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Morphology and Molecular Phylogeny of a New Hypotrich Ciliate, *Pseudourostyla guizhouensis* sp. nov. from Southern China, with Notes on a Chinese Population of *Hemicycliostyla franzi* (Foissner, 1987) Paiva et al., 2012 (Ciliophora, Hypotricha)

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ABSTRACT

The morphology and molecular phylogeny of a soil hypotrich ciliate, *Pseudourostyla guizhouensis* sp. nov., collected from southern China, were investigated. *Pseudourostyla guizhouensis* sp. nov. has an elongate elliptical body measuring 180–310 × 65–85 μm in vivo; invariably two right and three or four left marginal rows; six or seven dorsal kineties; adoral zone consisting of 57–70 membranelles; 12–16 frontal cirri, one buccal cirrus, 13–20 midventral pairs, two frontoterminal cirri, two pre-transverse ventral cirri, and five to seven transverse cirri. Morphogenesis during physiological regeneration indicates that the marginal rows of each side originate from a common anlage that differentiates into several rows. Molecular phylogenetic analysis based on SSU rDNA sequence data reveals that *P. guizhouensis* sp. nov. clusters with the type species *P. cristata* (Jerka-Dziadosz, 1964) Borror, 1972 and that the genus *Pseudourostyla* is monophyletic. The morphological characters of another soil hypotrich ciliate, *Hemicycliostyla franzi* (Foissner, 1987) Paiva et al., 2012, are also described based on a Chinese (Guizhou) population.

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Keywords
Ciliature; phylogeny; Pseudourostylidae; soil ciliate; taxonomy.

ACCORDING to Berger (2006), the family Pseudourostylidae Jankowski, 1979 comprises three genera, namely *Hemicycliostyla* Stokes, 1886, *Pseudourostyla* Borror, 1972, and *Trichototaxis* Stokes, 1891. One of the most important diagnostic features of the type genus *Pseudourostyla* is the formation mode of the marginal rows, i.e., groups of marginal cirri arise from a common primordium to the right of the rightmost row of each group (Berger 2006). Kumar et al. (2010) reported that, in a reorganizer of *Hemicycliostyla franzi* (Foissner, 1987) Paiva et al., 2012 (called *Pseudourostyla franzi* Foissner, 1987), the marginal rows develop in the usual manner for urostylids, that is, by intrakinetal (within-row) formation. Consequently, Paiva et al. (2012) transferred this species to *Hemicycliostyla*. To date, eight species have been assigned to *Pseudourostyla*, namely *P. cristata* (Jerka-Dziadosz, 1964) Borror, 1972, *P. cristatoides* Jung et al., 2012, *P. dimorpha* Foissner, 2016, *P. levis* Takahashi, 1973, *P. muscorum* (Kahl, 1932) Borror, 1972, *P. nova* Wiackowski, 1988, *P. pelotensis* Paiva and Silva-Neto, 2006, and *P. subtropica* Chen et al., 2014 (Berger 2006; Chen et al. 2014; Foissner 2016; Jung et al. 2012; Paiva and Silva-Neto 2006). Five species have been assigned to *Hemicycliostyla*, namely *H. concha* (Entz, 1884) Kahl, 1932, *H. franzi*, *H. lacustris* Gellért and Tamás, 1958, *H. marina* Kahl, 1932, and *H. sphagni* Stokes, 1886 (Berger 2006; Paiva et al. 2012).

The present paper reports the morphology of two hypotrich ciliates, *Pseudourostyla guizhouensis* sp. nov. and *Hemicycliostyla franzi*, isolated from soil in southern China. The molecular phylogeny of the novel species was investigated based on small subunit ribosomal DNA sequences data.

MATERIALS AND METHODS

Sampling and cultivation

Soil samples were collected on 19 July 2014 from the floodplain of a small river near the village of Zhonghuashan (27°34′48″N, 109°15′42″E, about 394 m above sea level), Aojiazhai Township, Tongren City, Guizhou Province, China. The annual average temperature of the sampling site is 15 °C and the annual average precipitation is 1,300 mm. About 1 kg of soil was air-dried in the laboratory. Ciliates were stimulated to excyst and emerge from the soil samples by employing the non-flooded Petri dish method described by Foissner (2016). Non-clonal cultures were established at room temperature (about 25 °C) in Petri dishes containing mineral water with squeezed rice grains to enrich the bacterial food.

Morphological investigations

Living cells were observed, using differential interference contrast microscopy and photographed using a digital camera (DP73, Olympus). The protargol silver staining method of Wilbert (1975) was used to reveal the nuclear apparatus and ciliature. Measurements of the stained specimens were carried out with an ocular micrometre. Drawings of stained specimens were performed at 1,250 × with the aid of a camera lucida. To illustrate the changes occurring during morphogenetic processes, the old (parental) ciliary structures are depicted by contour whereas new structures are shaded black. Terminology is mainly according to Berger (2006).

DNA extraction, PCR amplification, and gene sequencing

The genomic DNA of *Pseudourostyla guizhouensis* sp. nov. was extracted from single specimens, the procedure was conducted as previous studies (Chen et al. 2017; Luo et al. 2017; Shao et al. 2014). In short, each cell of the new species was isolated, washed repeatedly with sterile water to remove potential contamination, and transferred to a 2-ml microfuge tube with minimum volume of water. Genomic DNA was extracted, using the REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO), following the manufacturer's instructions. The SSU rDNA was amplified with the eukaryotic

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universal primers Forward (5'-AAC CTG GTT GAT CCT GCC AGT-3') and Reverse (5'-TGA TCC TTC TGC AGG TTC ACC TAC-3') (Huang et al. 2016; Medlin et al. 1988). High fidelity *Taq* polymerase (Takara Ex *Taq*, Takara Biotechnology, Dalian) was used to minimize the possibility of amplification errors. The amplification cycles were as follows: 5 min at 94 °C, followed by 30 cycles at 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 (Gao et al. 2016).

Phylogenetic analyses

The SSU rDNA sequence of *Pseudourostyla guizhouensis* sp. nov. was aligned to the sequences of 71 other spirotrich ciliates from the GenBank database, using the online program Muscle 3.7 (Edgar 2004). *Apodiophrys ovalis*, *Diophrys scutum*, *Paradiophrys zhangi*, and *Uronychia multicirrus* were selected as the outgroup taxa. Regions that could not be aligned unambiguously were removed and the ends were trimmed manually, resulting in a matrix of 1,632 characters. The program MrModeltest v.2.2 (Nylander 2004) selected the GTR + I (= 0.5618) + G (= 0.4527) as the best model with Akaike Information Criterion (AIC). The Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) which was run with two sets of four chains for 1,000,000 generations and a sampling frequency of 100 generations. The first 25% of sampled trees were discarded as burn-in prior to constructing 50% majority rule consensus trees. The Maximum-likelihood (ML) 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.

In order to determine the statistical probability of the hypothesis that the family Pseudourostylidae is monophyletic, constrained ML analyses were generated by PAUP v.4.0. Internal relationships within the constrained groups and relationships among the remaining taxa were unspecified. The reliability of the constrained tree was compared to the unconstrained ML topologies, using the approximately unbiased (AU) test (Shimodaira 2002) implemented in the CONSEL v 0.1 (Shimodaira and Hasegawa 2001).

Nomenclatural Acts

This article conforms to the requirements of the amended International Code of Zoological Nomenclature (ICZN 2012), and hence the new name contained herein is available under that Code. The published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix https://zoobank.org/. This work was published in a journal with an ISSN.

RESULTS

***Pseudourostyla guizhouensis* sp. nov.**

Description. Body 180–310 × 65–85 μm in vivo and 175–300 × 50–88 μm after protargol staining, ratio of length to width 3–4:1, elongate elliptical in outline, with lateral margins more or less parallel, both ends broadly rounded (Fig. 1A, 2A), dorsoventrally flattened about 2:1, very flexible but not contractile. Cytoplasm colorless and packed with granular inclusions. Posterior cell portion usually filled with food vacuoles rendering cell dark at low magnification (Fig. 2B). Usually one contractile vacuole near left body margin with two collecting canals, one extending anteriorly and one posteriorly (Fig. 1A, C); sometimes two contractile vacuoles positioned at about 35% and 70% of body length (Fig. 1B–D, 2B–D). Colorless cortical granules, ca. 0.5 μm in diameter, difficult to recognize, loosely and irregularly distributed throughout the cortex (Fig. 1E, 2E, F). On average 33 (26–40) ellipsoidal macronuclear nodules dispersed throughout cell, two to five micronuclei (Fig. 1G, 2G). Locomotion typically by swimming or crawling moderately fast on substrate.

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Adoral zone of membranelles about 38% of body length in fixed specimens, composed of 57–70 membranelles (Table 1) with cilia 12–15 μm long. Undulating membranes relatively short, slightly curved and optically intersecting. Pharyngeal fibers distinct after protargol staining, extending posteriorly. Frontal cirri forming a bicorona comprising six to eight pairs of cirri, with cilia 12–15 μm long. Single buccal cirrus, two frontoterminal cirri, and 13–20 midventral pairs in a zig-zag row extending almost to the two pre-transverse ventral cirri. Five to seven transverse cirri. Three or four left and invariably two right marginal rows. Six or seven longitudinal dorsal kineties of almost cell length and dorsal cilia about 5 μm long.

Physiological regeneration. Two reorganizers were found (Fig. 1H–J, 2I, J); 2). In these specimens: 1) the proximal portion of the parental adoral zone is resorbed and replaced by new membranelles (Fig. 1H, 2I); 2) the frontal-midventral-transverse (FVT) cirral streak I forms the leftmost cirrus in the anterior corona and the undulating membranes, streak II forms the second cirrus in the anterior corona and the buccal cirrus, streaks III to n–1 forms other cirri in bicorona and midventral pairs, the last five to seven streaks generate one transverse cirrus each, the last two streaks contribute one pretransverse cirrus each, and the last streak forms two frontoterminal cirri (Fig. 1H, J); 3) the new left and right marginal cirral rows develop from a common unit of anlage on each side, which form three or four left and invariably two right new marginal rows (Fig. 2I); and 4) the new dorsal kineties anlagen develop intrakinetally, then extend anteriorly and posteriorly to replace the old structures (Fig. 1I, 2J).

SSU rDNA sequences and phylogenetic analyses. The SSU rDNA sequence of *Pseudourostyla guizhouensis* sp. nov. (GenBank accession number KX139470) is 1,727 bp long and has a G+C content of 45.2%. The phylogenetic trees inferred from the SSU rDNA sequences using ML and BI, have similar topologies; therefore, a single topology from the ML tree is presented with support values from both algorithms indicated on branches (Fig. 4). *Pseudourostyla* spp. form a fully supported clade (ML 100%, BI 1.00), and *P. guizhouensis* sp. nov. is sister to the type *P. cristata* (DQ019318). The genera *Hemicycliostyla*, *Pseudourostyla*, and *Trichototaxis* are distinctly separated in the genealogies, and the hypothesis that these three genera form a monophyletic group is rejected by AU tests ($P = 0.012$).

***Hemicycliostyla franzi* (Foissner, 1987) Paiva et al., 2012**

Description of the Chinese (Guizhou) population. Body about 260 × 80 μm in vivo and 250 × 94 μm after protargol staining, elongate elliptical in outline, slightly narrowed at both ends and very flexible (Fig. 3A, F). One contractile vacuole left of buccal vertex (Fig. 3F). Posterior cell portion in some specimens full of captured prey, possibly the testate amoeba *Difflugia* (Fig. 3A, F). Cortical granules colorless, about 0.6 μm in diameter, scattering irregularly. 166–195 ellipsoidal macronuclear nodules, approximately 10 × 5 μm in size after protargol staining (Fig. 3C; Table 1). The number of micronuclei is uncertain (three micronuclei were observed in only one out of eight specimens). Locomotion usually by crawling moderately fast on debris and rotating about main body axis when swimming.

Adoral zone of membranelles occupying about 27% of cell length (in protargol preparations), question mark-shaped, with 55–66 membranelles. Undulating membranes in *Oxytricha*-pattern, i.e., paroral and endoral slightly curved and optically intersecting (Fig. 3D, K). Pharyngeal fibers distinct after protargol impregnation, extending posteriorly (Fig. 3I). Eleven to 15 frontal cirri arranged in a bicorona. About 12 midventral pairs in a zig-zag row extending to mid-body (Fig. 3D). Two or three buccal cirri, usually one parabuccal cirrus, and two or three frontoterminal cirri. Seven or eight left and six or seven right marginal rows with anterior portion of outermost marginal rows extending onto dorsal surface and terminating with dorsal bristles (Fig. 3E); marginal rows occupy large

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portion of cell surface. Five to eight transverse cirri (Fig. 3D, J). Three or four dorsal kineties extending almost entire cell length (Fig. 3E, L).

DISCUSSION

Pseudourostyla guizhouensis sp. nov.

Morphological comparison with related taxa. The major morphological features of the new species and its congeners are presented in Table 2. The key distinguishing feature of *Pseudourostyla guizhouensis* sp. nov. is that it invariably has two right marginal rows and thus it can be easily separated from those species with four or more right marginal rows, i.e., *P. cristata* (both the neotype and the Lake Biwa populations), *P. cristatoides*, *P. dimorpha*, *P. levis*, *P. pelotensis*, and *P. subtropica*. The two remaining *Pseudourostyla* species, namely *P. muscorum* and *P. nova*, have two right marginal rows, similarly as *P. guizhouensis* sp. nov.. The main differences between *P. guizhouensis* sp. nov. and *P. nova* are the numbers of frontal cirri (12–16 vs. 4–6), midventral pairs (13–20 vs. 22–36), left marginal rows (three or four vs. invariably two), and macronuclear nodules (26–40 vs. 9–25). Furthermore, *P. guizhouensis* sp. nov. has a bicorona that is typical in the genus *Pseudourostyla*, whereas the frontal cirri in *P. nova* are arranged in a single row or monocorona (Chen et al. 2014; Wiackowski 1988). *P. guizhouensis* sp. nov. can be distinguished from *P. muscroum* by the numbers of buccal cirri (one vs. nine) and transverse cirri (5–7 vs. 8–15) (Berger 2006; Kahl 1932).

Morphogenetic comparison. Very early reorganizers of *Pseudourostyla guizhouensis* sp. nov. were not available. From the longitudinal splitting of the marginal cirral anlagen of a middle stage reorganizer (Fig. 1H, J), we speculate that the new marginal cirral rows develop from one anlage on each side, each of which probably originated from the dedifferentiation of the rightmost parental marginal rows; these anlagen then split and form three left and two right new marginal rows (Fig. 2I). This process is similar to that in *P. cristata* and *P. cristatoides*, this is, the marginal rows of each side are formed from a single anlage which appears within the rightmost parental row (Chen et al. 2010; Jung et al. 2012). Further similarities of the cortical development in *P. guizhouensis* sp. nov. with that in *P. cristata* and *P. cristatoides* are that the dorsal kinety anlagen originate in parental rows and generate new dorsal kineties extending anteriorly and posteriorly (Chen et al. 2010; Jung et al. 2012).

Hemicycliostyla franzi (Foissner, 1987) Paiva et al., 2012

The Chinese population of *Hemicycliostyla franzi* was firstly found in subtropical soils from the Hunan province (Shen et al. 1992), but only a brief characterization the present species was provided. A more detailed description and illustrations of Chinese population are provided based on Guizhou specimens in present paper.

Comparison of Chinese (Guizhou) population with African and Indian population. The Chinese (Guizhou) population of *Hemicycliostyla franzi* matches well the African (Foissner 1987; Berger 2006) and Indian populations (Kumar et al. 2010) in the ciliary pattern and other unique characteristics, i.e., the numbers of marginal rows and macronuclear nodules, an anterior portion of the outermost marginal rows composed of dorsal bristles, marginal rows occupying a large portion of the cell surface, and the absence of caudal cirri. Furthermore, all these three populations were found in terrestrial habitats. There are, however, minor differences among these three populations: 1) the adoral zone of the Chinese (Guizhou) specimens of *Hemicycliostyla franzi* occupies 27% of body length, which matches its length in the African specimens (30%), whereas it is much longer in the Indian specimens (39%); 2) the numbers of buccal cirri, transverse cirri, and marginal rows in the Guizhou specimens are similar to those in the African specimens, but differ from those in the Indian specimens (Berger 2006; Foissner 1987; Kumar et al. 2010); 3) both the Chinese (Guizhou) and the Indian specimens possess 0.6 μm-sized, spherical cortical granules, whereas the African specimens

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possesses 1.0–2.0 × 1.5 μm-sized, ellipsoidal cortical granules which can be extruded (Berger 2006; Paiva et al. 2012); 4) the pharyngeal wall is without peculiarities in the Chinese (Guizhou) specimens, whereas the African specimens have many about 1 μm long rods (Berger 2006; Foissner 1987).

Phylogeny of the family Pseudourostylidae and *Pseudourostyla guizhouensis* sp. nov.

Members of the family Pseudourostylidae are characterized by having a bicorona, a midventral complex composed of cirral pairs only, and more than one left marginal cirral row (Berger 2006). Consequently, three genera, namely *Hemicycliostyla*, *Pseudourostyla*, and *Trichototaxis*, were assigned to the Pseudourostylidae by Berger (2006) who noted, however, that details of the cirral pattern of the latter two genera were lacking at that time; so, their placement in this family was tentative. Two new species of *Trichototaxis* have since been described, including details of their cirral pattern, i.e., *T. marina* Lu et al., 2014 and *T. songi* Chen et al., 2007. Although both species possess a bicorona, a midventral complex composed of cirral pairs only, and more than one left marginal row, morphogenetic and molecular data did not support the familial placement of *Trichototaxis* (Chen et al. 2007; Lu et al. 2014). In addition, the type species of *Hemicycliostyla*, *H. sphagni* Stokes, 1886, has since been redescribed, and an Indian population of *H. franzi* has been reported (Kumar et al. 2010; Paiva et al. 2012).

In the SSU rDNA tree presented here, *Pseudourostyla*, *Hemicycliostyla*, and *Trichototaxis* fall into three separate clades, which is consistent with previous findings (Lu et al. 2014). Furthermore, the AU test rejects the possibility that the family Pseudourostylidae is monophyletic. Differences in morphogenetic processes also suggest a more distant relationship among the three pseudourostylid genera. These include: 1) marginal cirral rows originate from individual anlagen in *Hemicycliostyla*, but from a common anlage in *Pseudourostyla*; 2) the macronuclear nodules fuse into a single mass during morphogenesis in *Pseudourostyla*, whereas they fuse into more than one mass in *Trichototaxis marina* (Lu et al. 2014). However, since morphogenetic and molecular data for the type species of *Trichototaxis*, *T. stagnatilis* Stokes, 1891, are not available, it would be premature to revise the family Pseudourostylidae.

In the present study, *Pseudourostyla guizhouensis* sp. nov. clustered with other *Pseudourostyla* species with moderate to high support, which corresponds well with the morphological data, i.e., a large body, a bicorona, midventral pairs, two or more marginal rows on each side, and the absence of caudal cirri. Furthermore, the morphogenetic pattern in *P. guizhouensis* sp. nov. resembles its congeners. The monophyly of *Pseudourostyla* is thus well supported by our analyses.

TAXONOMIC SUMMARY

Class Spirotrichea Bütschli, 1889
Order Urostylida Jankowski, 1979
Family Pseudourostylidae Jankowski, 1979
Genus *Pseudourostyla* Borror, 1972
Pseudourostyla guizhouensis sp. nov.

Diagnosis. Body 180–310 × 65–85 μm in vivo, elongate elliptical in outline, usually one contractile vacuole near left body margin. Cell with colorless, spherical (ca. 0.5 μm across) cortical granules, distributed irregularly (loosely spaced). 57–70 adoral membranelles; 12–16 cirri forming a bicorona, one buccal cirrus, two frontoterminal cirri, 13–20 midventral pairs, two pre-transverse cirri, and five to seven transverse cirri. Three or four left and invariably two right marginal rows. Six or seven dorsal kineties. 26–40 macronuclear nodules and two to five micronuclei.

Type locality. Village of Zhonghuashan, Aojiazhai Township, Tongren City, Guizhou Province, China (27°34′48″N, 109°15′42″E), soil sample.

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Type slides. A protargol slide with the holotype specimen marked by in ink circle (LFC2014080101A) (Fig. 1E, F) and one paratype slide (LFC2014080101B) are deposited in the collection of the Laboratory of Protozoology, Ocean University of China, China. A second paratype slide (LFC2014080101C) is deposited in the Natural History Museum, London with the registration number NHMUK 2017.1.6.1.

Etymology. The specific epithet *guizhouensis* refers to the Guizhou Province, China, where the new taxon had been discovered.

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FIGURE LEGENDS

Figure 1 Morphology of *Pseudourostyla guizhouensis* sp. nov. in vivo (**A**–**D**) and after protargol staining (**E**–**I**). (A) Ventral view of a typical individual. (B–D) Showing arrangement and variability of the contractile vacuoles. (E) Line diagram of dorsal surface showing the irregularly arranged cortical granules. (F, G) Ventral and dorsal view of the holotype specimen, showing the general ciliary pattern, macronuclear nodules, and micronuclei (arrowheads). (H, I) Ventral and dorsal view of a mid-stage reorganizer, arrowhead indicates the frontal-midventral-transverse cirral streak I, double-arrowhead indicates the frontal-midventral-transverse cirral streak n. (J) Ventral view of a late reorganizer, and arrow indicates newly formed buccal cirrus. AZM, adoral zone of membranelles; CV, contractile vacuole; E, endoral; FC, frontal cirri; FTC, frontoterminal cirri; LMA, anlage of left marginal rows; LMR 1–3, left marginal rows 1–3; Ma, macronuclear nodule; MP, midventral pairs; P, paroral; PT, pretransverse cirri; RMA, anlage of right marginal rows; RMR 1, 2, right marginal rows 1, 2; TC, transverse cirri; UMA, anlage of undulating membranes; 1–7, dorsal kineties 1–7. Scale bars = 60 μm.

Figure 2 Photomicrographs of *Pseudourostyla guizhouensis* sp. nov. in vivo (**A**–**F**) and after protargol staining (**G**–**J**). (A) Ventral view of a typical specimen. (B–D) Showing the granular inclusions (B) and the contractile vacuoles (C, D). (E, F) Showing the dorsal surface and the irregularly arranged cortical granules (arrowheads). (G, H) Ventral and dorsal view of an interphase specimen, showing the ciliary pattern. Arrows and arrowhead indicate the micronuclei and the buccal cirrus, respectively. (I, J) Ventral and dorsal views of a mid-stage reorganizer (arrowhead indicates the anlage of undulating membranes, arrow indicates the buccal cirrus). AZM, adoral zone of membranelles; CV, contractile vacuoles; FTC, frontoterminal cirri; LMA, anlage of left marginal rows; Ma, macronuclear nodules; RMA, anlage of right marginal rows; 1–7, dorsal kineties 1–7. Scale bars = 60 μm.

Figure 3 Morphology of Chinese population of *Hemicycliostyla franzi* in vivo (**A**, **F**) and after protargol staining (**B**–**E, G**–**K**). (A) Ventral view of a typical individual. (B) Dorsal view, showing the contractile vacuole. (C) Dorsal view showing the macronuclear nodules, and the micronuclei (arrowheads). (D, E) Ventral and dorsal views of the same specimen to show the ciliary pattern (arrowhead indicates the buccal cirri, arrow marks the parabuccal cirrus). (F) Showing ingested food (*Difflugia*) and the contractile vacuole. (G) Showing the cortical granules (arrows) which are darkly impregnated. (H) Early stage of formation of the oral primordium (arrows). (I) Showing the pharyngeal fibers (arrows). (J) Transverse cirri. (K, L) Ventral and dorsal views of the same specimen to show the ciliary pattern (arrow indicates the transverse cirri, arrowhead indicates the parabuccal cirrus). AZM, adoral zone of membranelles; CV, contractile vacuole; DK 1–4, dorsal kineties 1–4; E, endoral; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal rows; Ma, macronuclear nodules; P, paroral; RMR, right marginal rows; TC, transverse cirri. Scale bars = 60 μm.

Figure 4 Maximum-likelihood (ML) tree based on SSU rDNA sequences showing the phylogenetic relationships of *Pseudourostyla guizhouensis* sp. nov. (bold). Numbers near nodes are the non-parametric bootstrap values for maximum likelihood (ML) and the posterior probability values for Bayesian inference (BI). Fully supported (100%/1.00) branches are marked with solid circles. The asterisk (*) indicates topologies that differ in the ML and BI phylogenies. The scale bar corresponds to two substitutions per 100 nucleotide positions. GenBank accession numbers are given for each species.

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Table 1. Morphometric data of *Pseudourostyla guizhouensis* sp. nov. (Pg) and a population of *Hemicycliostyla franzi* (Hf) from China.

Character[a]	Species	Min	Max	Mean	Med	SD	CV	n
Body, length	Pg	175	300	243.3	247	39.0	16.0	18
	Hf	160	290	249.4	268	42.5	17.0	8
Body, width	Pg	50	88	71.8	75	9.9	13.8	18
	Hf	65	115	93.8	103	19.2	20.5	8
Length of adoral zone	Pg	50	110	91.4	90	17.2	18.9	18
	Hf	36	86	68.0	70	14.9	22.1	8
Proportion of body length occupied by AZM (%)	Pg	29	42	38.0	38	4.8	12.5	18
	Hf	23	32	27.0	28	0.0	10.3	8
Adoral membranelles, number	Pg	57	70	63.1	63	4.5	7.1	18
	Hf	55	66	61.3	62	3.8	6.2	6
Buccal cirri, number	Pg	1	1	1.0	1	0.0	0.0	18
	Hf	2	3	2.7	3	0.5	19.3	6
Frontal cirri, number	Pg	12	16	13.9	14	1.2	8.7	18
	Hf	11	15	13.6	14	1.4	10.3	8
Frontoterminal cirri, number	Pg	2	2	2.0	2	0.0	0.0	18
	Hf	2	3	2.8	3	0.4	14.4	6
Transverse cirri, number	Pg	5	7	6.1	6	0.8	12.4	18
	Hf	5	8	6.6	7	1.0	14.9	7
Pretransverse ventral cirri, number	Pg	2	2	2.0	2	0.0	0.0	18
Midventral pairs, number	Pg	13	20	17.1	17	2.0	11.8	18
	Hf	8	14	11.7	12	1.8	15.0	8
Left marginal cirral rows, number	Pg	3	4	3.3	3	—[b]	—	18
	Hf	7	8	7.8	8	—	—	8
Cirri in left marginal cirral row 1, number	Pg	22	36	25.6	25	4.3	16.7	15
Cirri in left marginal cirral	Pg	20	32	26.4	26	3.9	14.6	15

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row 2, number								
Cirri in left marginal cirral row 3, number	Pg	16	30	21.1	21	4.7	22.3	15
Cirri in left marginal cirral row 4, number	Pg	2	12	5.0	4	4.1	82.8	5
Right marginal cirral rows, number	Pg	2	2	2.0	2	0.0	0.0	18
	Hf	6	7	6.6	7	—	—	8
Cirri in right marginal cirral rows 1, number	Pg	20	35	28.0	28	4.7	16.7	15
Cirri in right marginal cirral rows 2, number	Pg	22	37	30.4	30	4.3	14.0	15
Dorsal kineties, number	Pg	6	7	6. 9	7	—	—	18
	Hf	3	4	3.5	4	—	—	8
Macronuclear nodules, number	Pg	26	40	32.8	32	3.9	11.8	18
	Hf	166	195	177.3	175	9.7	5.5	7
Macronucleus, length	Pg	8	16	12.4	13	2.5	20.2	18
	Hf	5	16	10.2	10	3.3	32.5	8
Macronucleus, width	Pg	4	7	5.5	5	1.1	20.3	18
	Hf	3	7	4.6	4	0.7	15.7	8
Micronuclei, number	Pg	2	5	3.7	4	0.8	21.0	18
	Hf	3	3	—	—	—	—	1
Micronuclei, length	Pg	5	9	6.2	6	1.3	20.4	18
Micronuclei, width	Pg	3	5	3.7	4	0.7	18.8	18

All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of specimens investigated; SD, standard deviation.

[a] Data based on randomly selected, protargol-stained specimens.
[b] Statistically senseless.

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Table 2. Morphometric comparison of *Pseudourostyla guizhouensis* sp. nov. with other *Pseudourostyla* species.

Character	*P. guizhouensis* sp. n.	*P. cristata* (neotype)	*P. cristata* (Lake Biwa)	*P. levis* (neotype)	*P. levis* （Indian）	*P. muscroum*	*P. nova*	*P. pelotensis*	*P. cristatoides*	*P. subtropica*	*P. dimorpha*[a]
Body size in vivo	180–310 × 65–85	221–324 × 59–147	280–400 × 90–130	150–300 × 25–100	246 × 76	250–350 × 65–95	140–200 × 45–55	190 × 80	220–265 × 85–125	300–450 × 120–200	120–230 × 25–65
Body size after protargol staining	175–300 × 50–88	181–303 × 40–111	270–380 × 100–220	192 × 49	189–266 × 58–83	/	200–375 × 60–125	145–195 × 51–70	210–290 × 65–125	300–600 × 160–360	106–198 × 21–57
Proportion of body length occupied by AZM (%)	38	40	40	38	40	31	35	36	33	25–33	22–38
Adoral membranelles, number	63 (57–70)	88	89 (80–94)	67	87 (79–100)	/	58 (52–64)	47 (38–54)	97 (84–115)	131 (104–173)	31–47
Buccal cirri, number	1	1 or 2	1	1	1	ca. 9	1	1	1	1	2
Frontal cirri, number	14 (12–16)	22 (18–26)	23 (20–24)	/	/	/	4 (4–6)	9–13	24 (20–30)	32 (26–44)	9 (6–12)
Midventral pairs, number	17 (13–20)	21.5 (on average)	22 (17–25)	19	34–42[b]	/	29 (22–36)	13 (10–15)	21 (17–25)	19 (14–28)	8 (6–11)
Left marginal rows, number	3 (3 or 4)	4–6	5 or 6	5	5	2	2	4–6	6 (5–7)	7–13	5 or 6
Right marginal rows, number	2	4 or 5	5	4	5	2	2	5–8	4 (4 or 5)	5–9	4–6
Transverse cirri, number	6 (5–7)	5–10	7–10	7	7–13	8–15	8 (7–12)	5–9	9 (6–12)	7–12	4–9
Dorsal kineties, number	7 (6 or 7)	8	8–10	7 or 8	7	/	8 (7–9)	7–11	11 (10–13)	11 (8–14)	2 or 3
Macronuclear nodules, number	33 (26–40)	41 (27–55)	28 (15–36)	43	74 (58–92)	/	16 (9–25）	88 (62–114)	78 (30–106)	114 (68–219)	67–160
Micronuclei, number	4 (2–5)	5 (3–7)	5 (2–9)	ca. 3	6 (4–9)	/	4 (2–8)	/	/	4 (2–6)	3–9
Data source	Present paper	Oberschmidleitner and Aescht (1996); Berger (2006)	Chen et al. (2010)	Berger (2006)	Kumar et al. (2010)	Kahl (1932); Berger (2006)	Chen et al. (2014)	Paiva and Silva-Neto (2006)	Jung et al. (2012)	Chen et al. (2014)	Foissner (2016)

All measurements in μm. AZM, adoral zone of membranelles; /, data not available.

[a] Calculated from the two morphs.
[b] Data combining the bicorona and midventral rows.

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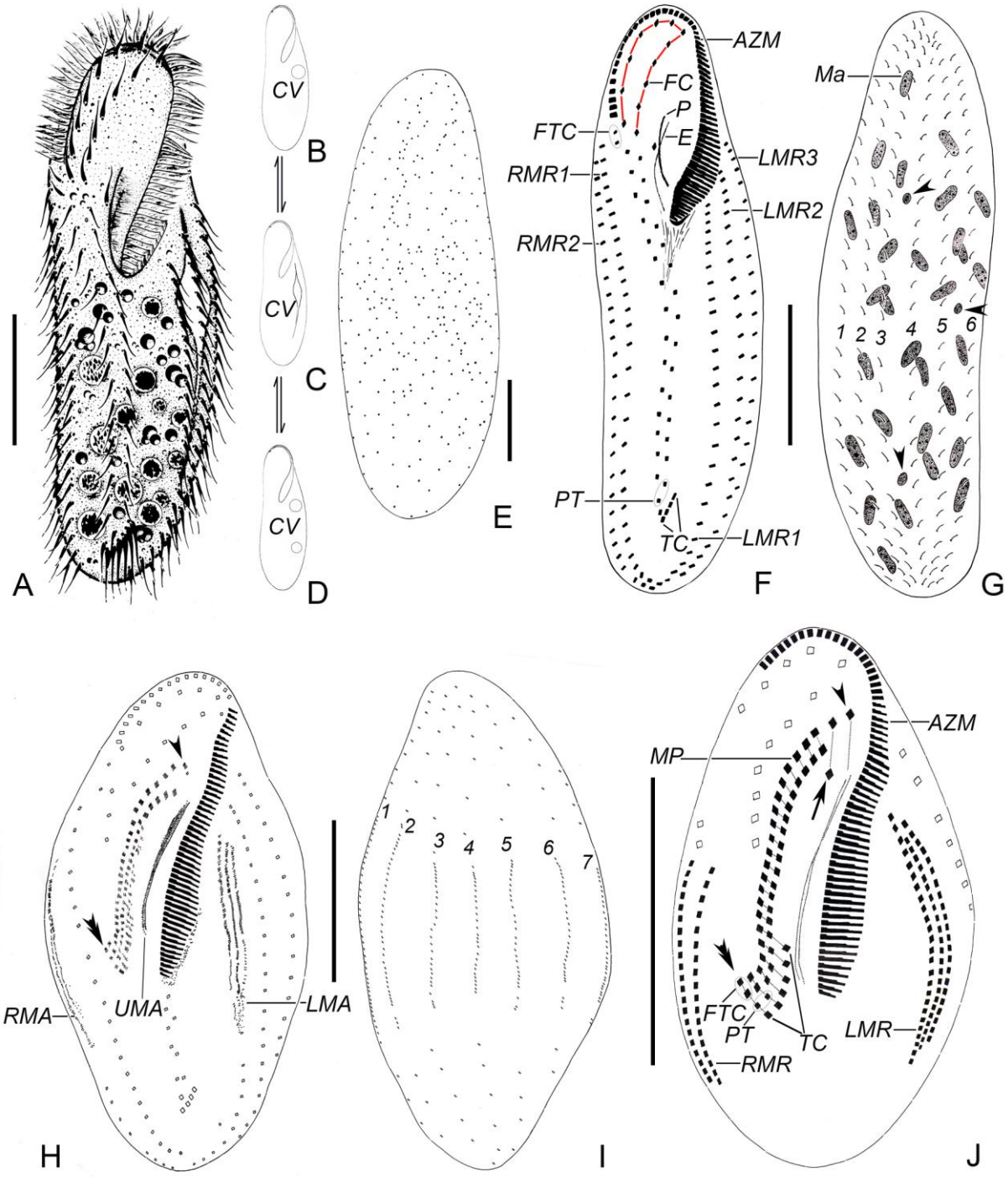

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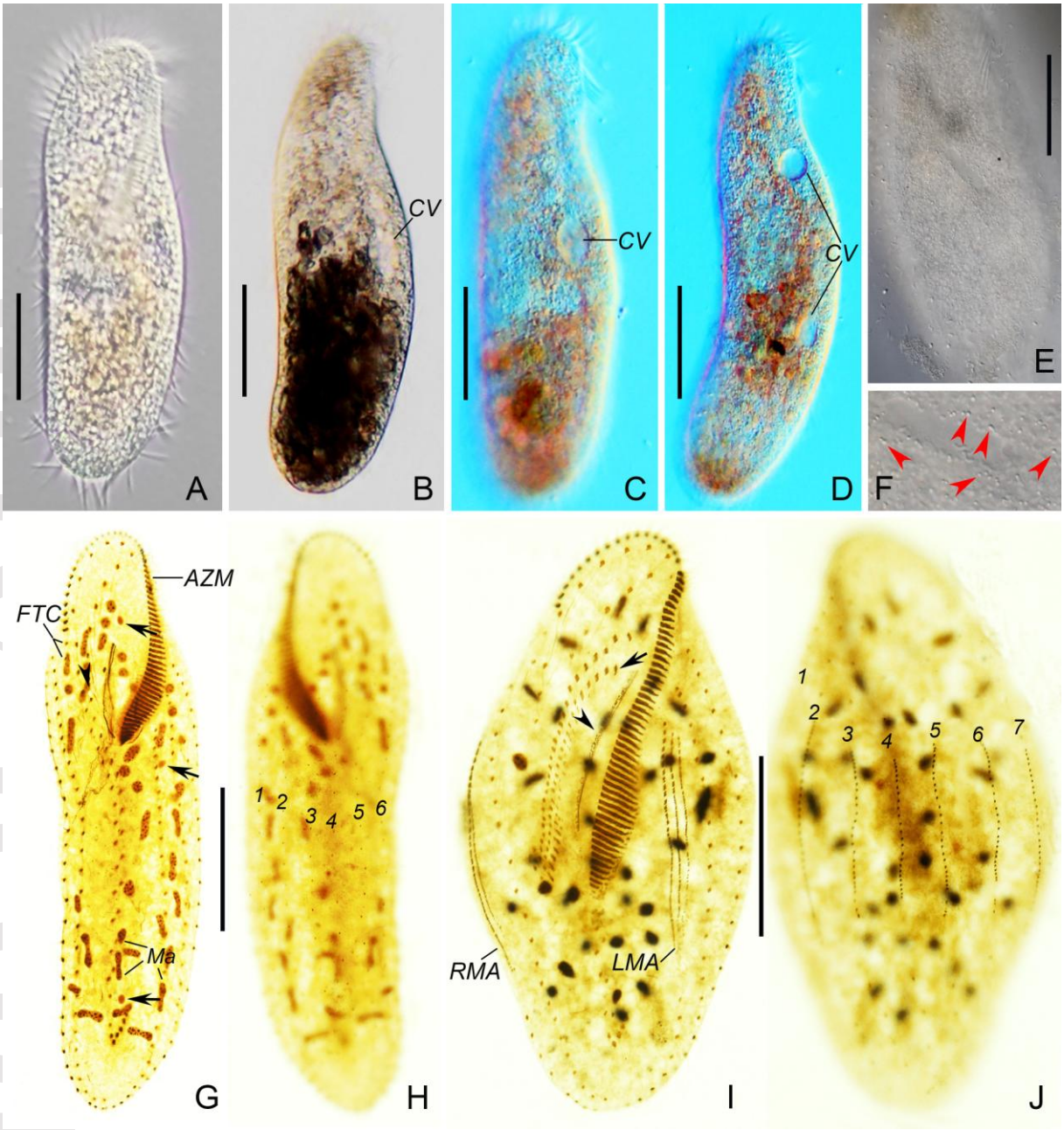

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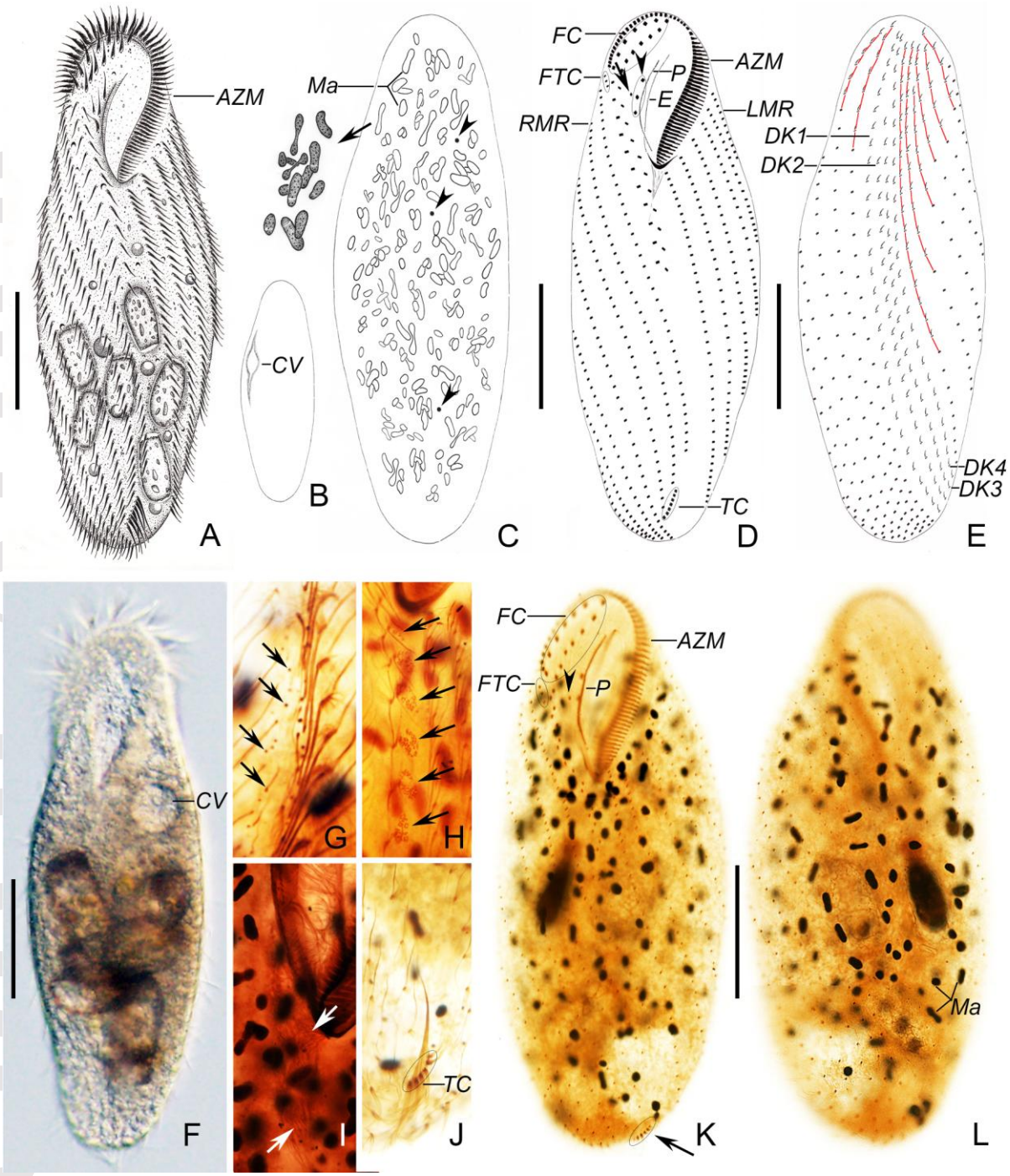

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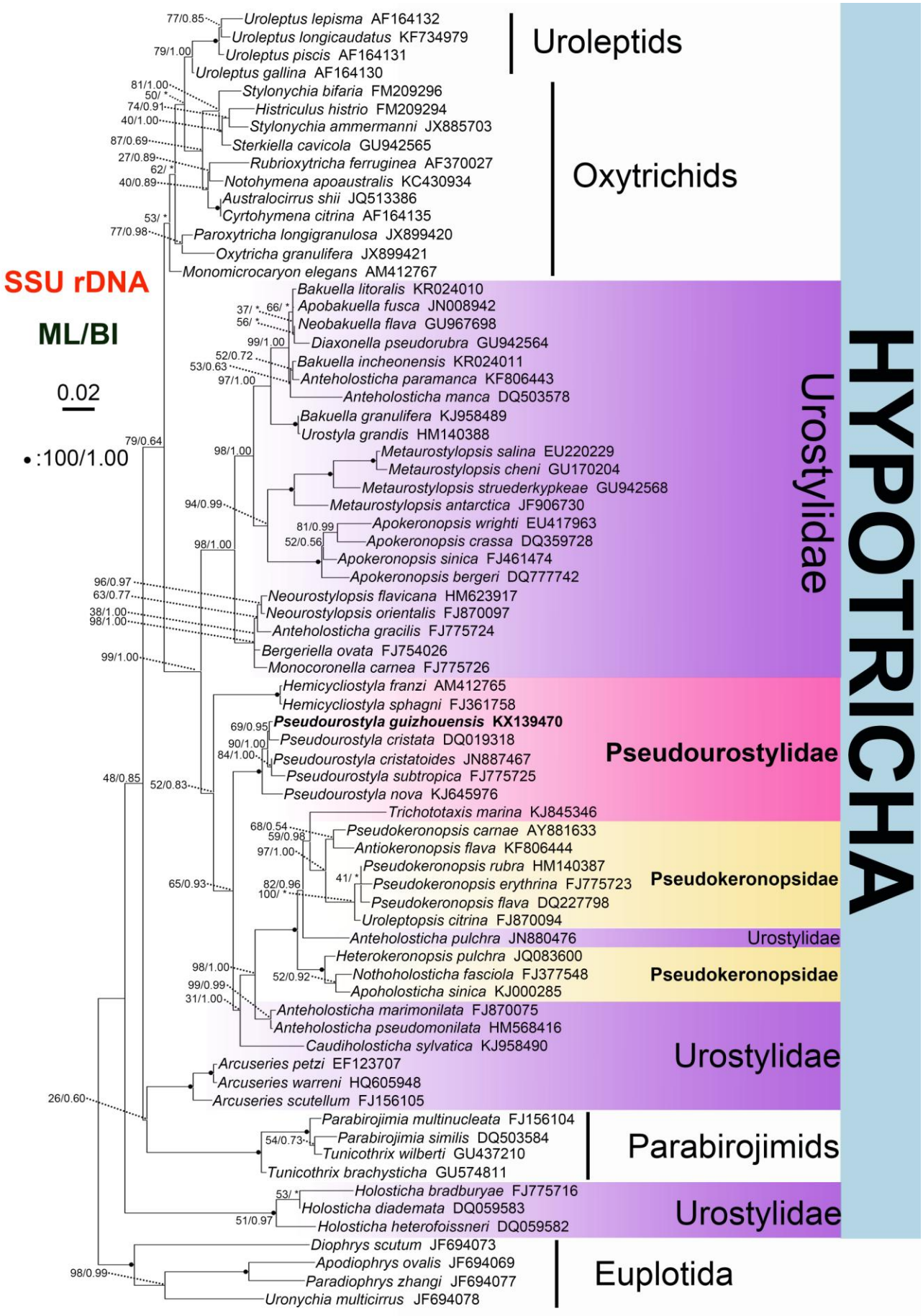

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