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Research article

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Systematic positions and taxonomy of two freshwater ciliates found in China, with establishments of one new family and two new species (Ciliophora, Oligohymenophorea)

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Wang X., Liu L., Wang J., Wang X., Wang J., Wang L., Zhao L. & Pan X. 2025. Systematic positions and taxonomy of two freshwater ciliates found in China, with establishments of one new family and two new species (Ciliophora, Oligohymenophorea). European Journal of Taxonomy 981: 1–20. https://doi.org/10.5852/ejt.2025.981.2813

The ciliated protozoans are morphologically complex groups with a huge variety of species and a worldwide distribution in terrestrial, freshwater, brackish, marine, and hypersaline habitats (Chen et al. 2017; Wu et al. 2020, 2023; Li et al. 2021, 2024; Liu et al. 2022, 2024; Song et al. 2022, 2024; Jiang et al. 2023; Chi et al. 2024; Hao et al. 2024). Ciliates show high biodiversity, with over 4000 free-living species described to date (Gao et al. 2017).

The class Oligohymenophorea #de Puytorac et al., 1974 was established by Puytorac in 1974, and contains seven subclasses, namely Apostomatia Chatton & Lwoff, 1928, Astomatia Schewiakoff, 1896, Hymenostomatia #Delage & Hérouard, 1896 , Peniculia Fauré-Fremiet in Corliss, 1956, Peritrichia Stein, 1859, Scuticociliatia #Small, 1967 , and Urocentria Wang et al., 2021. The cell size of Oligohymenophorea species is commonly small to medium, with the shape typically ovoid to elongate ovoid (Lynn 2008). In addition, species assigned to Oligohymenophorea are characterized by their oral apparatus consisting of four adoral membranelles: a distinct right paroral membrane composed of dikinetids and three left oral membranelles (polykinetids). In terms of taxonomy, ciliates in the class Oligohymenophorea have been studied for a long time. The first report of the class Oligohymenophorea was the description of #Müller, #### by Müller (Müller ####; Lynn ####). However, the taxonomy and systematics of Oligohymenophorea is still confused due to the limitations in techniques for species identification and the progress in molecular techniques (Gentekaki et al. 2014; Zhang et al. 2014; Wang et al. 2015). Therefore, it is necessary to carry out more work with an integrative method combining typical morphology and molecular approaches to resolve the taxonomic confusion in this complex group. The family Tetrahymenidae #Corliss, 1952 , belonging to Hymenostomatia #Delage & Herouard, 1896 , is mainly characterized by the following features: small, pyriform to elongate-ovoid cells that are free-swimming, and the oral structures (a paroral dikinetid that is ciliated along its entire length, and three oral polykinetids, each of equal number of rows of kinetosomes) (Lynn 2008).

The type genus #Furgason, 1940 is characterized by a four-part oral structure, and so far, over 80 species have been assigned to it (Quintela-Alonso et al. 2013; Liu et al. 2016; Lynn et al. 2018; Doerder 2019; Pan et al. 2019; Rataj & V#a#ný 2020; Zhang & V#a#ný 2021). Species of can be divided into three infrageneric groups based

on life cycle characteristics: the rostrata group, the patula group, and the pyriformis group (Corliss 1970; Lynn & Doerder 2012).

In recent years, many species and new taxa in the class Oligohymenophorea were discovered and reported from Northeast China, including ciliates in Hymenostomatia , Peniculia, and Scuticociliatia , indicating the potential diversity of undiscovered ciliates and highlighting the importance of conducting further research in the class Oligohymenophorea (Pan et al. 2017a, 2017b; Cai et al. 2018; Pan et al. 2019, 2020; Hao et al. 2022).

In the present study, two ciliates, # DEBUG->jats:named-contentgen. et sp. nov. and # DEBUG->jats:named-contentsp. nov. , are described using observation in vivo and silver staining techniques (Foissner 1991). SSU-rRNA gene sequence data are also supplied for both species and their phylogenetic positions in the SSU-rRNA gene tree are estimated.

DEBUG->jats:named-contentgen. et sp. nov. was collected on 11 October 2022 from a freshwater aquarium at Dafa international fish market in Harbin, Heilongjiang Province (45°44#9# N, 126°35#41# E), Northeast China (water temperature about 23#). # DEBUG->jats:named-contentsp. nov. was collected on 23 November 2022 from a freshwater aquarium at Harbin Normal University, Harbin, Heilongjiang Province (45°52#3# N, 126°33#2# E), Northeast China (water temperature about 22#). About 0.5 L of water was collected from 0.1 to 0.5 m below the surface using sterile sampling bottles (Fig. 1).

Samples of # DEBUG->jats:named-contentgen. et sp. nov. were kept in Petri dishes at room temperature with added unsterilized rice grains to enrich the growth of bacteria as a food source for the ciliates. Cells of # DEBUG->jats:named-contentsp. nov. were kept in monoclonal cultures in Petri dishes at room temperature (about 25#) with filtered habitat water supplemented with sterilized rice grains to enrich the growth of bacteria as food for ciliates. Cells from Petri dishes were observed and photographed using differential interference contrast and bright-field microscopy (Zeiss Axio Imager A2, German) (Foissner 1991).

The silver carbonate method revealed the infraciliature and nuclear apparatus (Fernandez-Galiano 1976; Foissner 1992). Counts and measurements of sliver-stained specimens were performed at magnifications of 100 × to 1000 ×. Drawings of live and stained specimens are based on microscope observations, freehand sketches, and photomicrographs. Drawings of stained specimens were made with drawing devices (line drawing pens and tracing papers). Classification and terminology are according to Lynn (2008).

About ten cells from the clonal culture of each species were washed with distilled water and genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The SSU-rRNA gene was amplified with the primers Euk A: 5'-AAC CTG GTT GAT CCT GCC AGT-3' and EukB: 5'-TGA TCC TTC TGC AGG TTC ACC TAC-3' (Medlin et al. 1988). PCR condition for amplification of the SSU-rRNA gene was: denaturation for 5 min at 94°C, followed by 5 cycles of denaturation for 45 s at 94°C, annealing for 1 min 45 s at 56°C, extension for 2 min at 72°C, and other 25 cycles of denaturation for 45 s at 94°C, annealing for 1 min 45 s at 60°C, extension for 2 min at 72°C, and a final extension for 8 min at 72°C (Li et al. 2022; Liu et al. 2023). Bidirectional sequencing was performed by the Shanghai Sangon Biotechnology Company (Shanghai, China).

Except for the two newly sequenced species, 61 SSU-rRNA gene sequences downloaded from the GenBank database were used in the analyses, as well as two species as the outgroup (for accession numbers, see Fig. 6). Sequences were aligned, and resulting alignments were refined by only trimming both ends using BioEdit ver. 7.0.5.2 (Hall 1999). Bayesian inference (BI) analysis was performed using MrBayes on XSEDE ver. 3.2.6 (Ronquist & Huelsenbeck 2003) on CIPRES Science Gateway (Miller et al. 2010) using the GTR+I+G evolutionary model as the best-fit model selected by MrModeltest ver. 2 (Nylander 2004) according to the Akaike Information Criterion (AIC). Markov Chain Monte Carlo (MCMC) simulations were run with two sets of four chains for 4 000 000 generations, with a sample frequency of every 100 th generation. The first 10 000 trees were discarded as burn-in. A maximum likelihood (ML) tree was constructed using RAxML-HPC2 ver. 8.2.10 (Stamatakis et al. 2008) on the CIPRES Science Gateway (Miller et al. 2010) with the GTR+I+G evolutionary model as the best-fit according to the AIC criterion selected by the program Modeltest ver. 3.4 (Posada & Crandall 1998). Nodal support came from 1000 bootstrap replicates. TreeView ver. 1.6.6 and MEGA ver. 7 (Tamura et al. 2013) were used to visualize tree topologies. For the interpretation of bootstrap values and Bayesian posterior probabilities, we consider values # 95 as high, from 71 to 94 as moderate, and from 50 to 70 as low (Hillis & Bull 1993; Alfaro et al. 2003).

Repository

HANU = Harbin Normal University

Abbreviations

M1 = membranelle 1

M2 = membranelle 2

M3 = membranelle 3

Ma = macronucleus

Mi = micronucleus

PK = postoral kineties

PM = paroral membrane

Sc = scutica

SK = somatic kineties

SK 1 = somatic kinety 1

SKn = somatic kinety n

Taxonomy

DEBUG :list -> | DEBUG :item -> | DEBUG :label -> Class | et al., 1974 | | DEBUG :item -> | DEBUG :label -> Subclass# Subclass Hymenostomatia #Delage & Hérouard, 1896 | DEBUG :item -> | DEBUG :label -> Order# Order Tetrahymenida #Fauré-Fremiet in Corliss, 1956 | DEBUG :floatingText -> | DEBUG :body ->

Family

urn:lsid:zoobank.org:act:A9FCE478-3C33-458D-AF4F-02505DF5BB74

three oral membranelles, M3 consists of several clusters, forming several parallel parts.

DEBUG ->jats:named-contentgen_nov

DEBUG <floatingText -> | DEBUG :body ->

Genus

urn:lsid:zoobank.org:act:D5CB9EA2-346B-4228-99EF-ABC86B4B9BE8

the buccal field is big and deep, approximately 40% of body the length; 19 or 20 somatic kineties; two postoral kineties; M1 and M2 are approximately equal length; M2 consists of three parts (M2a, b, c); M3 is shorter than M1 and M2; one macronucleus; micronucleus is not observed; freshwater habitat.

The genus-group name ‘ ’ highlights the difference of the oral apparatus and outline from that of the genus .

left

DEBUG :floatingText -> DEBUG :body ->

DEBUG->jats:named-content gen. et sp. nov. (monotypic genus)

urn:lsid:zoobank.org:act:AD200533-1E68-4CF5-BE38-1CB4104F26A8

Figs 2–3; Table 1.

Size in vivo 160–250 × 125–210 µm; anterior end pointed and posterior end broadly rounded; buccal field big and deep, approximately 40% of body length; 19 or 20 somatic kineties; two postoral kineties; M1 and M2 approximately equal length; M2 consists of three parts (M2a, b, c); M3 obviously shorter than M1 and M2; one macronucleus. Micronucleus not observed; freshwater habitat.

The species-group name ' ' (Latin adjective; eastern, occurring in the Orient) refer to the fact that this species was discovered in East Asia.

material

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A freshwater aquarium at Dafa international fish market in Harbin ( ), Northeast .

Size in vivo usually 160–250 × 125–210 µm; body shape flexible; anterior end narrowed and posterior end broadly rounded usually (Fig. 2A–B, E, H–I, K). When food vacuoles filled by food, 60% of posterior part of cell swells extremely (Fig. 2A, E). Ratio of length to width about 1.5:1. Buccal field usually big, deep, and oval, approximately 40% of body length (Fig. 2A, E, H–I; Table 1). Cortical granules spherical, arranged randomly on pellicle (Fig. 2H, J–K). Cytoplasm colorless to deep dark when food vacuoles filled with food (Fig. 2A, E, H–I, K), often containing several big food vacuoles filled with ingested (food vacuoles about 40 µm in diameter) (Figs 2K, 3G). Single contractile vacuole (approximately 25 µm in diameter) positioned close to posterior end (Fig. 2K). Extrusomes not observed; single ellipsoid macronucleus, approximately 60 µm across after staining, usually squeezed by food vacuoles, no micronucleus observed (Fig. 3A–D, H). Length of somatic cilia approximately 10 µm, densely arranged. Locomotion mainly by swimming slowly, sometimes rotating forwards. Preying on .

Altogether 19 or 20 SK, arranged latitudinally, extending the entire length of body, consisting of monokinetids (Figs 2C–D, 3A–D). Two PK: PK1 and PK2 commence anteriorly at level posterior of PM and extend posteriorly nearly to end of cell (Figs 2F, 3C). Oral structure consisting of one PM and three oral membranelles (Figs 2C, F–G, 3E–F). PM generally C-shaped, composed of paired basal bodies, organized in zigzagging pattern, extending anteriorly to M2. (Figs 2F–G, 3E–F). M1 and M2 approximately equal in length, composed of four or three rows of kinetids (Figs 2F–G; 3E–F). M2 consisting of three parts, namely M2a, b, c. M2a not obvious anteriorly and conspicuous posteriorly, composed of two rows of kinetids (Figs 2F–G, 3E–F). M2b obvious, consisting of four rows of kinetids (Figs 2F–G, 3E–F). M2c divided into two parts, each composed of four rows of kinetids (Figs 2F–G, 3E). M3 shorter than M1 and M2, containing three rows of kinetids (Fig. 3E–F).

The SSU rRNA gene sequence of # DEBUG->jats:named-content gen. et sp. nov. ahas been deposited in GenBank database with the accession number OR616813.

DEBUG :list -> DEBUG :item -> DEBUG :label -> Subclass# Subclass Scuticociliatia #Small, 1967 DEBUG :item -> DEBUG :label -> Order# Order Pleuronematida #Fauré-Fremiet in Corliss, 1956 DEBUG :item -> DEBUG :label -> Family# Family Cyclidiidae #Ehrenberg, 1838 DEBUG :item -> DEBUG :label -> Genus# Genus Cyclidium #Müller, 1773

DEBUG :floatingText -> DEBUG :body ->

urn:lsid:zoobank.org:act:EFC1DA92-72AD-4EA2-936F-12805A06A0EA

Figs 4–5; Tables 1–2

kinetics: SKn extends to posterior sub-terminally; one or two macronuclei and one micronucleus; M1 consists of two longitudinal rows and is obviously shorter than M2; M2 has a long-triangle shape; M3 consists of four basal bodies in one row; the scutica is mainly composed of two pairs of kinetosomes; single caudal cilium; freshwater habitat.

#, indicating its similarity to #Pan, 2020.

A freshwater aquarium at Harbin Normal University, Harbin (), Northeast.

4A, K–N). Ratio of length to width about 1.25:1. Buccal field about 40%–45% of body length, with prominent paroral membrane on ventral side (Fig. 4A, K–P; Table 1). Cortex smooth. Somatic cilia and cilia of paroral membrane about 6 μm long (Fig. 4K–N), single caudal cilium about 13 μm long (Fig. 4A, K, N, P). Cytoplasm colorless, containing several (approximately 1 μm in diameter) bacteria-filled food vacuoles and variable-sized (0.5–1 μm) granules (Fig. 4A, K, N). Contractile vacuole located near posterior end of cell, approximately 4 μm in diameter (Fig. 4A, K–L, N). Extrusomes not observed; one or two globular macronuclei approximately 7 μm in diameter (eight out of ## cells examined with two macronuclei); one micronucleus approximately # μm across after staining (Figs #E, #K–L). Locomotion by swimming fast, sometimes motionless for long periods.

of dikinetids in its anterior half and widely positioned monokinetids posteriorly (Fig. 5E, K). SKn consisting of 10 to 13 kinetids; SK 1 composed of 14 kinetids, PM generally L-shaped, occupies about 40%–45% of body length, composed of zigzagging structure (Figs 4D, 5C). M1 consists of two longitudinal rows of monokinetids, shorter than M2 (Figs 4D, 5D); M2 long-triangle shaped, composed of three longitudinal rows and 8 horizontal rows (Figs 4D, 5C); M3 obviously smaller and shorter than M1 and M2, bearing one row consisting of four basal bodies (Figs 4D, 5C). Scutica patterns diversified (Figs 4F–J, 5F–J), arranged in two groups mainly (Figs 4D, F, 5E), located near posterior end of PM; each part composed of two and four basal bodies.

database with the accession number OR466484.

Phylogenetic positions of and

maximum likelihood tree is shown here (Fig. 6).

periphery of the Tetrahymenida clade. # DEBUG->jats:named-contentsp. nov. clusters with the clades formed by sp. KX853100, Z22879, #EU 032356 and #KY 476313.

The order Tetrahymenida #Fauré-Fremiet in Corliss, 1956 is mainly characterized by small to medium-sized cell with typically ovoid outline and holotrichous somatic ciliation. The oral structures consists of a right paroral (undulating membrane) and three left oral polykinetids (membranelles) situated in the oral cavity. Members of this order exhibit a complex life cycle in histophagous and parasitic species, and are commonly found in freshwater habitats, sometimes terrestrial (Lynn 2008).

Pseudotetrahymenidae # DEBUG->jats:named-contentfam. nov. should be assigned to the Tetrahymenida based on its morphological characteristics and habitat. In terms of the structure of the oral apparatus and

morphology of the cell, the following families should be compared with Pseudotetrahymenidae : Glaucomidae #Corliss, 1971 , Spirozonidae #Kahl, 1926 , Tetrahymenidae #Corliss, 1952 , Turaniellidae #Didier, 1971 , Trichospiridae #Kahl, 1926 (Roux 1901; Corliss 1952, 1971; Foissner et al. 1981; Ganner & Foissner 1989).

Pseudotetrahymenidae # DEBUG->jats:named-contentfam. nov. differs from Glaucomidae by the following characters: the absence of a small group of kinetosomes (X group) (vs present in Glaucomidae), M1 and M2 composed of three or four rows of kinetids (vs more than three rows in Glaucomidae), and the cell size (165–230 µm vs usually 40–70 µm in Glaucomidae) (Corliss 1971; Lynn 2008; Pan et al. 2017b).

Pseudotetrahymenidae # DEBUG->jats:named-contentfam. nov. can be separated from Spirozonidae by: the somatic kineties arranged latitudinally (vs somatic kineties on left side and dorsal left mild torsional in Spirozonidae), the caudal cilia absent (vs caudal cilia present, forming a ring at the posterior end of cell in Spirozonidae), and the paroral membrane having continuous dikinetids (vs paroral membrane having isolated dikinetids at anterior end in Spirozonidae) (Foissner et al. 1981).

Though Pseudotetrahymenidae # DEBUG->jats:named-contentfam. nov. resembles Tetrahymenidae in the body shape and the position of the paroral membrane, which begins at the level of M2, it can be distinguished from Tetrahymenidae in having M2 composed of several parts (vs only one part in Tetrahymenidae) (Corliss 1952; Lynn 2008).

Pesudotetrahymenidae # DEBUG->jats:named-contentfam. nov. can be clearly distinguished from Turaniellidae by the following features: latitudinally arranged right ventral kineties (vs curving left, twisting anterior of the oral region, sometimes abruptly, to run parallel to the anterior suture in Turaniellidae), longitudinal somatic kineties (vs one or more somatic kineties interrupted by left edge of buccal cavity in Turaniellidae), and a paroral membrane entirely consisting of ciliated kinetosomes (vs anterior part consisting of ciliated and posterior part of non-ciliated kinetosomes in Turaniellidae) (Ganner & Foissner 1989; Lynn 2008).

When compared with Trichospiridae , Pesudotetrahymenidae # DEBUG->jats:named-contentfam. nov. can be separated by the following features: longitudinal somatic kineties (vs a special band of cilia associated with a pellicular ridge that spirals dextrally posteriorly, ending in a transverse ring of cilia in Trichospiridae), the absence of caudal cilia (vs caudal cilia forming a tuft in Trichospiridae), and the deep buccal cavity containing one paroral membrane and three oral membranelles (vs oral structures as anterior extensions of several somatic kineties invaginating into a shallow cavity in Trichospiridae) (Roux 1901; Lynn 2008).

The most important diagnostic criteria for species identification and separation in the genus are the pattern of oral membranelles 1–3, the ratio of oral field to the body length, the number of somatic kineties, the type of kineties constituting somatic ciliary rows, the number of macronuclei and micronuclei, and the termination position of the posterior end of somatic kineties (Borror 1972; Grolière 1980; Didier & Wilbert 1981; Agamaliev 1983; Alekperov 2005).

DEBUG->jats:named-contentsp. nov. can be separated from by its smaller body size (20–30 × 15–25 µm vs 35–40 × 18–20 µm in), M2 composed of three longitudinal lines and eight horizontal rows (vs four longitudinal lines and ten horizontal rows), and M3 having one row (vs two rows in) (Pan et al. 2020).

DEBUG->jats:named-contentsp. nov. can be distinguished from #Pan et al., 2017 by having nine or ten somatic kineties (vs stably 11 in), somatic kineties consisting of dikinetids in their anterior half and widely positioned monokinetids posteriorly (vs comprising loosely spaced monokinetids in), and M3 single-rowed (vs two-rowed in) (Pan et al. 2017).

DEBUG->jats:named-contentsp. nov. differs from Song, 2000 by having shorter buccal field (about 45% of body length vs 75% in), nine or ten somatic kineties (vs mostly 11 or 13 in), and occurring in a freshwater habitat (vs marine habitat for) (Song 2000).

DEBUG->jats:named-contentsp. nov. can be distinguished from #Grolière, 1980 by fewer somatic kineties (nine or ten vs 14–16 in), and a smaller ratio of buccal field relative to the body length (45% vs > 60% in) (Grolière 1980).

Compared with , # DEBUG->jats:named-contentsp. nov. differs in the following features: larger body size (20–30 × 15–25 µm vs 12–18 × 8–12 µm in), and smaller buccal field to body length ratio (45% vs 56% in) (Pan et al. 2020).

Scutica is a structure composed of non-ciliated kinetosomes in diverse patterns which exists widely in scuticociliates (Borror 1963; Thompson 1967; Lynn 2008; Fan et al. 2011; Foissner et al. 2014; Pan et al. 2020; Hao et al. 2022; Poláková et al. 2023). Scutica of ciliates assigned to the genus mainly consist of two pairs of basal bodies (Song 2000; Pan et al. 2017a; Pan et al. 2020; Poláková et al. 2023). In present study, five distinct patterns of scutica were observed in hereafter named types #–# (Figs 4F–J, 5F–J).

Scutica type # contains two parts composed of two and four kinetosomes, respectively (Figs 4F, 5F). Kinetosomes of type # are divided into three parts consisting of two, three and three kinetosomes each (Figs 4G, 5G). Type # is composed of four basal bodies divided into two pairs (Figs 4H, 5H). Type # consists of eight basal bodies divided into two parts, two pairs of kinetosomes forming one part, another four kinetosomes forming one row (Figs 4I, 5I). In type V, two rows of kinetosomes forms shape #, each row consisting of four basal bodies (Figs 4J, 5J). Such a phenomenon indicates the possibility of intraspecific diversity of scutica among scuticociliates. Therefore, it is necessary to get more morphological data to confirm the intraspecific diversity of scutica for scuticociliates, not just for the genus .

The newly obtained SSU-rDNA sequence of # DEBUG->jats:named-contentgen. et sp. nov. is most similar to #AF 364041, with which it shares a molecular similarity of about 99.28% but still exhibits a difference of 15 nucleotides, which support the validity of as a distinct species. Besides, Pseudotetrahymenidae # DEBUG->jats:named-contentfam. nov. emerges as the earliest-branching clade within Tetrahymenida and forms a sister clade to all the other Tetrahymenida species with high support (ML/BI, 99/1.00). This phylogenetic position, combined with the morphological characteristics of , further supports the establishment of Pseudotetrahymenidae .

DEBUG->jats:named-contentsp. nov. groups with sp. KX853100, Z22879, #EU 032356, and #KY 476313 with full support, indicating a close relationship between these species.

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The authors declare that they have no conflict of interest.

DEBUG :figure -> DEBUG :graphic ->

Map and sampling sites.. Map of China, showing the location of sampling sites in Harbin. . Collecting site of Dafa international fish market (45°44#9# N, 126°35#41# E) from which was collected. . Collecting site of Harbin Normal University (45°52#3# N. 126°33#2# E) where was collected.

DEBUG :figure -> DEBUG :graphic ->

Morphology and infraciliature of from life (A–B, E, H, I–K) and after carbonate-staining (C–D, F–G).. Ventral views of the representative cell, arrowhead in A indicates oral field. . Infraciliature of the holotype (HANU WX-20221011-01) showing the ciliary pattern and macronucleus. . Ventral view. . Dorsal view. . Dorsal view of representative cell. . Details of oral structure. . Arrowhead shows macronucleus, arrow indicates oral field. . Arrowhead shows oral cilia. . Details of cortical granules. . Arrow shows big food vacuole filled with ingested ; arrowhead marks contractile vacuole. M1 = membranelle 1; M2a = membranelle 2a; M2b = membranelle 2b; M2c = membranelle 2c; M3 = membranelle 3; PM = paroral membrane. Scale bars = 95 μm.

DEBUG :figure -> DEBUG :graphic ->

Photomicrographs of after silver carbonate staining, paratypes (HANU WX-20221011-02), showing the whole cell (A–D) and details of the buccal region (E–H). . Ventral view. . Dorsal view. . Different form of specimen exhibiting infraciliatures and macronucleus. . Details of the adoral zone. . Arrowhead shows stained in food vacuole. . Macronucleus. M1 = membranelle 1; M2a = membranelle 2a; M2b = membranelle 2b; M2c = membranelle 2b; M3 = membranelle 3; PM = paroral membrane; Ma = macronucleus; PK = postoral kineties. Scale bars = 95 μm; E–H not to scale.

DEBUG :table -> DEBUG :row -> DEBUG :cell -> Character | DEBUG :cell -> Species | DEBUG :cell -> Max | DEBUG :cell -> Min | DEBUG :cell -> Mean | DEBUG :cell -> M | DEBUG :cell -> CV | DEBUG :cell -> SD | DEBUG :cell -> N | | DEBUG :row -> DEBUG :cell -> Body length, μm | DEBUG :cell -> Po | DEBUG :cell -> 234 | DEBUG :cell -> 166 | DEBUG :cell -> 193.2 | DEBUG :cell -> 192 | DEBUG :cell -> 8.6 | DEBUG :cell -> 16.7 | DEBUG :cell -> 17 | | DEBUG :row -> DEBUG :cell -> | DEBUG :cell -> Cp | DEBUG :cell -> 30 | DEBUG :cell -> 20 | DEBUG :cell -> 24.6 | DEBUG :cell -> 24.0 | DEBUG :cell -> 13.2 | DEBUG :cell -> 3.3 | DEBUG :cell -> 24 | DEBUG :row -> DEBUG :cell -> | DEBUG :cell -> Cv | DEBUG :cell -> 34 | DEBUG :cell -> 45 | DEBUG :cell -> 39.3 | DEBUG :cell -> 40 | DEBUG :cell -> 7.8 | DEBUG :cell -> 3.1 | DEBUG :cell -> 15 | | DEBUG :row

left

-> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 18 | DEBUG :cell -> 12 | DEBUG :cell -> 13.2 | DEBUG :cell -> 13 | DEBUG :cell -> 14.4 | DEBUG :cell -> 1.9 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cs | DEBUG :cell -> 32 | DEBUG :cell -> 21 | DEBUG :cell -> 28.3 | DEBUG :cell -> 27 | DEBUG :cell -> 21.4 | DEBUG :cell -> 5.7 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cvb | DEBUG :cell -> 19 | DEBUG :cell -> 12 | DEBUG :cell -> 13.5 | DEBUG :cell -> – | DEBUG :cell -> 10.9 | DEBUG :cell -> 1.46 | DEBUG :cell -> 17 | | DEBUG :row -> | DEBUG :cell -> Body width, µm | | DEBUG :cell -> Po | DEBUG :cell -> 208 | DEBUG :cell -> 124 | DEBUG :cell -> 164.5 | DEBUG :cell -> 168 | DEBUG :cell -> 12.1 | DEBUG :cell -> 19.8 | DEBUG :cell -> 19 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cp | DEBUG :cell -> 25 | DEBUG :cell -> 15 | DEBUG :cell -> 19.7 | DEBUG :cell -> 19.5 | DEBUG :cell -> 15.2 | DEBUG :cell -> 3.0 | DEBUG :cell -> 24 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 34 | DEBUG :cell -> 25 | DEBUG :cell -> 28.5 | DEBUG :cell -> 29 | DEBUG :cell -> 8.5 | DEBUG :cell -> 2.4 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 12 | DEBUG :cell -> 8 | DEBUG :cell -> 10.3 | DEBUG :cell -> 1 | DEBUG :cell -> 7.1 | DEBUG :cell -> 0.7 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cs | DEBUG :cell -> 16 | DEBUG :cell -> 12 | DEBUG :cell -> 13.2 | DEBUG :cell -> 13 | DEBUG :cell -> 9.1 | DEBUG :cell -> 1.2 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cvb | DEBUG :cell -> 13 | DEBUG :cell -> 8 | DEBUG :cell -> 9.8 | DEBUG :cell -> – | DEBUG :cell -> 9.2 | DEBUG :cell -> 0.22 | DEBUG :cell -> 17 | | DEBUG :row -> | DEBUG :cell -> Buccal field length, µm | | DEBUG :cell -> Po | DEBUG :cell -> 94 | DEBUG :cell -> 64 | DEBUG :cell -> 76.8 | DEBUG :cell -> 75.0 | DEBUG :cell -> 10.2 | DEBUG :cell -> 7.9 | DEBUG :cell -> 20 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cp | DEBUG :cell -> 15 | DEBUG :cell -> 9 | DEBUG :cell -> 11.2 | DEBUG :cell -> 11.0 | DEBUG :cell -> 11.2 | DEBUG :cell -> 1.2 | DEBUG :cell -> 24 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 24 | DEBUG :cell -> 17 | DEBUG :cell -> 20.4 | DEBUG :cell -> 20 | DEBUG :cell -> 10.1 | DEBUG :cell -> 2.1 | DEBUG :cell -> 18 | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 8 | DEBUG :cell -> 6 | DEBUG :cell -> 7.4 | DEBUG :cell -> 7 | DEBUG :cell -> 6.1 | DEBUG :cell -> 0.43 | DEBUG :cell -> 12 | | DEBUG :row -> DEBUG :cell -> | | DEBUG :cell -> Cs | DEBUG :cell -> 15 | DEBUG :cell -> 10 | DEBUG :cell -> 12.2 | DEBUG :cell -> 12 | DEBUG :cell -> 9.1 | DEBUG :cell -> 1.1 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | DEBUG :cell -> Cvb | DEBUG :cell -> 13 | DEBUG :cell -> 9 | DEBUG :cell -> 9.7 | DEBUG :cell -> – | DEBUG :cell -> 6.8 | DEBUG :cell -> 0.66 | DEBUG :cell -> 17 | | DEBUG :row -> | DEBUG :cell -> Somatic kineties, number | DEBUG :cell -> Po | DEBUG :cell -> 22 | DEBUG :cell -> 18 | DEBUG :cell -> 19.7 | DEBUG :cell -> 20 | DEBUG :cell -> 5.4 | DEBUG :cell -> 1.1 | DEBUG :cell -> 9 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cp | DEBUG :cell -> 10 | DEBUG :cell -> 9 | DEBUG :cell -> 9.2 | DEBUG :cell -> 9.0 | DEBUG :cell -> 4.4 | DEBUG :cell -> 0.4 | DEBUG :cell -> 24 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 10 | DEBUG :cell -> 9 | DEBUG :cell -> 9.6 | DEBUG :cell -> 10 | DEBUG :cell -> 13.6 | DEBUG :cell -> 0.5 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 9 | DEBUG :cell -> 8 | DEBUG :cell -> 8.3 | DEBUG :cell -> 8 | DEBUG :cell -> 3.7 | DEBUG :cell -> 0.3 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cs | DEBUG :cell -> 11 | DEBUG :cell -> 11 | DEBUG :cell -> 11 | DEBUG :cell -> 11 | DEBUG :cell -> 0 | DEBUG :cell -> 0 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cvb | DEBUG :cell -> 13 | DEBUG :cell -> 11 | DEBUG :cell -> 11.7 | DEBUG :cell -> – | DEBUG :cell -> 5.1 | DEBUG :cell -> 0.6 | DEBUG :cell -> 16 | | DEBUG :row -> | DEBUG :cell -> Postoral kineties, number | | DEBUG :cell -> Po | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> 2.0 | DEBUG :cell -> 2.0 | DEBUG :cell -> 0.0 | DEBUG :cell -> 0.0 | DEBUG :cell -> 10 | | DEBUG :row -> | DEBUG :cell -> Macronucleus length, µm | | DEBUG :cell -> Po | DEBUG :cell -> 88 | DEBUG :cell -> 48 | DEBUG :cell -> 60.0 | DEBUG :cell -> 55.0 | DEBUG :cell -> 16.1 | DEBUG :cell -> 11.4 | DEBUG :cell -> 14 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cp | DEBUG :cell -> 11 | DEBUG :cell -> 5 | DEBUG :cell -> 6.9 | DEBUG :cell -> 7.0 | DEBUG :cell -> 19.2 | DEBUG :cell -> 1.3 | DEBUG :cell -> 16 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 14 | DEBUG :cell -> 8 | DEBUG :cell -> 11 | DEBUG :cell -> 11 | DEBUG :cell -> 16.5 | DEBUG :cell -> 2 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 5 | DEBUG :cell -> 3 | DEBUG :cell -> 4.1 | DEBUG :cell -> 4 | DEBUG :cell -> 2.5 | DEBUG :cell -> 0.1 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cvb | DEBUG :cell -> 5 | DEBUG :cell -> 3 | DEBUG :cell -> 3.9 | DEBUG :cell -> – | DEBUG :cell -> 18.5 | DEBUG :cell -> 0.72 | DEBUG :cell -> 16 | | DEBUG :row -> DEBUG :cell -> Macronucleus, number | | DEBUG :cell -> Po | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 0 | DEBUG :cell -> 0 | DEBUG :cell -> 22 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cp | DEBUG :cell -> 2 | DEBUG :cell -> 1 | DEBUG :cell -> 1.3 | DEBUG :cell -> 1 | DEBUG :cell -> 35.3 | DEBUG :cell -> 0.5 | DEBUG :cell -> 23 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 2 | DEBUG :cell -> 1 | DEBUG :cell -> 1.5 | DEBUG :cell -> 1 | DEBUG :cell -> 33.7 | DEBUG :cell -> 0.5 | DEBUG :cell -> 27 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 1 | DEBUG :cell -> 0 | DEBUG :cell -> 0 | DEBUG :cell -> 15 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cs | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> 0 | DEBUG :cell -> 0 | DEBUG :cell -> 13 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cvb | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> 2 | DEBUG :cell -> – | DEBUG :cell -> 0 | DEBUG :cell -> 0 | DEBUG :cell -> > 100 | | DEBUG :row -> | DEBUG :cell -> Basal bodies in SKn, number | | DEBUG :cell -> Cp | DEBUG :cell -> 13 | DEBUG :cell -> 10 | DEBUG :cell -> 11.4 | DEBUG :cell -> 11.0 | DEBUG :cell -> 9.3 | DEBUG :cell -> 1.1 | DEBUG :cell -> 18 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cv | DEBUG :cell -> 13 | DEBUG :cell -> 11 | DEBUG :cell -> 11.7 | DEBUG :cell -> 12 | DEBUG :cell -> 8.2 | DEBUG :cell -> 0.9 | DEBUG :cell -> 11 | | DEBUG :row -> | DEBUG :cell -> | | DEBUG :cell -> Cg | DEBUG :cell -> 12 | DEBUG :cell -> 11 | DEBUG :cell -> 11.1 | DEBUG :cell -> 11 | DEBUG :cell -> 1.8 | DEBUG :cell -> 0.2 | DEBUG :cell -> 11 |

DEBUG :row -> DEBUG :cell -> DEBUG :cell -> Cs DEBUG :cell -> 13 DEBUG :cell -> 12 DEBUG :cell -> 12.6 DEBUG :cell -> 13 DEBUG :cell -> 3.8 DEBUG :cell -> 0.4 DEBUG :cell -> 14 DEBUG :row -> DEBUG :cell -> DEBUG :cell -> Cvb DEBUG :cell -> 11 DEBUG :cell -> 10 DEBUG :cell -> 10.4 DEBUG :cell -> – DEBUG :cell -> 4.9 DEBUG :cell -> 0.51 DEBUG :cell -> 11 DEBUG :row -> DEBUG :cell -> Basal bodies in SK 1, number DEBUG :cell -> Cp DEBUG :cell -> 14 DEBUG :cell -> 13 DEBUG :cell -> 13.1 DEBUG :cell -> 13 DEBUG :cell -> 2.6 DEBUG :cell -> 0.3 DEBUG :cell -> 15 DEBUG :row -> DEBUG :cell -> DEBUG :cell -> Cs DEBUG :cell -> 13 DEBUG :cell -> 12 DEBUG :cell -> 12.6 DEBUG :cell -> 13 DEBUG :cell -> 4.6 DEBUG :cell -> 0.5 DEBUG :cell -> 14 DEBUG :row -> DEBUG :cell -> DEBUG :cell -> Cvb DEBUG :cell -> 16 DEBUG :cell -> 14 DEBUG :cell -> 15.2 DEBUG :cell -> – DEBUG :cell -> 3.9 DEBUG :cell -> 0.58 DEBUG :cell -> 13 DEBUG :figure -> DEBUG :graphic ->

Morphology and infraciliature of . Ventral views of representative specimens (paratytes; HANU WX-20221123-02); arrowhead in L marks contractile vacuole, in M depicts paroral membrane and in N indicates caudal cilium. . Infraciliature of the holotype (HANU WX-20221123-01) to show the ciliary pattern. . Ventral view. . Dorsal view. . Details of oral structure. . Macronuclei and micronucleus. . Patterns of scutica. . Arrowhead shows flat section, arrow shows paroal membrane. . Arrowhead marks caudal cilium, arrow show paroal membrane. M1 = membranelle 1; M2 = membranelle 2; M3 = membranelle 3; Ma = macronucleus; Mi = micronucleus; PM = paroral membrane; Sc = scutica. Scale bars = 15 μm.

DEBUG :figure -> DEBUG :graphic ->

Photomicrographs of after silver carbonate staining; paratytes (HANU WX-20221123-02). . Whole cell. . Ventral View. . Dorsal view. . Details of the buccal region.. Details of buccal area. . Arrowhead indicate dikinetid. . Patterns of scutica after staining. . Macronuclei and micronucleus. . Arrowhead indicates dikinetid, arrow shows micronucleus. . Arrowhead marks micronucleus. M1 = membranelle 1; M2 = membranelle 2; M3 = membranelle 3; Ma = macronucleus; PM = paroral membrane; Sc = scutica. Scale bars = 15 μm.

DEBUG :table -> DEBUG :row -> DEBUG :cell -> Character DEBUG :cell -> DEBUG :cell -> DEBUG :row -> DEBUG :cell -> Body size, μ m (base on silver staining) DEBUG :cell -> 20–30 × 15–25 DEBUG :cell -> 18–28 × 11–18 DEBUG :row -> DEBUG :cell -> Length of buccal field/cell length DEBUG :cell -> 4.5/10 DEBUG :cell -> 3/4 DEBUG :row -> DEBUG :cell -> Somatic kineties, number DEBUG :cell -> 9–10 DEBUG :cell -> 14–16 DEBUG :row -> DEBUG :cell -> Macronucleus, number DEBUG :cell -> 1–2 DEBUG :cell -> 2 DEBUG :row -> DEBUG :cell -> Basal bodies in SKn, number DEBUG :cell -> 10–13 DEBUG :cell -> 16 DEBUG :figure -> DEBUG :graphic ->

The maximum likelihood tree inferred from the SSU-475 rRNA gene sequences, showing the positions of and (in red). Numbers at nodes represent the bootstrap values of maximum likelihood out of 1000 replicates and the inference. Fully supported (100%/1.00) branches are marked with solid circles. The scale bar corresponds to five substitutions per 100 nucleotide positions.