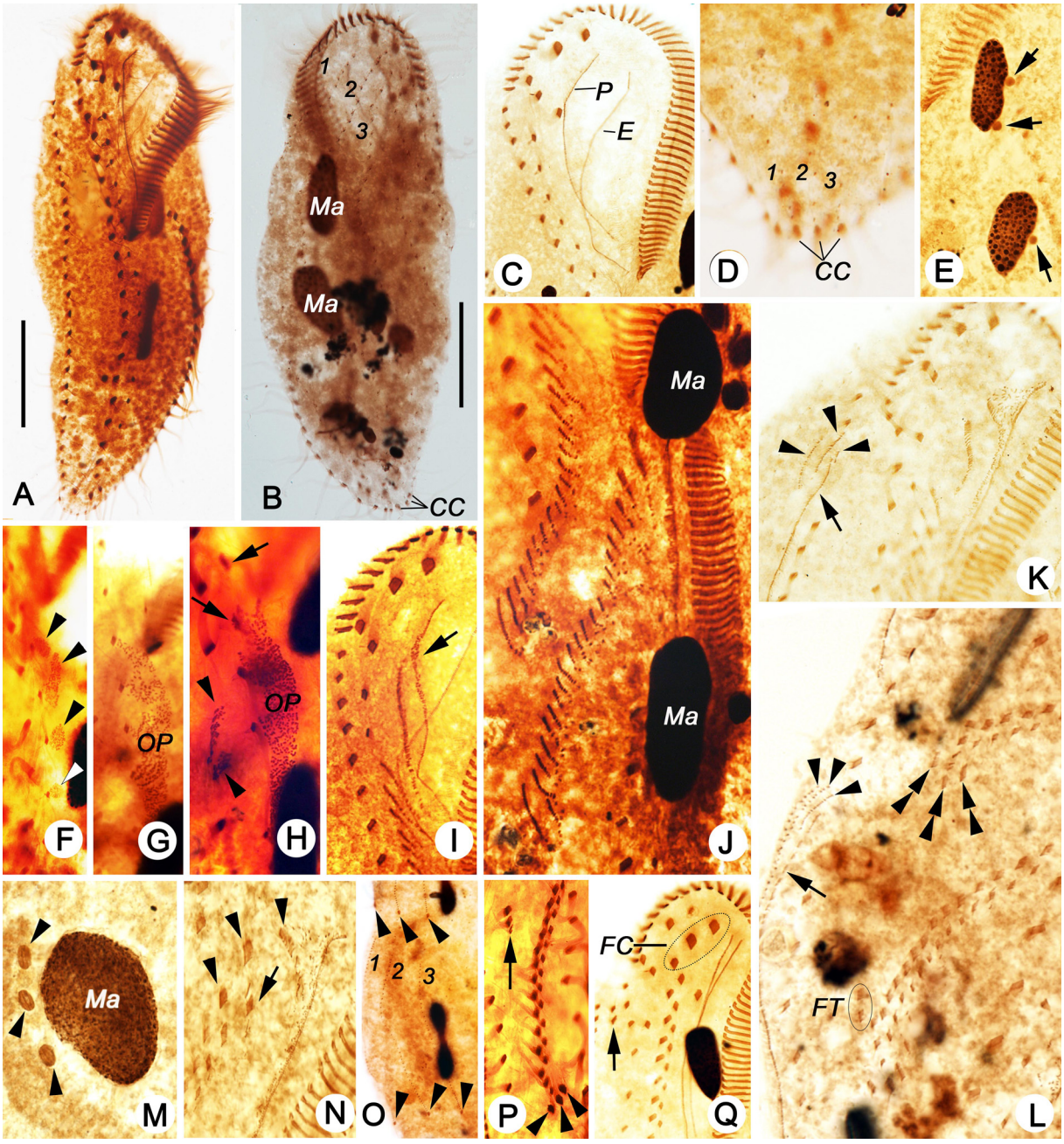

Fig. 4A–P. Morphology and morphogenesis of Chinese population of *Uroleptus (Caudiholosticha) stueberi* comb. nov. after protargol preparation. **A, B:** Ventral and dorsal side of different specimens to show ventral infraciliature, dorsal kineties, caudal cirri and macronuclear nodules. **C:** Ventral side of anterior portion. **D:** Dorsal side of posterior portion showing caudal cirri and dorsal kineties. **E:** Macronuclear nodules and micronuclei (arrows) in ventral view. **F:** Postoral region of very early divider showing newly formed groups of basal bodies (arrowheads). **G, H:** Oral primordium of opisthe in early divider and ventral view of a somewhat later divider; arrows and arrowheads mark a few midventral cirri disaggregated and dedifferentiated into anlagen. **I, J:** Ventral views of same early divider showing dedifferentiation of parental paroral (arrow in I; anlage I), formation of anlage II from parental buccal cirrus, and the newly formed oblique frontoventral-transverse cirri anlagen (J). **K:** Ventral view of a middle divider showing formation of dorsomarginal kineties (arrowheads) and right marginal row anlage (arrow). **L:** Ventral view of a middle divider showing the newly formed transverse cirri of the proter (double-arrowheads), as well as the dorsomarginal kineties anlagen (arrowheads), the right marginal row anlage (arrow), and the frontoterminal cirri of the opisthe. **M:** Micronuclei (arrowheads) and fused macronucleus of a middle divider. **N:** Ventral view of a middle divider showing newly formed frontal cirri (arrowheads) and buccal cirrus (arrow) of opisthe. **O:** Dorsal side of late divider with newly formed caudal (arrowheads) both in proter and opisthe. **P:** Ventral view of a late proter, showing the new transverse cirri (arrowheads) and frontoterminal cirri (arrow). **Q:** Ventral view of proter of late stage showing the new frontal cirri (encircled) and frontoterminal cirri (arrow). CC, caudal cirri; E, endoral; FC, frontal cirri; FT, frontoterminal cirri; Ma, macronuclear nodules; OP, oral primordium; P, paroral; 1–3, dorsal kineties. Bars, 50 μm.

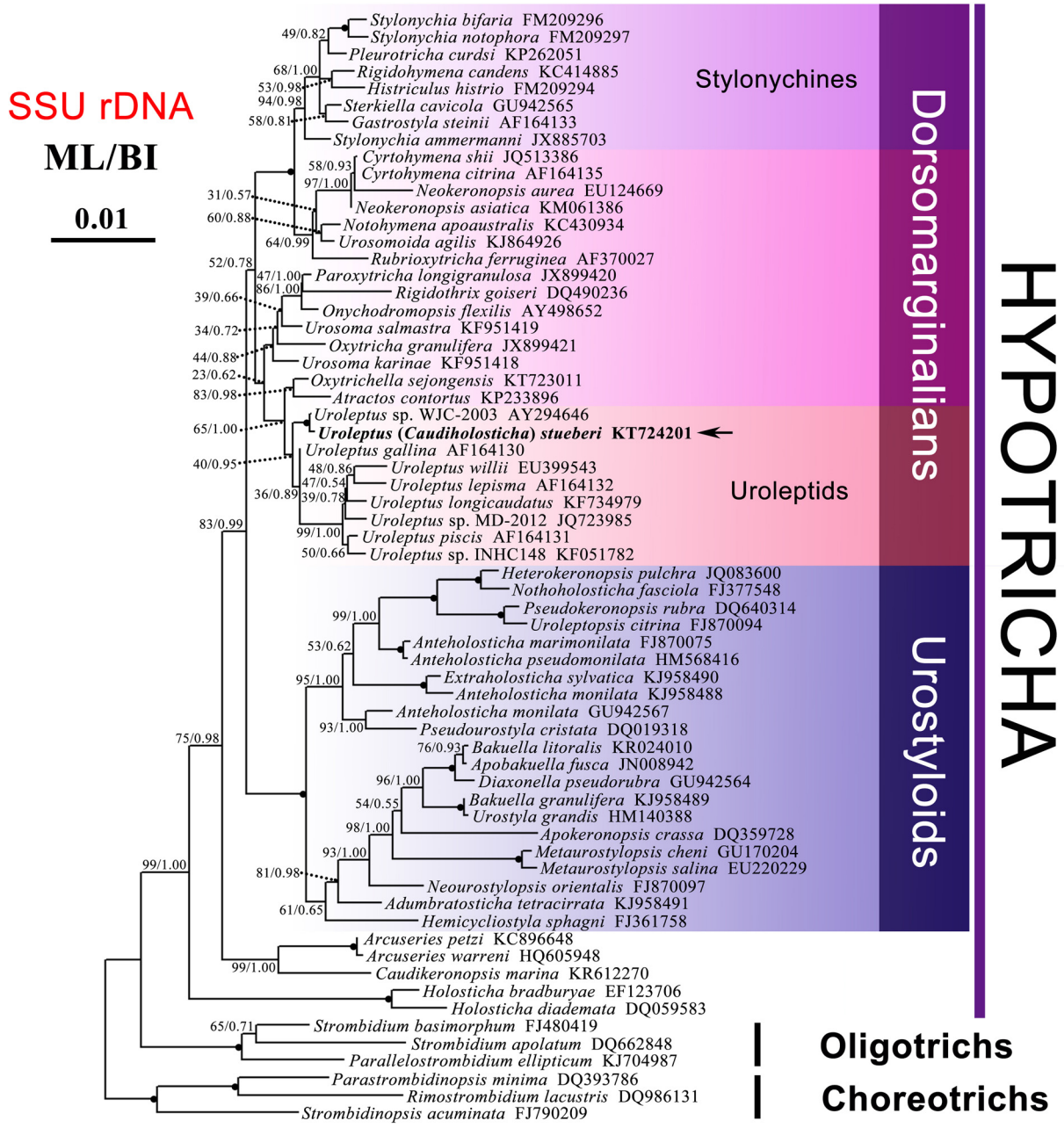

Fig. 5. Maximum-likelihood (ML) tree based on the SSU rDNA gene sequences showing the position of the Chinese population of *Uroleptus (Caudiholosticha) stueberi* (arrow). Numbers near nodes are nonparametric bootstrap values for maximum likelihood (ML) and posterior probability values for Bayesian inference (BI). Fully supported (100/1.00) branches are marked with filled circles. The scale bar corresponds to 0.01 expected substitutions per site. Note that we use the most recent names which sometimes do not agree with those found in GenBank.

Morphogenesis of Chinese population during cell division (Figs 3A–J, 4F–Q)

Stomatogenesis and development of frontoventral-transverse cirri. Stomatogenesis commences with the appearance of several small groups of basal bodies immediately left of the left midventral cirri in the postoral region (Figs 3A, 4F). These basal bodies merge by further proliferation of basal bodies forming a wedge-shaped oral primordium (Figs 3B, 4G).

Subsequently, the oral primordium becomes larger by further proliferation of basal bodies, and several membranelles are formed in the anterior portion. During this process, some left midventral cirri are dedifferentiated, indicating that parental basal bodies are incorporated into the oral primordium (Figs 3B, 4H).

Later, the second quarter of the parental paroral begins to disintegrate and the formation of membranelles in the opisthe is in progress. Simultaneously, the parental buccal cirrus (II/2) is modified to anlage II for the proter, and about

Table 1. Morphometric characterization of Chinese population of *Uroleptus (Caudiholosticha) stueberi* comb. nov. (*stu*) and *Caudiholosticha tetracirrata* sensu Lv et al. (2015, *tet*).[a]

Character	Species	Mean	M	SD	CV	Min	Max	n
Body, length	*stu*	226.1	225	19.3	8.5	200	250	15
	tet	158.6	161	21.4	13.5	124	183	7
Body, width	*stu*	81.4	80	10.2	12.6	65	100	15
	tet	54.0	52	5.8	10.8	46	63	7
AZM, length	*stu*	84.3	85	8.5	10.0	70	100	15
	tet	46.3	46	5.3	11.3	38	53	7
Length of AZM:body length, ratio[b]	*stu*	37.5	37	4.5	11.9	29	43	15
	tet	29.0	30	0.0	5.2	26	31	7
Adoral membranelles, number	*stu*	47.0	47	4.1	8.8	39	52	15
	tet	34.4	34	3.1	9.0	31	39	7
Frontal cirri, number	*stu*	3.0	3	0.0	0.0	3	3	15
	tet	3.0	3	0.0	0.0	3	3	7
Buccal cirri, number	*stu*	1.0	1	0.0	0.0	1	1	15
	tet	1.0	1	0.0	0.0	1	1	7
Frontoterminal cirri, number	*stu*	2.2	2	0.4	19.6	2	3	15
	tet	2.0	2	0.0	0.0	2	2	7
Midventral pairs, number	*stu*[d]	17.2	17	1.6	9.4	14	20	15
	tet	15.6	16	1.7	11.0	13	18	7
Anterior body end to last midventral pair, distance	*tet*	95.6	91	10.3	10.8	86	116	7
Transverse cirri, number	*stu*	3.0	3	0.0	0.0	3	3	15
	tet	6.9	7	0.7	10.1	6	8	7
Left marginal cirri, number	*stu*	30.5	30	3.4	10.1	24	36	15
	tet	48.1	48	1.1	2.2	47	50	7
Right marginal cirri, number	*stu*	28.3	28	1.9	6.8	26	32	15
	tet	51.7	52	1.3	2.4	50	54	7
Dorsal and dorsomarginal kineties, number	*stu*	7.4	7	0.5	6.9	7	8	15
	tet[c]	4.9	5	0.4	7.8	4	5	7
Caudal cirri, number	*stu*	3.0	3	0.0	0.0	3	3	15
	tet	4.7	5	0.5	10.4	4	5	7
Macronuclear nodules, number	*stu*	2.2	2	0.6	28.8	2	4	15
	tet	37.6	38	4.5	12.1	32	44	7
Macronuclear nodules, length	*stu*	33.3	32	7.9	23.7	20	45	15
	tet	12.0	12	0.8	6.8	11	13	7
Macronuclear nodules, width	*stu*	9.8	10	1.6	16.6	7	12	15
	tet	6.9	7	0.9	13.1	6	8	7
Micronuclei, number	*stu*	3.7	3	1.0	26.6	3	6	15
	tet	1.7	1	1.0	55.5	1	3	7
Micronuclei, diameter	*stu*	2.7	3	0.5	17.1	2	3	15
	tet	4.9	5	0.9	18.5	4	6	7

[a] All data are based on protargol-prepared specimens. Measurements in μm. Abbreviations: AZM, adoral zone of membranelles; CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of cells analyzed; SD, standard deviation.

[b] In %.

[c] No dorsomarginal kineties present.

[d] Rearmost pair not included; this pair feigns, together with the corresponding pretransverse ventral cirrus (arrow in Fig. 2D), a short, longitudinal midventral row.

20 oblique frontoventral-transverse cirri anlagen are formed for the proter and opisthe, respectively (Figs 3C, 4I, J). This anlagen area crosses the parental midventral complex in the central body portion. We did not find somewhat earlier dividers and thus it remains obscure whether the anlagen for the proter and the opisthe developed separately or via so-called primary primordia.

In middle dividers, the undulating membranes anlage (anlage I) in each daughter cell is clearly differentiated and, as usual, the left frontal cirrus develops from its anterior end (Fig. 3E). The parental adoral zone in the proter is more or less unchanged, while in the adoral zone of the opisthe all membranelles are formed and its anterior portion is curved rightwards (Fig. 3E). The segregation of cirri from the frontoventral-transverse cirri anlagen is complete (Figs 3E, 4L). Anlage I contributes the left frontal cirrus; anlage II forms the middle frontal cirrus and the buccal cirrus; anlage III produces the right frontal cirrus and the parabuccal cir-

rus (III/2); anlagen IV to n–3 contribute each a midventral pair; anlage n–2 produces a midventral pair and the leftmost transverse cirrus; anlage n–1 generates a longitudinally arranged midventral pair, a pretransverse ventral cirrus (likely homologous to V/2 of the 18-cirri hypotrichs), and the middle transverse cirrus (the midventral pair and the pretransverse ventral cirrus feign a short midventral row); and anlage n develops two (rarely three) frontoterminal cirri and the right transverse cirrus (Figs 3E, 4L, N).

In late stages, the anterior end of the new adoral zone starts with the final shaping, that is, the distal end continues with the bending to the right. The undulating membrane anlage of both the proter and the opisthe splits longitudinally and forms the new paroral and endoral. The frontoterminal cirri migrate anteriorly near to the distal end of the adoral zone. The dividing cell begins to elongate and the new ciliary structures move further apart as they migrate toward their final positions. The resorption of the remaining parental cirri continues (Figs 3G, I, 4P, Q).

Formation of the marginal cirri, dorsal kineties, and caudal cirri. In an early stage and within each parental marginal row, a few cirri near the anterior end and a few others below the mid-body dedifferentiate to form the marginal row anlagen for the proter and the opisthe respectively (Figs 3C, 4K). Later, these anlagen elongate right of the parental rows by adding basal bodies mostly posteriorly and by developing into cirri. Most parental marginal cirri are still present in late dividers indicating that they disappear very late (Fig. 3E, G, I).

Development of dorsal kineties anlagen commences in early dividers (Fig. 3D). The ciliature develops according to type 2 of Foissner and Adam (1983; = *Urosomoida*-type according to Berger and Foissner 1997 and Berger 1999), that is, new kineties 1–3 are produced at two levels within parental rows 1–3 (Fig. 3D, F). The remaining four or five rows are dorsomarginal kineties which originate, as is usual, close to/from the right anterior end of the right marginal row anlagen (Figs 3E, 4K, L). In late dividers, the dorsomarginal kineties increase in length and migrate onto the dorsal side to form the dorsal kineties 4–7 or 4–8 (Fig. 3H, J). One caudal cirrus each is formed at the posterior end of kineties 1–3 (Figs 3H, J, 4O).

Division of nuclear apparatus. The nuclear apparatus divides in the usual way, that is, the macronuclear nodules fuse to a single mass during middle stages. Later, this mass divides amitotically so that each one nodule is in the proter and the opisthe. During late stages, the nodules divide again so that each filial product has two macronuclear nodules. The micronuclei obviously divide mitotically (Figs 3F, H, J, 4J, M, O).

SSU rDNA gene sequence analysis and phylogenetic analyses (Fig. 5)

The SSU rDNA sequence of the Chinese population of *Caudiholosticha stueberi* (GenBank accession number KT724201) has a length of 1728 bp and a G + C content of 45.36%. The topologies of the ML and BI trees are similar and therefore only the ML tree is shown (Fig. 5). According to the phylogenetic analyses of the 64-taxon alignment, *C. stueberi* is sister of an unidentified *Uroleptus* population (*Uroleptus* sp. WJC-2003; AY294646) in both trees with full support (ML/BI, 100/1.00). The SSU rDNA sequence similarity between these two taxa is 99.0%. The other *Uroleptus* species sequenced so far form a cluster, which is, however, not supported statistically (36/0.89). *Uroleptus gallina* AF164130 is distinctly set off within this cluster. These two groups (*C. stueberi* + *Uroleptus* sp., respectively, other *Uroleptus* species) form a cluster with high support in the BI tree (40/0.95). The next relatives are *Oxytrichella sejongensis* (for explanation of name, see discussion) and *Atractos contortus*, two dorsomarginalians which, however, lack a midventral complex. The Dorsomarginalia Berger, 2006 form a monophyletic group in our tree, however, the support is weak (Fig. 5).

Caudiholosticha tetracirrata sensu Lv et al. (2015) (Fig. 6A, B; Table 1)

Remarks: Lv et al. (2015) sequenced the SSU rDNA (GenBank accession number KJ958491) of a Chinese freshwater population of *C. tetracirrata* (*C. tetracirra* in this paper is an incorrect subsequent spelling). In the supporting information, they provided two micrographs, which, however, do not show relevant details. Thus, we made two illustrations showing the ventral and dorsal ciliature and the macronuclear apparatus and provide a morphometric characterization of this population, which was very likely misidentified by Lv et al. (2015; see discussion of *Adumbratosticha* gen. nov.).

Discussion

Identification of Chinese population as *Caudiholosticha stueberi*

The morphology of the Chinese population from a floodplain soil agrees very well with that of the type population from a soil in a mountain valley (Fuscher Tal) in Salzburg, Austria (Foissner 1987; for revision, see Berger 2006, p. 235). Thus, the identification is beyond reasonable doubt.

Caudiholosticha, now a monotypic subgenus of *Uroleptus*

Caudiholosticha was established for those *Holosticha* species which have caudal cirri and which lack the prominent apomorphies of *Holosticha* sensu Berger (2003). He fixed *Holosticha stueberi* as type species because it is very well defined morphologically via the original description (Foissner 1987) and the caudal cirri are rather distinct.

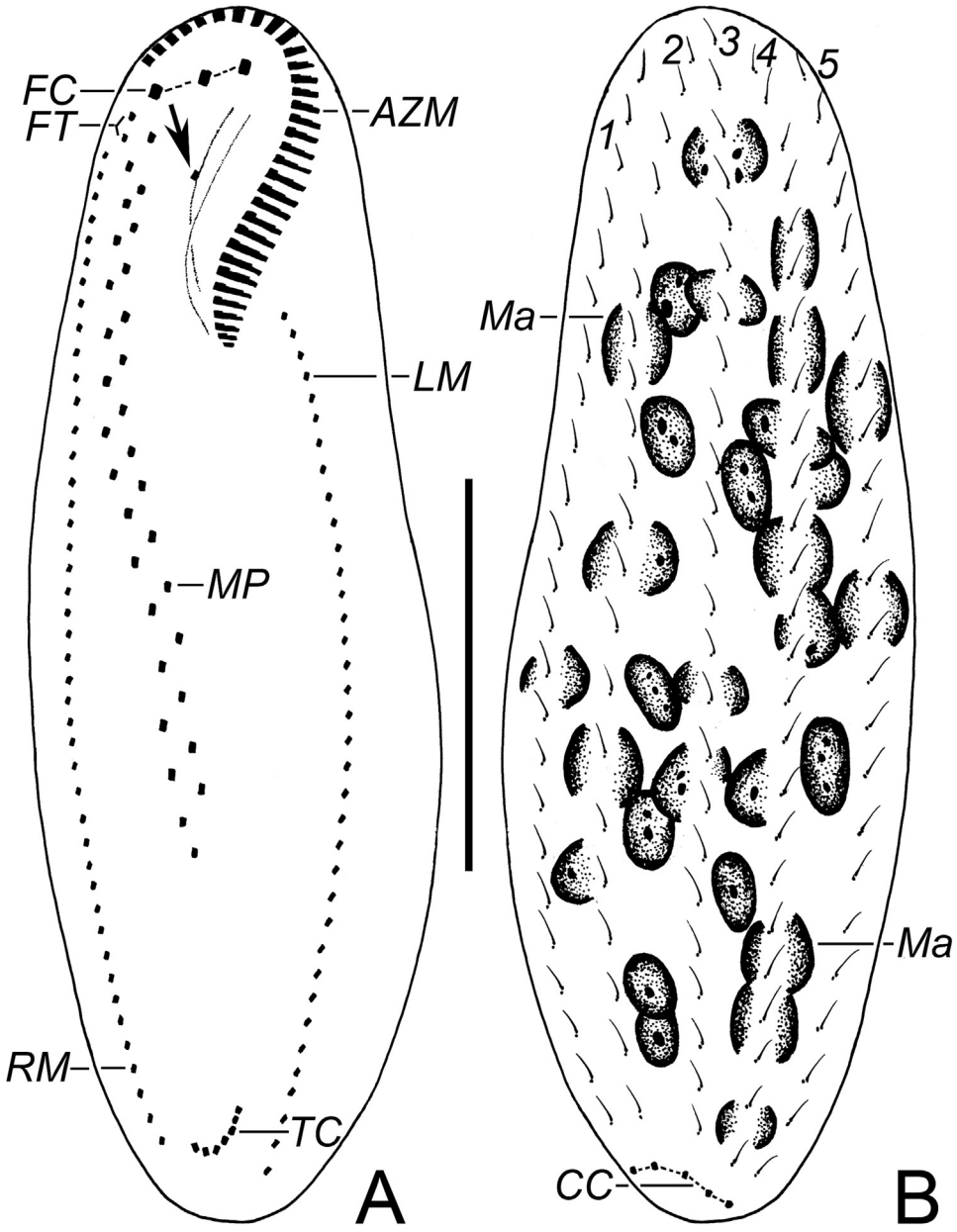

Fig. 6A, B. *Caudiholosticha tetracirrata* sensu Lv et al. (2015) after protargol preparation (originals). Infraciliature of ventral and dorsal side and macronuclear apparatus. Arrow marks buccal cirrus. AZM, adoral zone of membranelles; CC, caudal cirri; FC, frontal cirri; FT, frontoterminal cirri; LM, left marginal row; Ma, macronuclear nodules; MP, midventral pairs forming the midventral complex; RM, right marginal row; TC, transverse cirri; 1–5, dorsal kineties. Bar, 50 μm.

Berger (2003) already stated that *Caudiholosticha* is non-monophyletic due to the lack of an apomorphy. Somewhat later, Berger (2006) and Foissner and Stoeck (2006) hypothesized that *C. stueberi* is a *Uroleptus* species because both taxa have the same ventral and dorsal ciliature. The present morphogenetic data, especially the validation of dorsomarginal kineties (Figs 3E, G, H, J, 4K,L), as well as the molecular analyses (Fig. 5) support this assumption.

Apart from the type species, Berger (2003, 2006), Foissner (2016), and Li et al. (2016) assigned 16 species to *Caudiholosticha* (see next chapter). However, the available, partly sparse data on these 16 species more or less strongly indicate that they are misclassified in this genus (see next chapter). Consequently, only the type species remains in *Caudiholosticha*.

At present, two groups can be clearly distinguished in *Uroleptus* from the morphological point of view (Berger, unpublished data; Chen et al. 2016). The first group comprises those species which have distinctly more than five, conspicuous transverse cirri, for example, *Uroleptus piscis* (Müller, 1773) Ehrenberg, 1831 (e.g., Foissner et al. 1991, p. 252). By contrast, the second group includes all species which

have five or less, inconspicuous transverse cirri, for example *U. longicaudatus* Stokes, 1886, a member of the *U. limnetis* complex (Chen et al. 2016). The species of both *Uroleptus* groups have a more or less distinct tail (e.g., Foissner et al. 1991; He et al. 2011; Kahl 1932). The ventral ciliature of *Caudiholosticha stueberi* resembles the species of the second *Uroleptus* group because only three transverse cirri are present (Table 1; Foissner 1987). However, *C. stueberi* has a broadly rounded rear end (Fig. 2H–K) and thus a close relationship of this species with *Uroleptus* is unobvious.

Phylogenetic analyses inferred from the SSU rDNA gene sequence support the infraciliature-based classification of *C. stueberi* in *Uroleptus*, which is a monophyletic group according to this molecular marker in almost all studies (Fig. 5; e.g., Chen et al. 2016; Foissner et al. 2004; Sonntag et al. 2008). *Caudiholosticha stueberi* is closely related with an unidentified *Uroleptus* population (AY294646) which is, according to Chang et al. (2005), morphologically similar to *U. gallina* (Müller, 1786) Foissner et al., 1991. *Uroleptus gallina* (AF164130), also analyzed by Chang et al. (2005), is the first sequence branching off in the sister group composed of the remaining *Uroleptus* species sequenced so far (Fig. 5). However, morphologically *U. gallina* belongs, in contrast to *C. stueberi*, to the group with many transverse cirri (for review of *U. gallina*, see Foissner et al. 1991, p. 244). Unfortunately, this is not the sole inconsistency with our morphology-based hypothesis that species with few, respectively, many transverse cirri form separate groups because *U. piscis* (AF164131) – another species with many transverse cirri (Foissner et al. 1991, p. 252) – is more closely related with species having few transverse cirri, for example *U. willii* (Sonntag et al. 2008) or *U. longicaudatus* (Chen et al. 2016), than with *U. gallina*. This problem is perhaps due to inaccuracies by the American workers (Chang et al. 2005; direct submission by Prescott and co-workers in 1999; see NCBI database) who obviously did not check the identifications by protargol preparations (Foissner et al. 2004), a prerequisite when dealing with such a difficult group.

Just recently, two phylogenetic analyses inferred from SSU rDNA sequences have been published which disturb the monophyly of *Uroleptus* (Bourland 2015, Fig. 10, but not his Fig. 9; Jung et al. 2016). Both species described and sequenced, *Atractos contortus* Vörösváry, 1950 and *Urosomoida sejongensis* Jung et al., 2016 (now *Oxytrichella sejongensis*, see below), are – like *Uroleptus* – non-oxytrichid dorsomarginalians (Berger 2008, p. 46), but lack the zigzag pattern formed by midventral pairs, a feature which is so characteristic for *Uroleptus* species (He et al. 2011). Whether such trees (roughly) reflect the true relationships or whether they are misclassifications due to various problems remains obscure (for brief discussion of some problems, see Bourland 2015, p. 370). In our tree, these two species form a cluster, which is sister to *Uroleptus* (Fig. 5; a discussion whether these two species are indeed closely related or whether this is an error is beyond the scope of the present paper). Anyhow, during the further course of the discussion, we assume that *A. contortus* and *O. sejongensis* do not belong to the genus *Uroleptus*. In the tree published by Jung et al. (2016) and in our tree (Fig. 5), *Urosomoida sejongensis* is distinctly separated from *U. agilis* (Engelmann, 1862) Hemberger in Foissner, 1982, type species of *Urosomoida* Hemberger in Foissner, 1982, indicating that these two species are not closely related and thus non-congeneric. Thus, we transfer it to *Oxytrichella*, a genus just recently established by Foissner (2016, p. 776) for *Urosomoida*-like species with a single micronucleus between the two macronuclear nodules: *Oxytrichella sejongensis* (Jung, Baek, Kim and Choi, 2016) comb. nov.

In spite of the discrepancies between the classifications based on morphological, respectively, molecular data, we propose to divide *Uroleptus* into three subgenera using the number of transverse cirri and the presence/absence of a tail as relevant morphological features. In addition, the subgenera seem to be characterised by different habitats. The classification as subgenera is mainly indicated by the phylogenetic analyses based on molecular data (Fig. 5). However, it cannot be excluded that a classification of *Paruroleptus* and *Caudiholosticha* in genus rank in the uroleptids is more appropriate when more gene sequences based on unambiguously identified populations are available. In that case, the Uroleptidae Foissner and Stoeck, 2008 would not be longer monotypic. At present, this group contains only the type genus and is therefore redundant.

Uroleptus Ehrenberg, 1831

Improved diagnosis: Flexible non-oxytrichid Dorsomarginalia (i.e., kinety fragmentation lacking, but dorsomarginal rows present) with midventral complex composed of pairs only; right (= anterior) cirrus of each midventral pair distinctly larger than left one. More or less distinct tail present or lacking. Three frontal cirri, one buccal cirrus, and cirrus III/2 present. Five or less (subgenera *U. (Paruroleptus)* and *U. (Caudiholosticha)*) or more than five (7–22; subgenus *U. (Uroleptus)*) transverse cirri present. Three bipolar dorsal kineties and one, two, or more (up to seven in *U. magnificus*!) dorsomarginal kineties. Three caudal cirri present, each one attached to rear end of bipolar kineties. Freshwater and soil.

Type species (by subsequent designation by Borror 1972, p. 12): *Trichoda musculus* Müller, 1773 (*Uroleptus musculus* (Müller, 1773) Ehrenberg, 1831).

Subgenera assigned: *U. (Uroleptus)* Ehrenberg; *U. (Paruroleptus)* Wenzel; *U. (Caudiholosticha)* Berger.

Remarks: A list of species assignable to *Uroleptus* has been provided by He et al. (2011); a detailed revision will be published elsewhere (Berger, Monograph part 5, in preparation). As explained by Chen et al. (2016), there is no unambiguous record of a *Uroleptus* species which lacks transverse cirri. In addition, no serious record of a marine population has been published.

Rigidothrix with *R. goiseri* as single species resembles *Uroleptus* roughly in body outline and ventral and dorsal ciliature (Foissner and Stoeck 2006). However, the rigid body and a clear separation from *Uroleptus* in molecular trees (e.g., Foissner and Stoeck 2006, 2008; Kim et al. 2014; Fig. 5) demonstrate that the resemblance is obviously due to convergent evolution. For exclusion of *Atractos contortus* (Bourland 2015) and *Oxytrichella sejongensis* (Jung et al. 2016) from *Uroleptus*, see above.

Uroleptus (Uroleptus) Ehrenberg, 1831

Diagnosis: *Uroleptus* with more than five transverse cirri; more or less distinct tail present.

Type species (same as for genus): *Trichoda musculus* Müller, 1773 (now *Uroleptus (Uroleptus) musculus* (Müller, 1773) Ehrenberg, 1831).

Remarks: This is the nominotypical subgenus. *Uroleptus* species with more than five transverse cirri have been reliably recorded only from limnetic habitats.

Uroleptus (Paruroleptus) Wenzel, 1953

Diagnosis: *Uroleptus* with five or less transverse cirri; more or less distinct tail present.

Type species (by original designation): *Holosticha caudata* Stokes, 1886 (now *Uroleptus (Paruroleptus) caudatus* (Stokes, 1886) Borror, 1972).

Remarks: For some details on complicated nomenclature and history of "*Paruroleptus*", including the invalidity of *Paruroleptus* Kahl, 1932, see He et al. (2011). A detailed survey of all records published so far shows that species belonging to this subgenus have been reliably recorded from limnetic and terrestrial habitats (Berger, unpublished).

Uroleptus (Caudiholosticha) Berger, 2003 stat. nov.

Diagnosis: *Uroleptus* with five or less transverse cirri; posterior body end broadly rounded, that is, tail lacking.

Type species: *Holosticha stueberi* Foissner, 1987.

Species assignable: *Uroleptus (Caudiholosticha) stueberi* (Foissner, 1987) comb. nov.

Remarks: At present this subgenus is monotypic, that is, comprises only the type species. It has been recorded from terrestrial and semiterrestrial habitats only.

Classification of remaining *Caudiholosticha* species

Berger (2003, 2006) classified 13 species in *Caudiholosticha*, namely, *C. stueberi* (type species; see previous chapter); *C. sylvatica* (Foissner, 1982) Berger, 2003 (for revision, see Berger 2006, p. 239); *C. tetracirrata* (Buitkamp and Wilbert, 1974) Berger, 2003 (Berger 2006, p. 246); *C. islandica* (Berger and Foissner, 1989) Berger, 2003 (Berger 2006, p. 252); *C. paranotabilis* (Foissner et al., 2002) Berger, 2006 (Berger 2006, p. 254); *C. notabilis* (Foissner, 1982) Berger, 2006 (Berger 2006, p. 260); *C. gracilis* (Foissner, 1982) Berger, 2006 (Berger 2006, p. 266); *C. algivora* (Kahl, 1932) Berger, 2003 (Berger 2006, p. 270); *C. viridis* (Kahl, 1932) Berger, 2003 (Berger 2006, p. 272); *C. navicularum* (Kahl, 1932) Berger, 2003 (Berger 2006, p. 274); *C. multicaudicirrus* (Song and Wilbert, 1989) Berger, 2003 (Berger 2006, p. 276); *C. interrupta* (Dragesco, 1966) Berger, 2003 (Berger 2006, p. 279); and *C. setifera* (Kahl, 1932) Berger, 2003 (Berger 2006, p. 281). Only recently, Li et al. (2016) described the sole marine *Caudiholosticha* species, *C. marina*. Likewise quite recently, Foissner (2016, p. 531ff) described three terrestrial *Caudiholosticha* species, namely from Germany (*C. silvicola*), Venezuela (*C. halophila*), and the Virgin Islands (*C. virginensis*).

As detailed above, all these species, except the type species, do not belong to *Caudiholosticha* and therefore have to be transferred to other genera. On the basis of the available data, we preliminary classify the remaining 16 species in six new urostyloid genera, which are, at least partly, very likely non-monophyletic due to the lack of convincing apomorphies. Since not all taxonomically relevant details are known at the individual (type) species, the diagnoses are partly imprecise and thus have to be improved when further data become available.

Extraholosticha gen. nov.

Diagnosis: Urostyloid with a short cirral row formed by anlage I between left frontal cirrus and undulating membranes. Three frontal cirri. Buccal cirrus, parabuccal cirrus, frontoterminal cirri, pretransverse ventral cirri, and transverse cirri present. Midventral complex composed of pairs only. One left and one right marginal row. More than three dorsal kineties. Caudal cirri present, originating from rightmost kinety only in sole species included.

Etymology: *Extraholosticha* is a composite of the Latin adjective *extra* (Brown 1954, p. 312) and the genus-group name *Holosticha* (for derivation see Berger 2006, p. 89). The word *extra* refers to the additional (=extra) cirri behind the left frontal cirrus, and *Holosticha* alludes to the fact that the type species was originally classified in *Holosticha*. Feminine gender.

Type species: *Holosticha sylvatica* Foissner, 1982.

Species assignable: *Extraholosticha sylvatica* (Foissner, 1982) comb. nov.

Remarks: The lack of dorsomarginal kineties in combination with the presence of a midventral complex as well as the placement within the urostyloids in molecular trees (Lv et al. 2015; Li et al. 2016; Fig. 5) require the removal of *C. sylvatica* from *Caudiholosticha*, respectively, *Uroleptus (Caudiholosticha)*. It clusters with a terrestrial population

(KJ958488) of *Anteholosticha monilata* (Kahl, 1928) Berger, 2003 (type species of *Anteholosticha* Berger, 2003) in Lv et al. (2015) and in our tree (Fig. 5). Interestingly, *A. monilata* KJ958488 from China does not cluster with *A. monilata* GU942567 from a Korean brackish water in Lv et al. (2015) and the present tree (Fig. 5), indicating that at least one of these two identifications is incorrect. In Li et al. (2016), *E. sylvatica* has a somewhat isolated position in the core urostyloids. Anyhow, a classification of *C. sylvatica* (with caudal cirri) in *Anteholosticha* (without caudal cirri) or another genus seems not justified at the present state of knowledge. Thus, we establish *Extraholosticha* gen. nov. Since only a small part of the hypotrich diversity is known at present (Foissner 2016, p. 487), we are confident that this new genus will not be monotypic forever.

Extraholosticha sylvatica has two interesting features, namely some (2–4) cirri behind the left frontal cirrus and the caudal cirri are posterior to the rightmost dorsal kinety. Berger (2006, p. 240, 241) hypothesized that the short frontal row originates from anlage I while the caudal cirri develop from the rear end of the rightmost dorsal kinety. Kumar et al. (2010) confirmed both assumptions on an Indian population.

Bicoronella costaricana Foissner, 1995 – type of the monotypic genus–also has some cirri (5–7) between the left frontal cirrus and the anterior portion of the undulating membranes. In addition, it has 3–6 caudal cirri between the rear end of the marginal rows, but it is unknown from which dorsal kinety/kineties the caudal cirri originate. However, there is a crucial difference between *E. sylvatica* and *B. costaricana*, namely in the frontal ciliature: the former has three frontal cirri while the latter has a distinct bicorona. This disagreement strongly suggests that they are not closely related, that is, it would be unwise to transfer *Holosticha sylvatica* to *Bicoronella*.

Adumbratosticha gen. nov.

Diagnosis: Elongate rectangular urostyloids with rounded posterior end. Number of caudal cirri (often two) lower than number of dorsal kineties; caudal cirri in two species vestigial. Undulating membranes relatively short, almost straight, and not distinctly intersecting. Three frontal cirri. Buccal cirrus (near anterior end of undulating membranes), parabuccal cirrus, frontoterminal cirri, and transverse cirri present; pretransverse ventral cirri lacking or present. Midventral complex composed of pairs only. One left and one right marginal row. More than three dorsal kineties. Many macronuclear nodules. Likely terrestrial.

Etymology: *Adumbratosticha* is a composite of the Latin adjective *adumbrat·us* (hazy, vague, indistinct; Brown 1954, p. 70), the thematic vowel *·o-*, the Greek noun *stich-* (row, line; Werner 1972, p. 390), and the inflectional ending *·a*. The first part of the name refers to the indistinct caudal cirri present in two species included. To avoid that the name is too long, only the second part of *Holosticha* was used, alluding to the fact that two species were originally classified in *Holosticha*. Feminine gender.

Type species: *Holosticha tetracirrata* Buitkamp and Wilbert, 1974.

Species assignable: *Adumbratosticha tetracirrata* (Buitkamp and Wilbert, 1974) comb. nov. (type species); *Adumbratosticha islandica* (Berger and Foissner, 1989) comb. nov. (original combination: *Holosticha islandica*); *Adumbratosticha virginensis* (Foissner, 2016) comb. nov. (original combination: *Caudiholosticha virginensis*).

Remarks: At present, this genus comprises three species that agree, inter alia, in their reduced number of caudal cirri (in addition, these cirri are very inconspicuous in *A. tetracirrata* and *A. islandica*), their broadly rounded posterior end (in contrast to the members of the next genus), and the terrestrial habitat (in contrast to the members of *Limnoholosticha* gen. nov., see below). Since caudal cirri are very likely part of the ground pattern of the hypotrichs (Berger 2008, p. 28), we have to hypothesize that the indistinct caudal cirri of *A. tetracirrata* and *A. islandica* are rudiments. Ontogenetic data and gene sequence analyses are needed for a better interpretation of this feature and to estimate the position of the type species in the urostyloids as well as to show whether or not the three species preliminary included cluster together.

We do not classify the three species mentioned above in *Anteholosticha*, a rather large, non-monophyletic group of urostyloid species which lacks caudal cirri (Berger 2003, 2006), because this would make this genus even more inhomogeneous.

Lv et al. (2015) sequenced *C. tetracirrata* (GenBank accession number KJ958491). A detailed comparison of this Chinese population (Fig. 6A, B, Table 1) with the populations reviewed by Berger (2006, p. 246) revealed differences in the following features: (i) habitat (limnetic vs. terrestrial); (ii) shape of undulating membranes and position of buccal cirrus (long, curved, and intersecting and buccal cirrus distinctly behind anterior end of membranes vs. shorter and not distinctly curved, that is, more or less parallel, and buccal cirrus at/close to anterior end of membranes); (iii) number of adoral membranelles (34 on average vs. 24–26 on average; see p. 285 in Berger 2006); (iv) number of midventral pairs (about 16 on average vs. about 10); (v) number of marginal cirri (48 left and 52 right vs. about 20–29 left and 26–30 right); (vi) number of dorsal kineties (five vs. four); and (vii) number of caudal cirri (four or five vs. usually two or less). In addition, the number of transverse cirri is higher in the limnetic population from China than in the populations described by Buitkamp and Wilbert (1974) and Foissner (1982) (on average seven vs. on average 2.7–4.0). This comparison demonstrates that *C. tetracirrata* (KJ958491) sensu Lv et al. (2015) is a misidentification and therefore this sequence cannot be used for the estimation of the phylogenetic position of *Adumbratosticha*. The population does not belong to another species previously assigned to *Caudiholosticha* indicating that it represents a new species. However, since live observations are lacking and the protargol preparations are of mediocre quality we

refrain from such a step. Since the number of caudal cirri is identical with the number of dorsal kineties it does not fit very well the diagnosis of *Adumbratosticha* proposed above.

Acuholosticha gen. nov.

Diagnosis: Slender urostyloids with narrowly rounded posterior body end. Three frontal cirri. Buccal cirrus, parabuccal cirrus, frontoterminal cirri, and transverse cirri present; pretransverse ventral cirri present or lacking. Midventral complex composed of pairs only. One left and one right marginal row. 2–4 dorsal kineties; caudal cirri present (see remarks). Many macronuclear nodules. Likely terrestrial.

Etymology: *Acuholosticha* is a composite of the Latin noun *acus* (needle, pin; Brown 1954, p. 69) and the genus-group name *Holosticha* (for derivation see Berger 2006, p. 89). It alludes to the fact that the posterior end of the species included is narrowly rounded (acute, pointed) and that they were previously classified in (*Caudi*)*holosticha*. Feminine gender.

Type species: *Uroleptus paranotabilis* Foissner, Agatha and Berger, 2002.

Species assignable: *Acuholosticha paranotabilis* (Foissner, Agatha and Berger, 2002) comb. nov. (type species); *Acuholosticha gracilis* (Foissner, 1982) comb. nov. (original combination: *Perisincirra gracilis*); *Acuholosticha notabilis* (Foissner, 1982) comb. nov. (original combination: *Paruroleptus notabilis*); *Acuholosticha silvicola* (Foissner, 2016) comb. nov. (original combination: *Caudiholosticha silvicola*); *Acuholosticha halophila* (Foissner, 2016) comb. nov. (original combination: *Caudiholosticha halophila*).

Remarks: *Acuholosticha* is not a very homogenous group of slender, lanceolate, soil hypotrichs. All species have many macronuclear nodules. The dorsal ciliature is composed of two (*A. gracilis*) to five (*A. halophila*) kineties which are partly made of few and widely spaced bristles. However, the available data rather clearly show that dorsomarginal kineties are lacking, indicating that all are urostyloids. Ontogenetic data for all species are needed for a correct interpretation of the dorsal kinety and caudal cirri pattern.

According to the original description (Foissner 2016, p. 532), *Acuholosticha halophila* has a midventral row at the posterior end of the midventral complex. However, the unequal distances between these cirri indicate that these are also cirral pairs feigning a true row. Ontogenetic data are needed to support or reject this hypothesis.

For three species (*A. notabilis*, *A. silvicola*, *A. halophila*) a pharynx with short (about 1–2 μm) oblong structures has been described (Berger 2006; Foissner 2016). Interestingly, these species differ from the other two species assigned to *Acuholosticha* (*A. paranotabilis*, *A. gracilis*) also in the undulating membrane pattern and the position of the buccal cirrus (membranes relatively long, curved, and intersecting; buccal cirrus distinctly behind anterior end of membranes vs. membranes relatively short and rather straight, not distinctly intersecting; buccal cirrus near anterior end of membranes). Further studies, including molecular ones on reliably identified populations, will show whether or not these differences require the separation at genus level. Interestingly, some other urostyloids (e.g., *Birojimia terricola* Berger and Foissner, 1989; *Pseudobirojimia muscorum* (Kahl, 1932) Foissner, 2016; *Pseudourostyla dimorpha* Foissner, 2016) have the same undulating membrane pattern and pharynx as *A. notabilis*, *A. silvicola*, and *A. halophila* (Berger 2006; Foissner 2016). Further studies are needed to decide whether this similarity is a convergence or whether it indicates a close relationship.

Limnoholosticha gen. nov.

Diagnosis: Urostyloids with elliptical to wide elliptical outline. Three frontal cirri. Buccal cirrus, parabuccal cirrus, and about 5–8 transverse cirri present; presence/absence of frontoterminal and pretransverse ventral cirri not known. Midventral complex likely composed of pairs only. One left and one right marginal row. Caudal cirri present, in some species rather long. Two macronuclear nodules. Stagnant(?) inland waters.

Etymology: *Limnoholosticha* is a composite of the Greek noun *he limne* (stagnant water, pond, swamp; Hentschel and Wagner 1996, p. 366) and the genus-group name *Holosticha* (for derivation see Berger 2006, p. 89). It alludes to the fact that the species of this group occur in stagnant, limnetic (non-marine) habitats and were originally classified in *Holosticha*. Feminine gender.

Type species: *Holosticha (Holosticha) navicularum* Kahl, 1932.

Species assignable: *Limnoholosticha navicularum* (Kahl, 1932) comb. nov. (type species); *Limnoholosticha algivora* (Kahl, 1932) comb. nov. (original combination: *Holosticha (Holosticha) algivora*); *Limnoholosticha viridis* (Kahl, 1932) comb. nov. (original combination: *Holosticha (Holosticha) viridis*); *Limnoholosticha setifera* (Kahl, 1932) comb. nov. (original combination: *Holosticha (Holosticha) setifera*).

Remarks: Kahl (1932) described all species assigned to *Limnoholosticha*. They are small (about 75 μm, *L. algivora*) to moderately large (about 200 μm, *L. navicularum*) species from freshwater, one species (*L. setifera*) was discovered in inland waters with high salinity (Kahl 1932). They have a rather similar general appearance, including two macronuclear nodules. None of the species is described in detail and therefore the characterization of *Limnoholosticha* is unspecific and has to be improved when more data of the individual species become available. We select *L. navicularum* as type species because it has two rather distinct species features, namely, (i) a single, prominent micronucleus in-between the two macronuclear nodules and (ii) conspicuous transverse cirri which are displaced very far anteriorly (for revision, see Berger 2006, p. 274).

Multiholosticha gen. nov.

Diagnosis: Urostyloids with elliptical outline. Three frontal cirri. Buccal cirrus, parabuccal cirrus, pretransverse ventral cirri, and about 7–12 transverse cirri present; presence/absence of frontoterminal questionable. Midventral complex composed of pairs only. Perhaps all (IV to n) or at least almost all anlagen forming a midventral pair produce a transverse cirrus. One left and one right marginal row. Six dorsal kineties in both species included. Type species with six, second species with 14–16 caudal cirri forming bow-shaped (pseudo?)row. Many macronuclear nodules. Freshwater.

Etymology: *Multiholosticha* is a composite of *multi-* (Latin, indefinite numeral adjective; many; Hentschel and Wagner 1996, p. 409) and the genus-group name *Holosticha* (for derivation see Berger 2006, p. 89). It alludes to the fact that the species of this group have many caudal cirri and were originally classified in *Holosticha*. Feminine gender.

Type species: *Holosticha multicaudicirrus* Song and Wilbert, 1989.

Species assignable: *Multiholosticha multicaudicirrus* (Song and Wilbert, 1989) comb. nov. (type species); *Multiholosticha interrupta* (Dragesco, 1966) comb. nov. (original combination: *Holosticha interrupta*).

Remarks: The two species included have a very similar ventral and dorsal infraciliature indicating that they are closely related (for revision, see Berger 2006, p. 276ff). Ontogenetic data are needed for a correct interpretation of the ventral and dorsal infraciliature and gene sequence analyses would be useful for a rough estimation of the phylogenetic position of this group.

The rather high number of transverse cirri, which corresponds more or less the number of midventral pairs, as well as the relatively high number of dorsal kineties (six in both species) are somewhat reminiscent of pseudoamphisiellids, a group of marine hypotrichs with a conspicuous alveolar layer bearing prominent extrusomes. In addition, the midventral pairs do not form a distinct zigzag pattern as in *Multiholosticha* spp., but two distinctly separated pseudorows (e.g., Berger 2006, p. 191; Li et al. 2010). In molecular trees, the pseudoamphisiellids branch off as one of the first groups of hypotrichs, that is, not within the core urostyloids (e.g., Li et al. 2016); sometimes they branch off earlier than the oligotrichs (e.g., Li et al. 2010).

Caudikeronopsis gen. nov.

Diagnosis: Non-dorsomarginalian hypotrichs (pseudokeronopsids?) with three frontal cirri. Buccal cirrus, parabuccal cirrus, frontoterminal cirri, pretransverse ventral cirri, and transverse cirri present. Midventral complex composed of pairs only. One left and one right marginal row. More than three dorsal kineties, but not all form a caudal cirrus. Many macronuclear nodules. Marine.

Etymology: *Caudikeronopsis* is a composite of the Latin noun *caud·a* (the tail, meaning caudal cirri in present case; Hentschel and Wagner 1996, p. 157), the thematic vowel *·i-*, and the genus-group name *Keronopsis*, the second part of *Pseudokeronopsis* (for derivation, see Berger 2006, p. 887). It alludes to the fact that the sole species of this genus resembles *Pseudokeronopsis* species, but has caudal cirri. Feminine gender because ending with *-opsis* (ICZN 1999, Article 30.1.2).

Type species: *Caudiholosticha marina* Li, Chen and Xu, 2016.

Species assignable: *Caudikeronopsis marina* (Li, Chen and Xu, 2016) comb. nov.

Remarks: The habitus, the ventral and dorsal ciliature, and the marine habitat of *Caudikeronopsis marina* are somewhat reminiscent of a pseudokeronopsid (for revision, see Berger 2006). Interestingly, *C. marina* is closely related with the trachelostylids according to the molecular tree published by Li et al. (2016). Trachelostylids are marine, more or less distinctly cephalised 18-cirri hypotrichs which have a rather specific dorsal morphogenesis (for revision, see Berger 2008, p. 471; Shao et al. 2007) so that a close relationship between *Caudikeronopsis marina* and the trachelostylids is hardly imaginable from the morphological point of view. The next relative is *Arcuseries* Huang et al., 2014, a rather homogenous group composed of four marine, non-dorsomarginalian hypotrichs previously classified in *Anteholosticha*. They have, inter alia, U-shaped-arranged transverse cirri, a midventral complex composed of pairs only, three bipolar dorsal kineties, and the lack of caudal cirri in common (Li et al. 2016; Fig. 5). According to Fig. 1A, F in Li et al. (2016), the proximal portion of the adoral zone is widened. Interestingly, this feature is not mentioned in the text by Li et al. (2016). Since *Caudikeronopsis* is monotypic it is not known whether this feature is species- or genus-specific. A detailed redescription of *C. marina*, including details of the oral apparatus (endoral not observed by Li et al. 2016) and ontogenesis, are needed for a better understanding of this taxon.

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References

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

Berger, H., 2003. Redefinition of *Holosticha* Wrzesniowski, 1877 (Ciliophora, Hypotricha). Eur. J. Protistol. 39, 373–379.

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. 90, 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. (Zool.) 55, 19–46.

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

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

Bourland, W.A., 2015. Morphology, ontogenesis and molecular characterization of *Atractos contortus* Vörösváry, 1950 and *Stichotricha aculeata* Wrzesniowskiego, 1866 (Ciliophora, Stichotrichida) with consideration of their systematic positions. Eur. J. Protistol. 51, 351–373.

Brown, R.W., 1954. Composition of Scientific Words. A Manual of Methods and a Lexicon of Materials for the Practice of Logotechnics. Brown, Baltimore.

Buitkamp, U., Wilbert, N., 1974. Morphologie und Taxonomie einiger Ciliaten eines kanadischen Prärieboden. Acta Protzool. 13, 201–210.

Chang, W.-J., Bryson, P.D., Liang, H., Shin, M.K., Landweber, L.F., 2005. The evolutionary origin of a scrambled gene. Proc. Natl. Acad. Sci. U. S. A. 102, 15149–15154.

Chen, L., Lv, Z., Shao, C., Al-Farraj, S.A., Song, W., Berger, H., 2016. Morphology, cell division, and phylogeny of *Uroleptus longicaudatus* (Ciliophora, Hypotricha), a species of the *Uroleptus limnetis* complex. J. Eukaryot. Microbiol. 63, 349–362.

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

Dragesco, J., 1970. Ciliés libres du Cameroun. Ann. Fac. Sci. Univ. féd. Cameroun (Numéro hors-série), 1–141.

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, pp. 1–154.

Engelmann, T.W., 1862. Zur Naturgeschichte der Infusionsthiere. Z. wiss. Zool. 11, 347–393.

Foissner, W., 1982. Ökologie und Taxonomie der Hypotrichida (Protozoa: Ciliophora) einiger österreichischer Böden. Arch. Protistenk 126, 19–143.

Foissner, W., 1987. Faunistische und taxonomische Notizen über die Protozoen des Fuscher Tales (Salzburg, Österreich). Jber. Haus Nat. Salzburg 10, 56–68.

Foissner, W., 1995. Tropical protozoan diversity: 80 ciliate species (Protozoa, Ciliophora) in a soil sample from a tropical dry forest of Costa Rica, with description of four new genera and seven new species. Arch. Protistenk. 145, 37–79.

Foissner, W., 2016. Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. Denisia 35, 1–912.

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., Stoeck, T., 2006. *Rigidothrix goiseri* nov. gen., nov. spec. (Rigidotrichidae nov. fam.), a new "flagship" ciliate from the Niger floodplain breaks the flexibility-dogma in the classification of stichotrichine spirotrichs (Ciliophora, Spirotrichea). Eur. J. Protistol. 42, 249–267.

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.

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., Agatha, S., Berger, H., 2002. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Part I: text and line drawings. Part II: photographs. Denisia 5, 1–1459.

Foissner, W., Moon-van der Stay, S.Y., van der Stay, G.W.M., Hackstein, J.H.P., Krautgartner, W.-D., 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.

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.

Hentschel, E.J., Wagner, G.H., 1996. Zoologisches Wörterbuch. Tiernamen, allgemeinbiologische, anatomische, physiologische Termini und Kurzbiographien, 6th ed. Gustav Fischer Verlag, Jena.

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. Biodivers. 8, 409–416.

Huang, J., Chen, Z., Song, W., Berger, H., 2014. Three-gene based phylogeny of the Urostyloidea (Protista, Ciliophora,

Hypotricha), with notes on classfication of some core taxa. Mol. Phylogenet. Evol. 70, 337–347.
ICZN (International Commission on Zoological Nomenclature), 1999. International Code of Zoological Nomenclature. International Trust for Zoological Nomenclature, London.
Jung, J.H., Baek, Y.S., Kim, S., Choi, H.G., 2016. Morphology and molecular phylogeny of a new freshwater ciliate *Urosomoida sejongensis* n. sp. (Ciliophora, Sporadotrichida, Oxytrichidae) from King George Island, Antarctica. Zootaxa 4072, 254–262.
Kahl, A., 1928. Die Infusorien (Ciliata) der Oldesloer Salzwasserstellen. Arch. Hydrobiol. 19, 189–246.
Kahl, A., 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Dtl. 25, 399–650.
Kim, J.H., Vd'ačný, P., Shazib, S.U.A., Shin, M.K., 2014. Morphology and molecular phylogeny of *Apoterritricha lutea* n. g., n. sp. (Ciliophora, Spirotrichea, Hypotrichia): a putative missing link connecting *Cyrtohymena* and *Afrokeronopsis*. J. Eukaryot. Microbiol. 61, 520–536.
Kumar, S., Kamra, K., Sapra, G.R., 2010. Ciliates of the Silent Valley National Park, India: urostyloid hypotrichs of the region with a note on the habitat. Acta Protozool. 49, 339–364.
Li, J., Chen, X., Xu, K., 2016. Morphology and small subunit rDNA phylogeny of two new marine urostylid ciliates, *Caudiholosticha marina* sp. nov. and *Nothoholosticha flava* sp. nov. (Ciliophora, Hypotrichia). J. Eukaryot. Microbiol. 63, 460–470.
Li, L., Song, W., Al-Rasheid, K.A.S., Warren, A., Li, Z., Xu, Y., Shao, C., 2010. Morphology and morphogenesis of a new marine hypotrichous ciliate (Protozoa, Ciliophora, Pseudoamphisiellidae), including a report on the small subunit rRNA gene sequence. Zool. J. Linn. Soc. 158, 231–243.
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, Dordrecht.
Martin, J., 1982. Évolution des patrons morphogénétiques et phylogenèse dans le sous-ordre des Sporadotrichina (Ciliophora, Hypotrichida). Protistologica 18, 431–447.
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.
Müller, O.F., 1786. Animalcula Infusoria Fluviatilia et Marina, quae Detexit, Sytematice Descripsit et ad Vivum Delineari Curavit. Hauniae, Mölleri.
Nylander, J.A.A., 2004. MrModeltest, Version 2.2. Evolutionary Biology Centre, Uppsala University, Uppsala.
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.
Shao, C., Song, W., Yi, Z., Gong, J., Li, J., Lin, X., 2007. Morphogenesis of the marine spirotrichous ciliate *Trachelostyla pediculiformis* (Cohn, 1866) Borror, 1972 (Ciliophora, Stichotricha), with consideration of its phylogenetic position. Eur. J. Protistol. 43, 255–264.
Shao, C., Gao, F., Hu, X., Al-Rasheid, K.A.S., Warren, A., 2011. Ontogenesis and molecular phylogeny of a new marine urostylid Ciliate, *Anteholosticha petzi* n. sp. (Ciliophora, Urostylida). J. Eukaryot. Microbiol. 58, 254–265.
Song, W., Wilbert, N., 1989. Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. Lauterbornia Heft 3, 2–221.
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.
Stokes, A.C., 1886. Some new hypotrichous infusoria. Proc. Am. Phil. Soc. 23, 21–30.
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.
Vörösváry, B., 1950. A Kalános patak csillós véglényei (Die Ciliaten des Kalános-Baches). Annls biol. Univ. szeged. 1, 343–387.
Warren, A., Fyda, J., Song, W., 2002. The morphology of the poorly known freshwater urostylid ciliate *Neokeronopsis spectabilis* (Kahl, 1932) nov. gen., nov. comb. (Ciliophora: Urostylidae), with notes on its morphogenesis. Eur. J. Protistol. 38, 195–206.
Wenzel, F., 1953. Die Ciliaten der Moosrasen trockner Standorte. Arch. Protistenk. 99, 70–141.
Werner, F.C., 1972. Wortelemente lateinisch-griechischer Fachausdrücke in den biologischen Wissenschaften. Suhrkamp, Baden-Baden.
Wilbert, N., 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos 64, 171–179.
Wrzesniowski, A., 1877. Beiträge zur Naturgeschichte der Infusorien. Z. wiss. Zool. 29, 267–323.
Yi, Z., Song, W., 2011. Evolution of the order Urostylida (Protozoa, Ciliophora): new hypotheses based on multi-gene information and identification of localized incongruence. PLoS One 6, e17471.