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Three novel species of *Microglena* (Chlamydomonadales, Chlorophyceae) from China revealed by an integrative approach

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Abstract

The taxonomic status of *Microglena* as a genus within Chlamydomonadales was reconfirmed. Many strains previously assigned to *Chlamydomonas* have been reclassified as members of *Microglena*, and new species continue to be described under this genus. In this study, we investigated three strains isolated from China using an integrative approach. Phylogenetic analyses based on 18S and ITS rDNA sequences, combined with comparisons of compensatory base changes (CBCs) for the conserved regions of ITS-2 secondary structure revealed that the three strains represent three independent species included in *Microglena*, and they are distinct from all previously described *Microglena* species, as well as from two closely related strains SAG 96.80 and SAG 22.95. Furthermore, morphological comparisons demonstrated that none of the three strains match known species sharing similar features. . Based on these results, three new *Microglena* species, including one lacking pyrenoids, are described here. Our results enrich the understanding of morphological diversity within *Microglena*, especially regarding the loss of pyrenoids.

Keywords

18S rDNA, ITS-2 rDNA, Chlamydomonadales, *Microglena monadinia*, molecular phylogeny, morphology, new species

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Introduction

Microglena is a biflagellate genus belonging to Chlamydomonadales (Chlorophyceae). This genus was originally established by Ehrenberg (1832), including only two species *M. monadina* and *M. volvocina*. The latter was reclassified to the genus *Trachelomonas* as *Trachelomonas volvocina* by Ehrenberg (1834), while the former was transferred to *Chlamydomonas* as *C. monadina* by Stein (1878). Because six species of Chrysophyceae had been described under the name *Microglena* (see Ettl, 1978), *Microglena* was treated as a chrysophycean genus during the last century. Demchenko *et al.* (2012) reconfirmed the taxonomic status of *Microglena* as a member of Chlorophyceae. The phylogenetic position of *Microglena* was determined to correspond to the clade *Monadinia* sensu Nakada *et al.* (2008) based on the type species *M. monadina*, and many species previously assigned to *Chlamydomonas* (containing 11 new combinations and two new species) were reclassified as members of this genus (Demchenko *et al.*, 2012). In addition, *Microglena* species are widely distributed in diverse habitats, and especially, multiple psychrophilic strains isolated from snow or ice fields form a "polar-clade" within this genus (Demchenko *et al.*, 2012; Eddie *et al.*, 2008; Liu *et al.*, 2006). A new species, *M. antarctica*, was recognized based on four strains included in this polar-clade (Trentin *et al.*, 2022). The strain SAG 18.89 collected from brackish water previously assigned to *C. subehrenbergii* was also reclassified as a new species, named *M. redcarensis* (Nakada *et al.*, 2018). Moreover, the strain SAG 16.90 was considered to correspond to the diagnosis of *C. nova* nom. illegal. (Skuja, 1956) and was renamed as *M. skujae* by Demchenko *et al.* (2012). However, Nakada & Tomita (2014) believed this strain

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was actually identical to *Chlamydomonas media* (Klebs, 1986) and renamed it as a new combination *M. media*.

Given the widespread distribution of *Microglena* species and their morphological similarity to traditional *Chlamydomonas* sensu Ettl (1983), there are likely many undiscovered species in *Microglena*, and some known species may need to be transferred into this genus. In this study, we investigated three strains isolated from different cities in China based on an integrative approach, including morphological observation, 18S and ITS rDNA phylogeny and comparison of ITS-2 secondary structures. In addition, since a phylogenetic analysis by Fulnečková *et al.* (2012) showed two strains identified as *Tetracystis* species, SAG 96.80 and SAG 22.95, are closely related to *Microglena* species, we included these two strains in our phylogenetic analyses and secondary structure comparisons. Three novel *Microglena* species are proposed based on these results. Our findings enrich the species number of *Microglena* and provide a deeper insight into the morphological diversity within this genus.

Materials and methods

Sampling, isolation and cultivation: The three strains examined in this study were isolated from water samples collected at different areas: FACHB-3857 and FACHB-3854 were obtained from the Wushui and Wei Rivers, respectively, both of which are large, slow-moving, eutrophic lowland rivers in Weifang, Shandong. FACHB-3856 was isolated from an unnamed small, slow-moving, eutrophic lowland river in Xiantao, Hubei. Sampling was conducted on July 5, 2022, in Weifang and on March 30, 2022, in Xiantao. All water samples were collected using a plankton net. Unialgal cultures were established by serial dilution (Hoshaw and Rosowski, 1973). The strains were grown in 50 ml glass Erlenmeyer flasks containing 20 ml of AF-6 medium (Watanabe & Hiroki, 1997) at 20 °C under a light:dark cycle of 14:10 h with 50 µmol photons·m^{-2}·s^{-1}.

Light and transmission electron microscopy: The morphologies of the strains were observed using an Olympus BX53 light microscopy. Cells were fixed with Lugol's solution to observe the flagella. The micrographs were taken using an Olympus DP80 microscope digital camera with a U-HGLPS fluorescence light source (Olympus, Tokyo, Japan). The ultrastructure of cells was studied using transmission electron microscopy (TEM). For TEM, algal cells in the logarithmic growth phase were fixed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.2) at 4 °C for 2 h. After washing three times with the same buffer, cells were postfixed with 1% OsO_4 prepared in 0.1 M phosphate buffer for 1 h. Following three additional washes with buffer, cells were stained with 1% aqueous uranyl acetate overnight at 4 °C. Dehydration was carried out through a graded concentration increasing ethanol series. The dehydrated samples were embedded in epoxy resin. After curing, serial sections were prepared by a Leica UC7 Ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and stained with uranyl acetate and lead citrate. TEM observations and imaging were performed using a Hitachi HT 7800 transmission electron microscope (Hitachi High Technologies, Tokyo, Japan).

DNA extraction, PCR and sequencing: Genomic DNA was extracted using the AxyPrep MAG Plant Genomic DNA Extraction Kit (Corning Inc., Corning, New York, USA). The 18S rDNA was amplified using the primers 18S-FA and 18S-RB (Nakazawa & Nozaki, 2004) with the following PCR protocol: 94 °C for 5 min, followed by 32 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The whole ITS region was amplified using the primers S21ch and L5pl (Pawlowski *et al.*, 2001) with the following PCR protocol: 94 °C for 5 min, followed by 34 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 2 min, and a final extension at 72 °C for 10 min. The purification of PCR-amplified DNA fragments was carried out using the FastPure Gel DNA Extraction Mini Kit (Vazyme, Nanjing, Jiangsu, China). Then the purified DNA fragments were sequenced with an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA) using the primers mentioned above.

Phylogenetic analyses: Two datasets, 18S rDNA and concatenated 18S+ITS rDNA (containing 18S, ITS-1, 5.8S and ITS-2), were used for phylogenetic reconstructions. The 18S rDNA dataset consisted of 60 sequences with six *Caudivolvoxa* taxa as outgroups, and the concatenated dataset comprised 32 sequences with two *Moewusinia* taxa as outgroups. The accession numbers and taxa information for all sequences used here are listed in supplementary Table S2. The 18S , ITS-1, 5.8S and ITS-2 rDNA sequences were aligned separately using MAFFT 7.3 (Katoh & Standley, 2013) and trimmed by trimAl (Capella-Gutierrez *et al.*, 2009) with the parameters block = 6, gt = 0.9, st = 0.4 and sw = 3. The phylogenetic tree was inferred by Bayesian inference (BI) using MrBayes 3.2.7 (Ronquist *et al.*, 2012) and maximum likelihood (ML) method using IQ-TREE2 (Minh *et al.*, 2020). The determination of the best-fit evolutionary model was carried out using ModelFinder (Kalyaanamoorthy *et al.*, 2017), where the concatenated dataset was partitioned by genes (see Table S3). For BI method, Monte Carlo Markov Chain (MCMC) iterations were performed by two runs with four chains (three heated and one cold) for 2,000,000 generations, with trees sampled every 1,000 generations and the first 25% of trees discarded. A stationary distribution was assumed when the average standard deviation of split frequencies was lower than 0.01. For ML analysis, the robustness of branches was assessed by branch support values using bootstrap tests with 200 replicates and Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT, Guindon *et al.*, 2010) with 1000 replicates.

ITS-2 secondary structure and CBCs: The ITS sequences of the three strains, plus two additional strains SAG 96.80 and SAG 22.95, were aligned with available ITS-2 sequences of *Microglena* species (Demchenko *et al.*, 2012) using MARNA (Siebert & Backofen, 2005). The ITS-2 regions were extracted manually and folded using RNAstructure (Reuter & Mathews, 2010). The predicted secondary structures were manually adjusted by StructureEditor of RNAstructure, as shown in supplementary Figures S1-S5, according to the available ITS-2 secondary structures of *Microglena* species. All ITS-2 sequences and corresponding secondary structures were aligned in MARNA. The conserved regions, containing the 15 bp of the 5.8S-LSU stem, the first 5 bp of Helix I, the first 11 bp of Helix II (including the pyrimidine–pyrimidine mismatch) and all base-pairs of Helix III (only select alignable base-pairs for *M. antarctica*), were extracted manually from the alignment and aligned again in MARNA. The alignment was used to detect compensatory base changes (CBCs) and hemi compensatory base changes (hCBCs) using CBCAnalyzer (Wolf *et al.*, 2005). The uncorrected *p*-distances were calculated using MEGA12 (Kumar *et al.*, 2024). The CBCs and p-distances among the species are shown in a matrix (Table S1).

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Results

Molecular phylogeny

In this study, a branch with one of Bayesian posterior probabilities (PP) ≥ 0.95, SH-aLRT values ≥ 80% and bootstrap (BP) values ≥ 70% was considered to be significantly supported. The branch meeting all three criteria was considered strongly supported. Phylogenetic analysis based on 18S rDNA sequences revealed that all three strains examined in this study were included in *Microglena* (Figure 1). The strain FACHB-3854 was closely related to "*Chlamydomonas*" sp. KNUA040 and *T. vinatzeri* SAG 22.95, forming a strongly supported clade (1.00, 97.6%, 94%, respectively). The strain FACHB-3856 was positioned as a sister to this clade, and this phylogenetic relationship was also strongly supported (1.00, 99.5%, 96%, respectively). The strain FACHB-3857 was strongly supported as a sister to the lineage consisting of *M. redcarensis*, "*Chlamydomonas*" sp. CCMP 233 and the Polar-clade *sensu* Eddie *et al.* (2008). However, this Polar-clade did not receive significant support here. Furthermore, we investigated phylogenetic relationships within *Microglena* using a concatenated 18S+ITS rDNA dataset. *Microglena* was divided into two lineages in this phylogenetic analysis (Figure 2). Two strains, FACHB-3854 and FACHB-3856, formed a strongly supported lineage with *M. basinucleata*, *T. pampae* SAG 96.80 and *T. vinatzeri* SAG 22.95 (1.00, 84.7%, 70%, respectively). By contrast, the other lineage was only significantly supported by PP value. The strain FACHB-3857 was included in this lineage as a sister to the lineage consisting of *M. redcarensis* and the Polar-clade. However, compared to 18S rDNA phylogeny, this sister relationship was only significantly supported by SH-aLRT value in this analysis.

ITS-2 secondary structures and CBCs

According to secondary structures, the conserved regions of ITS-2 sequences were extracted to detect the number of CBCs (including CBC and hCBC) among the three strains investigated in this study and 21 strains representing 15 known *Microglena* species plus two *Tetracystis* strains (Table S1). For different strains of the same *Microglena* species, CBC difference is either one hCBC or no CBC (e.g. *M. opisthopyren* SAG 8.87, SAG 54.90 and NIES-2744, and *M. media* SAG 16.90 and NIES-2743). Among 15 described *Microglena* species, independent species differ by at least one CBC, with the fewest differences observed between *M. coccifera* and *M. opisthopyren* (SAG 8.87 and SAG 54.90). There are 2-19 CBCs between FACHB-3854 and other *Microglena* strains, with nine and one CBC differences from *T. pampae* SAG 96.80 and *T. vinatzeri* SAG 22.95, respectively. Similarly, the strain FACHB-3856 exhibits 2-17 CBCs relative to other *Microglena* strains, and nine and two CBCs from *T. pampae* SAG 96.80 and *T. vinatzeri* SAG 22.95, respectively. Compared with the above two strains, FACHB-3857 displayed many more CBCs, with at least 16 CBCs, up to 21. Moreover, the *p*-distances among the three strains and 15 *Microglena* species plus two *Tetracystis* strains were also calculated, as shown in Table S1.

Morphological observations

The three strains share several morphological similarities. Their vegetative cells are ellipsoidal or ellipsoidal-cylindrical and sometimes asymmetrical, possessing a cup-shaped chloroplast without a thick basal region, two flagella as long as (or slightly longer/shorter than) the cell length, two apical contractile vacuoles and a papilla. Despite these similarities, they display some distinct differences in morphology, the most obvious being the presence or absence of a pyrenoid. Two strains, FACHB-3857 and FACHB-3854, were clearly observed to possess an ellipsoidal or spherical pyrenoid under optical microscopy (Figures 3, 5, 6, 9, 11, 14), which was not found in FACHB-3856 (Figures 15–20). To determine whether a pyrenoid is indeed absent in this strains and explore the structure of the pyrenoid, these three strains were further investigated using TEM. FACHB-3857 and FACHB-3854 have a typical pyrenoid, which is surrounded by several discontinuous starch plates and penetrated by thylakoid membranes (Figures 21–24).In contrast, no potential pyrenoid was observed in FACHB-3856 (Figures 25–26). Other key morphological features of the three strains were presented in Table 1.

Taxonomic treatments

According to our findings, the three strains investigated in this study are recognized as three new species belonging to the genus *Microglena*. The revised generic concept for *Microglena* and detailed descriptions for these new species are provided below:

Microglena Ehrenberg 1832 emend. Y.L.Chen *et* G.X.Liu

Emended description: Motile unicellular green alga with two equal flagella. Cells ellipsoidal to broadly ellipsoidal, ellipsoidal-cylindrical or spherical, sometimes asymmetrical. Cell wall thick or moderately thick, with a trapezoidal or dome-shaped papilla. Chloroplast basically cup-shaped, with or without a thickening basal part; pyrenoid absent or variable in shape and number, from a single spherical, ellipsoidal, horseshoe-like, half-ring-shaped or ring-shaped pyrenoid to several ellipsoid or spherical pyrenoids, surrounded by several starch plates or naked, located at the thick basal region or at the lateral thickening region in basal, middle or upper positions of the chloroplast. Stigma elliptical, rod-like, oval or fusiform, in the anterior, middle, or posterior part of the cell. Two apical contractile vacuoles. Nucleus middle to anterior or basal. Asexual reproduction by sporulation into two or four zoospores, cell division with or without rotation of protoplast by 90°.

Diagnosis: Distinguished from other unicellular biflagellate

Table 1. Morphological features of three investigated strains and other *Microglena* species.

Species	strains	Cell shape, size	papilla	Chloroplast	Pyrenoid	Stigma	Nucleus	Flagellar length
M. wushuiensis	FACHB-3857	ellipsoidal to broadly ellipsoidal, ellipsoidal-cylindrical or ovoid, 11–17×6–13 µm	dome-shaped	deep cup-shaped with small incisions, without a thick basal part	spherical or ellipsoidal	rod-like, in middle to anterior region	in anterior region	as long as the cell
M. weihensis	FACHB-3854	ovoid, ellipsoidal or ellipsoidal-cylindrical, 8–12×5–8 µm	trapezoidal	deep cup-shaped with small incisions, without a thick basal part	ellipsoidal or spherical	oval, in anterior 1/2-1/4 region	in basal region	as long or slightly longer as the cell
M. apyrenoides	FACHB-3856	broadly ellipsoidal or ellipsoidal-cylindrical, 9.5–14.5×5–10.5 µm	low trapezoidal	deep cup-shaped, dissected in several lobes, without a thick basal part	irregular, without associated starch plates	elliptical, in anterior 1/2–1/4 region	in basal region	as long or slightly shorter as the cell
M. antarctica	IMA076A	ovoid, 11.9–24.4×7.1–19.1 µm	broad trapezoidal	cup-shaped, with a thick basal part	spherical to ellipsoidal	shape unknown, in middle region	in middle-anterior region	unknown
M. basinucleata	SAG 67.72	ellipsoidal to wide ellipsoidal, 15–22×12–20 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	halfring-shaped, without associated starch plates	elongated, in anterior-middle region	in basal region	as long as the cell
M. braunii	SAG 50.86	ellipsoidal to wide ellipsoidal, almost spherical, 17–25×11–23 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	horseshoe-like, halfring-shaped or widely ellipsoidal	short rod-like, in anterior-middle region	in central region	as long as the cell
M. charkoviensis	ACKU 267-03 ACKU 274-03	ellipsoidal to wide ellipsoidal, 10–16.5×8–14.5 µm	broad trapezoidal	cup-shaped, with a thick basal part	widely ellipsoidal	rod-like to fusiform, in anterior-middle region	in central region	as long as the cell
M. coccifera	SAG 54.91 SAG 55.91	wide ellipsoidal, 22–24×19–23 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	1 to 4-5 (8), widely ellipsoidal or spherical	rod-like to fusiform, in anterior-middle region	in central region	as long as the cell
M. globulifera	CCAC 0015 CCAC 0017	wide ellipsoidal to spherical, 16–22×10–19 µm	broad trapezoidal	deep cup-shaped perforated by small fissures, without a thick basal part	horseshoe-like or halfring-shaped	rod-like to fusiform, in anterior-middle region	in central region	as long as the cell
M. indica	SAG 46.96	wide ellipsoidal to spherical, 14–18×13–17 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	halfring to almost ring-shaped	rod-like, in anterior-middle region	in central region	as long as the cell
M. lobata	SAG 31.72	ellipsoidal to wide ellipsoidal, 17–23×13–20 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	halfring-shaped	rod-like, in anterior-middle region	in central region	as long as the cell
M. longirubra	SAG 5.92	ellipsoidal to wide ellipsoidal, 20–26×17–20 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	halfring-shaped	elongated to fusiform, in anterior-middle region	in central region	as long as the cell
M. media	NIES-2743 SAG 16.90	ellipsoidal to wide ellipsoidal, 18–23×14–20 µm	broad trapezoidal	deep cup-shaped dissected in several lobes, without a thick basal part	widely ellipsoidal to quarterring-shaped	rod-like, in anterior-middle region	in central region	as long as the cell

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Table 1 continued

Species	strains	Cell shape, size	papilla	Chloroplast	Pyrenoid	Stigma	Nucleus	Flagellar length
M. monadina	SAG 55.72	ellipsoidal to wide ellipsoidal, 19–23×12–18 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	halfring-shaped	rod-like, in anterior-middle region	in central region	as long as the cell
M. opisthopyren	SAG 8.87 SAG 54.90	ellipsoidal to wide ellipsoidal, 10–20×11–17 µm	broad trapezoidal	deep cup-shaped, without a thick basal part	horseshoe-like, halfring-shaped or widely ellipsoidal	elongated, in anterior-middle region	in central region	as long as the cell
M. redcarensis	SAG 18.89	ovoid to ellipsoidal, 10–23×9–20 µm	low trapezoidal	cup-shaped, with a thick basal part	oblate ellipsoidal to nearly spherical	semicircular, central or slightly anterior region	in anterior region	almost as long as the cell
M. reginae	SAG 17.89	ellipsoidal to ovoid, wide ovoid to almost spherical, 15–22×13–18 µm	broad trapezoidal	cup-shaped, with a thick basal part	widely ellipsoidal	elongated, in anterior-middle region	in central region	as long as the cell
M. uva-maris	SAG 19.89	ellipsoidal to wide ellipsoidal, 8–17×6–13 µm	broad trapezoidal	cup-shaped, with a thick basal part	widely ellipsoidal	ellipsoidal to elongated, in anterior-middle region	in central region	as long as the cell

genera by 18S and ITS rDNA sequences.

Type species: *Microglena monadina* Ehrenberg emend. Demchenko, Mikhailyuk *et* Pröschold.

Comments: Compared to the generic concept of *Microglena* revised by Demchenko *et al.* (2012), one additional morphological feature, the absence of pyrenoids, is supplemented.

Microglena wushuiensis Y.L.Chen *et* G.X.Liu *sp. nov.* (Figures 3–8)

Description: Cells ellipsoidal to broadly ellipsoidal, ellipsoidal-cylindrical or ovoid, young cells often asymmetrical, 11–17×6-13 µm. Cell wall moderately thick, with a dome-shaped papilla. Flagella as long as the cell. Chloroplast deep cup-shaped, sometimes with unobvious and shallow incisions at basal and lateral regions; a single pyrenoid, spherical or ellipsoidal, surrounded by several starch plates and penetrated by thylakoid membrane bundles, in lateral thickenings of the chloroplast in the medial position. Stigma rod-like, in the middle to upper part of the cell, sometimes lower in the middle part. Two apical contractile vacuoles. Nucleus at the 1/3 height of the cell, above the pyrenoid, near one side. Asexual reproduction by sporulation producing two or four zoospores, cell longitudinal division without rotation of protoplast by 90°. Sexual reproduction not observed.

Diagnosis: Similar to *Chlamydomonas pseudogloeogama* Gerloff (1940), *C. terricola* Gerloff (1940), *C. botulus* Ettl (1965) and *C. eucallosa* Ettl (1965) in the shape and size of cells as well as chloroplast morphology, but differing from *C. pseudogloeogama* by dome-shaped papilla, ellipsoidal pyrenoid and higher position of the nucleus, from *C. terricola* by dome-shaped papilla and chloroplast with less and indistinct incisions, from *C. botulus* by cell length-to-width ratio, larger pyrenoid and chloroplast with less and indistinct incisions, and from *C. eucallosa* by thinner wall, dome-shaped papilla and chloroplast without irregularly furrowed incisions and elongated perforations. Also similar to *C. noctigama* Pascher (1927) and *C. humophilos* Gerloff (1940), however, differing from *C. noctigama* by dome-shaped papilla, shorter flagella and slightly smaller cells, and from *C. humophilos* by dome-shaped papilla and apparently smaller cells. Distinguished from other *Microglena* species by 18S rDNA sequence (PX278842) and at least 16 CBCs in conserved regions of ITS-2 sequence (Table S1).

Holotype: HBI WUHA-1 (formaldehyde-fixed material), collected by Yangliang Chen, Jul 5, 2022; permanently preserved in the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China.

Reference strain: The living strain is deposited in the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China (FACHB) as FACHB-3857.

Type locality: Wushui River , Weifang City, Shandong Province, China (36°22'25.59"N, 119°24'27.04"E).

Habitat: planktic

Etymology: The specific epithet reflects the type locality of

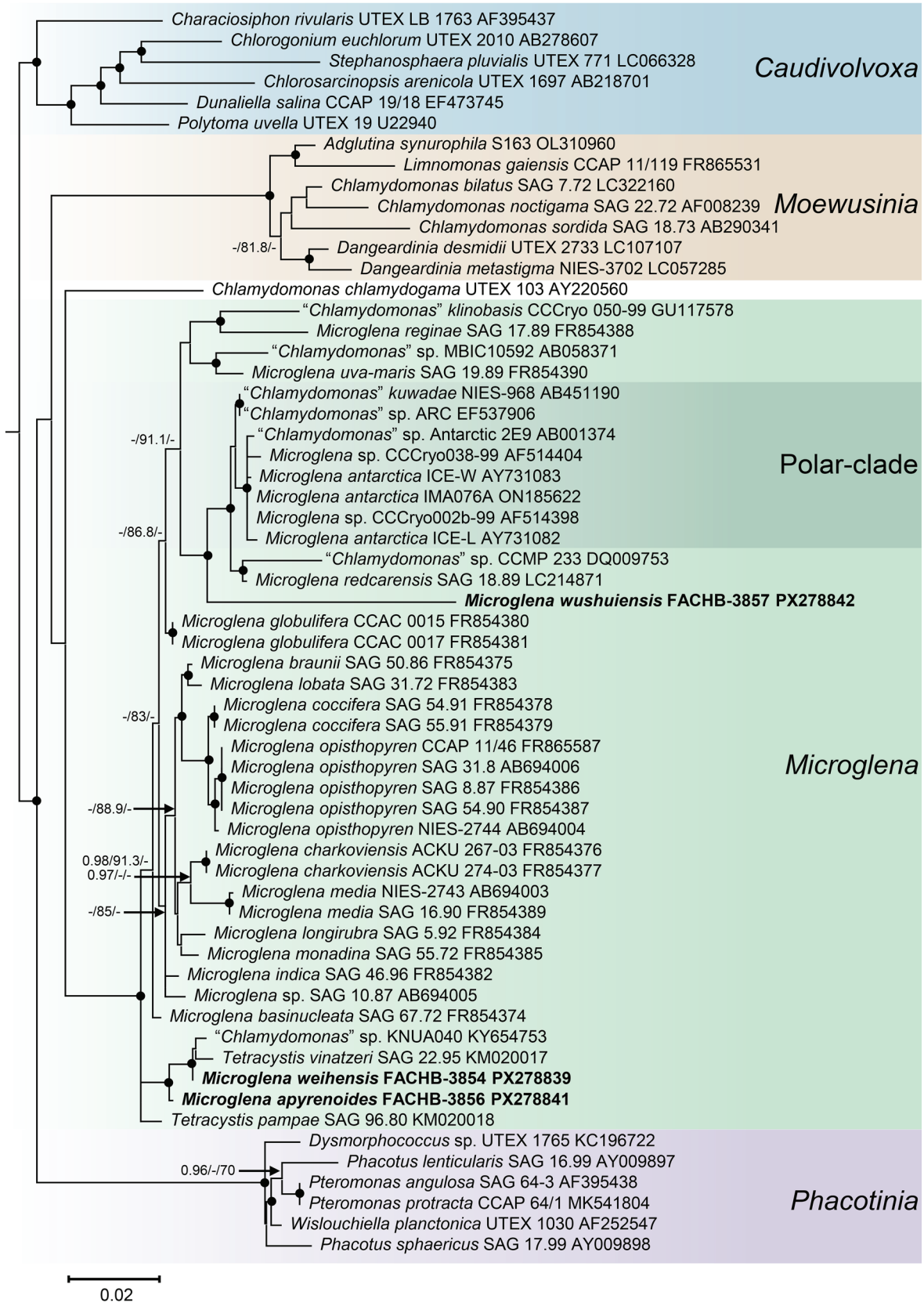


Figure 1. Maximum likelihood tree of the clade *Xenovolvoxa* sensu Nakada *et al.* (2008) based on 18S rDNA sequences with six taxa representing the *Caudivolvoxa* clade as outgroups. Support values are shown only for branches with Bayesian posterior probabilities ≥0.95, SH-aLRT values ≥ 80% or bootstrap values ≥ 70% from left to right. A dot indicates that the corresponding branch received significant support from all three of the above. Newly sequenced taxa are represented in bold.

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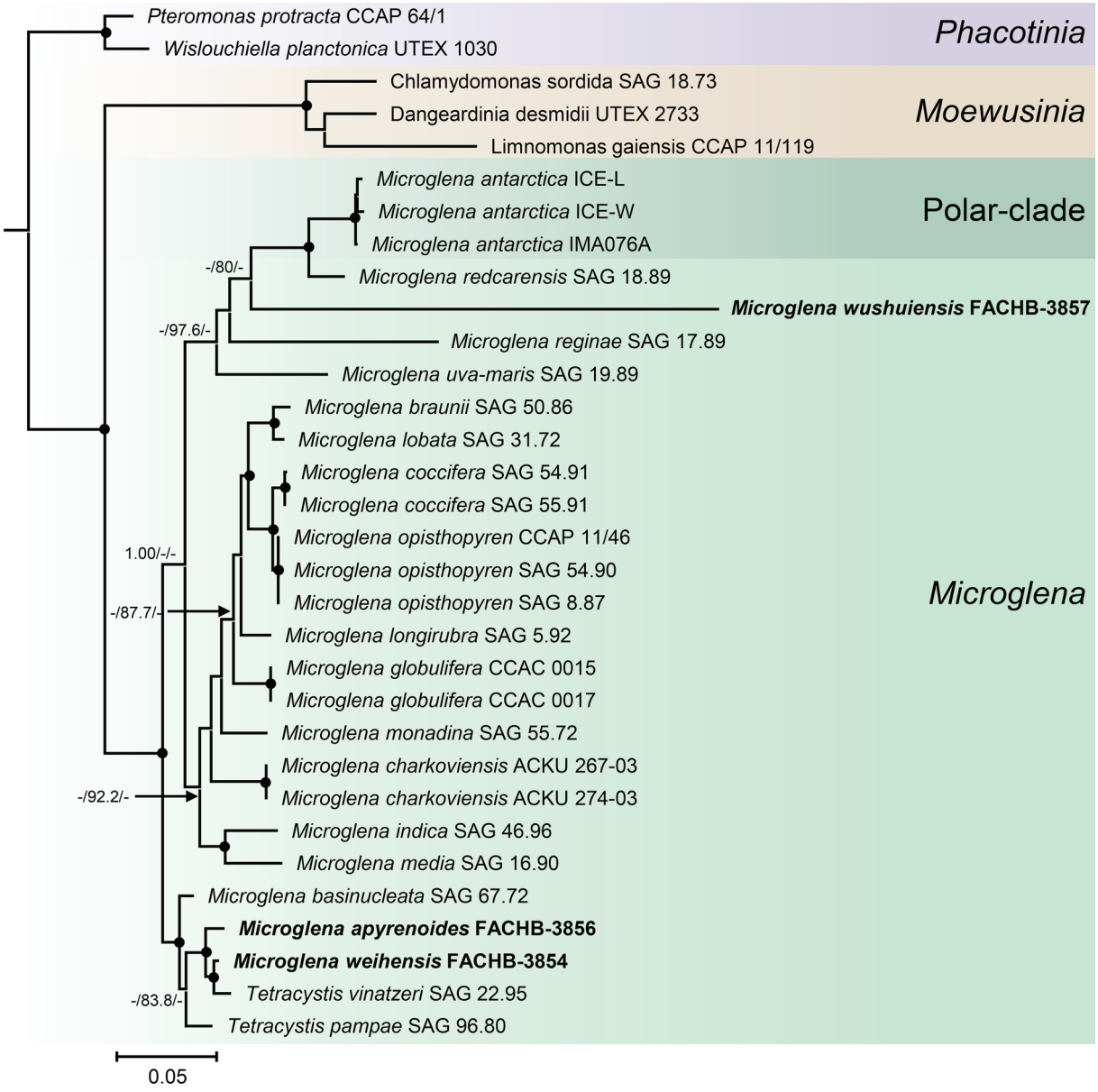


Figure 2. Maximum likelihood tree of *Microglena* based on concatenated 18S and ITS rDNA sequences with two taxa representing the *Phacotinia* clade sensu Nakada *et al.* (2008) as outgroups. Support values are shown only for branches with Bayesian posterior probabilities ≥0.95, SH-aLRT values ≥ 80% or bootstrap values ≥ 70% from left to right. A dot indicates that the corresponding branch received significant support from all three of the above. Newly sequenced taxa are represented in bold.

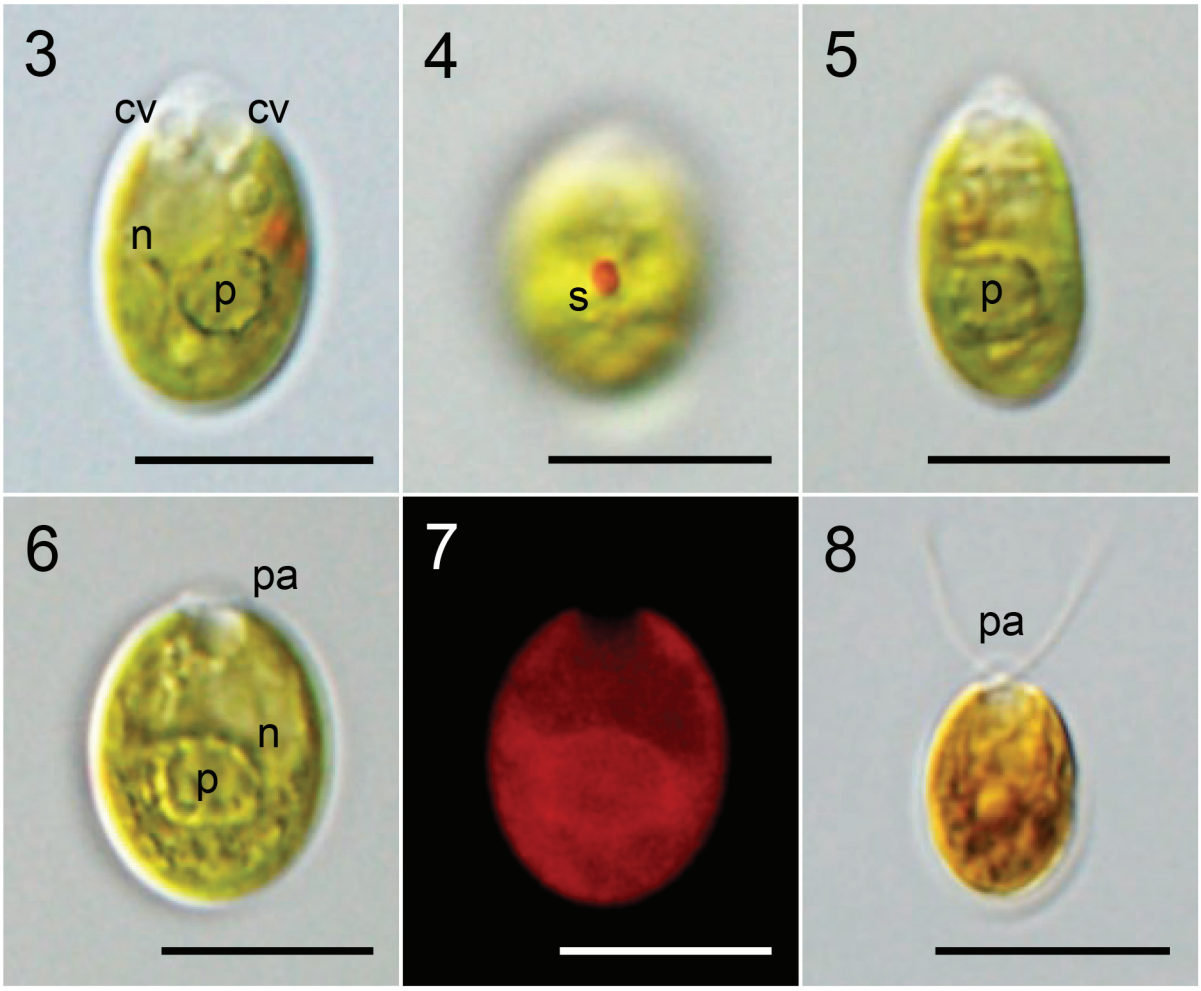
this species, the Wushui River of Shandong Province, China.

GenBank accession numbers: 18S rDNA: PX278842; ITS rDNA: PX396285.

***Microglena weihensis* Y.L.Chen *et* G.X.Liu *sp. nov.* (Figures 9–14)**

Description: Cells ovoid, ellipsoidal or ellipsoidal-cylindrical, sometimes asymmetrical, one side slightly convex, 8–12×5–8 µm. Cell wall moderately thick, with a trapezoidal papilla. Flagella as long as or slightly longer than the cell. Chloroplast deep cup-shaped, with indistinct incisions at basal and lateral parts; a single pyrenoid, ellipsoidal or spherical, with several discontinuous starch plates and penetrating thylakoid membrane bundles, in lateral thickenings of the chloroplast in the medial position. Stigma oval, in the anterior 1/2-1/4 of the cell. Two apical contractile vacuoles. Nucleus in the posterior lateral part of the pyrenoid, sometimes at the same height as the pyrenoid. Asexual reproduction by sporulation producing two or four zoospores, cell longitudinal division with or without rotation of protoplast by 90°. Sexual reproduction not observed.

Diagnosis: Similar to *Chlamydomonas kniepii* Moewus (1931), *C. sphagnophila* Pascher (1930), *C. moewusii* var. *eumetabletos* Gerloff (1940), *C. parvula* Gerloff (1940) and *C. elliptica* var. *britannica* Fritsch & John (1942) in cell shape, the position of the nucleus and chloroplast morphology, differing from *C. kniepii* by the presence



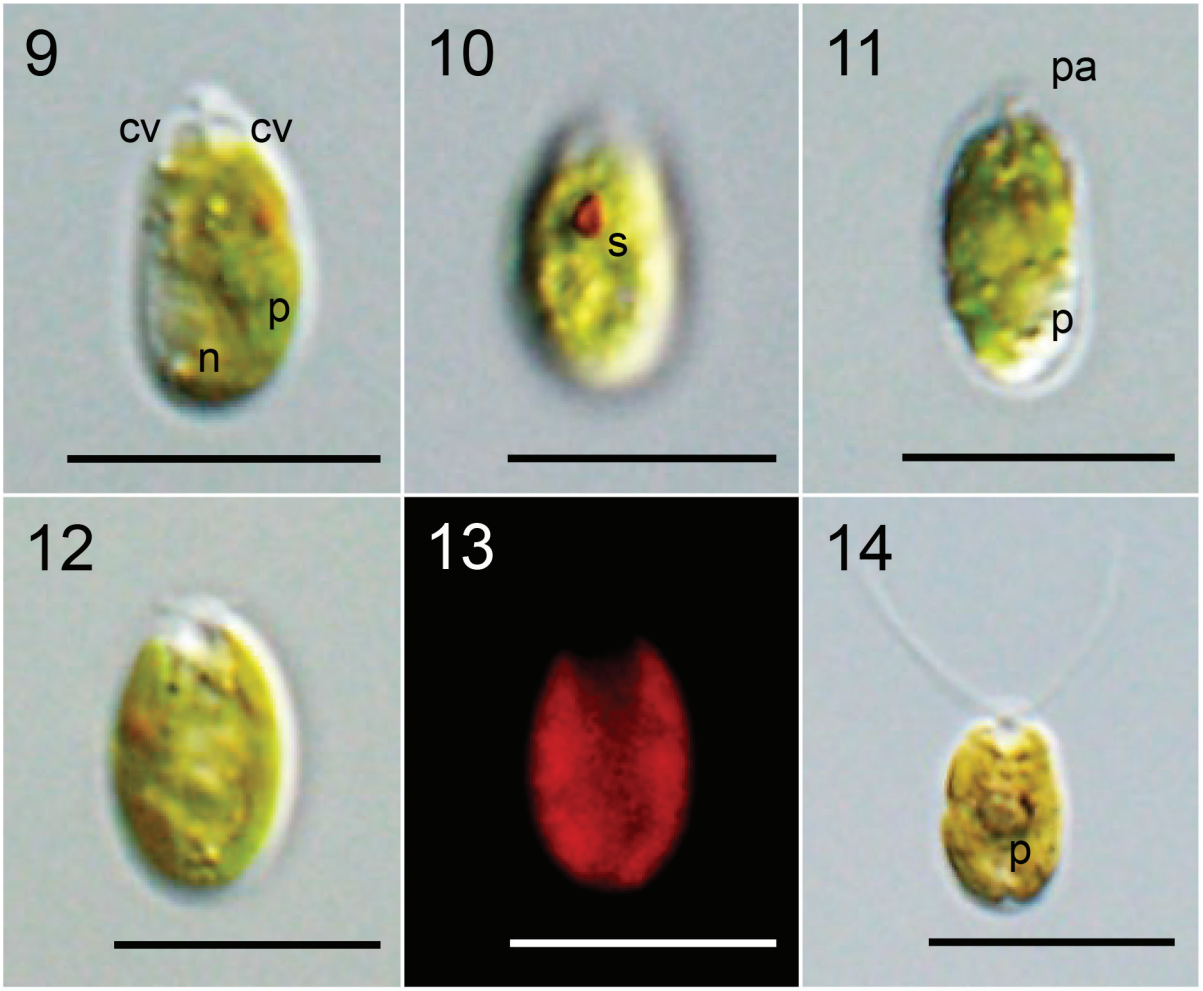
Figures 3–8. Morphology of *Microglena wushuiensis* FACHB-3857. **(3)** Optical section of a vegetative cell. **(4)** Surface view of a vegetative cell, showing a stigma. **(5)** A young vegetative cell, asymmetrical on the dorsal and ventral sides. **(6, 7)** Morphology of chloroplast structure, Figure 7, fluorescent image. **(8)** A vegetative cell fixed with Lugol's solution, showing two flagella. cv, contractile vacuole; n, nucleus (not conspicuous); p, pyrenoid; pa, papilla; s, stigma. Scale bars 10 µm.

of papilla and oval stigma, from *C. sphagnophila* by the presence of papilla, shorter flagella, oval stigma and slightly smaller cells, from *C. moewusii* var. *eumetabletos* by trapezoidal papilla, oval stigma and significantly smaller cells, from *C. parvula* by the presence of papilla and oval stigma, and from *C. elliptica* var. *britannica* by trapezoidal papilla, oval stigma and slightly smaller cells. Also similar to *C. oblongella* Lund (1947), but the stigma is not observed and the position and number of contractile vacuoles are uncertain in *C. oblongella*. Distinguished from other *Microglena* species by 18S rDNA sequence (PX278839) and at least two CBCs in conserved regions of ITS-2 sequence (Table S1).

Holotype: HBI WEHA-1 (formaldehyde-fixed material), collected by Yangliang Chen, Jul 5, 2022; permanently preserved in the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China.
Reference strain: The living strain is deposited in the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China (FACHB) as FACHB-3854.
Type locality: Weihe River, Weifang City, Shandong Province, China (36°19'7.20"N, 119°28'45.54"E).
Habitat: planktic
Etymology: The specific epithet reflects the type locality of this species, the Weihe River of Shandong Province, China.
GenBank accession numbers: 18S rDNA: PX278839; ITS rDNA: PX396282.

***Microglena apyrenoides* Y.L.Chen *et* G.X.Liu *sp. nov.* (Figures 15–20)**
Description: Cells ellipsoidal-cylindrical or broadly ellipsoidal, sometimes asymmetrical with one side flattened and the other slightly convex, 9.5–14.5×5–10.5 µm. Cell wall moderately thick, with a low trapezoidal papilla. Flagella as long as or slightly shorter than the cell. Chloroplast deep cup-shaped, with obvious incisions at basal and lateral parts, sometimes divided into several lobes; pyrenoids absent. Stigma elliptical, in the

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Figures 9–14. Morphology of *Microglena weihensis* FACHB-3854. **(9)** Optical section of a vegetative cell. **(10)** Surface view of a vegetative cell, showing a stigma. **(11)** Optical section of a vegetative cell, showing a papilla. **(12, 13)** Morphology of chloroplast structure, Figure 13, fluorescent image. **(14)** A vegetative cell fixed with Lugol's solution, showing two flagella. cv, contractile vacuole; n, nucleus (not conspicuous); p, pyrenoid; pa, papilla; s, stigma. Scale bars 10 µm.

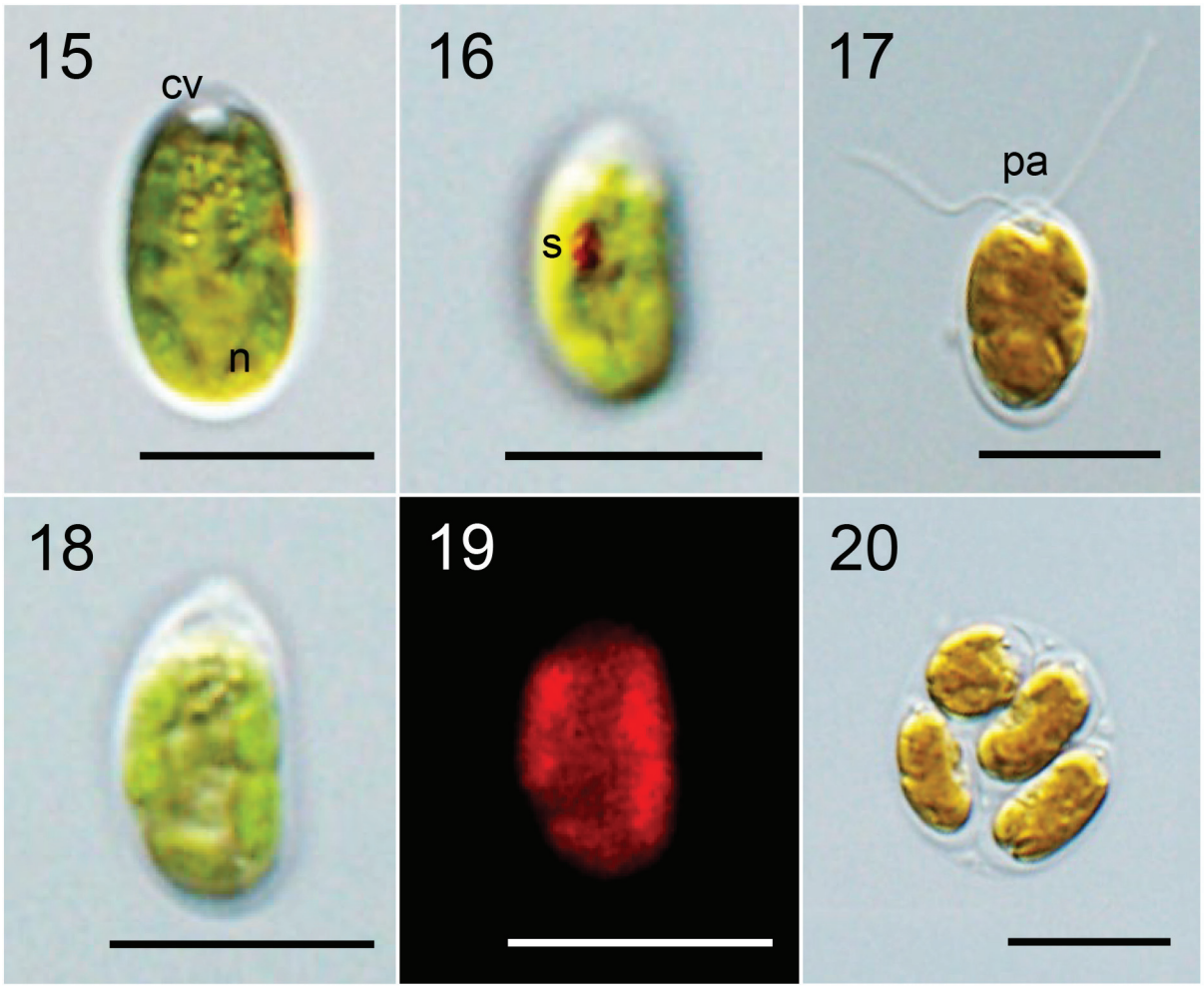
anterior ½–¼ of the cell. Two apical contractile vacuoles. Nucleus at the basal part of the cell. Asexual reproduction by sporulation producing two or four zoospores, cell longitudinal division without rotation of protoplast by 90°. Sexual reproduction not observed.

Diagnosis: Similar to *Mictoglena basinucleata* according to having a pyrenoid matrix without starch sheaths covered, however, differing from *M. basinucleata* distinctly smaller cell size and five CBCs in ITS-2 conserved region. Distinguished from other *Microglena* species by 18S rDNA sequence (PX278841) and at least two CBCs in conserved regions of ITS-2 sequence (Table S1).

Holotype: Specimen HBI R-4 (formaldehyde-fixed material), collected by Yangliang Chen, Mar 30, 2022; permanently preserved in the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China.
Reference strain: The living strain is deposited in the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China (FACHB) as FACHB-3856.
Type locality: A nameless little river, Xiantao City, Hubei Province, China (30°25'34.04"N, 113°8'45.32"E). Habitat: planktic
Etymology: The specific epithet reflects the absence of a pyrenoid in this species.
GenBank accession numbers: 18S rDNA: PX278841; ITS rDNA: PX396284.

Discussion

Phylogenetic analyses based on 18S rDNA and concatenated 18S and ITS rDNA showed these three strains belong to the genus *Microglena* (Figures 1–2). In terms of the CBC, a maximum of one hCBC exists among different strains of the same *Microglena* species; by contrast, independent *Microglena* species differ by at least one CBC. A previous study has also shown that in the vast

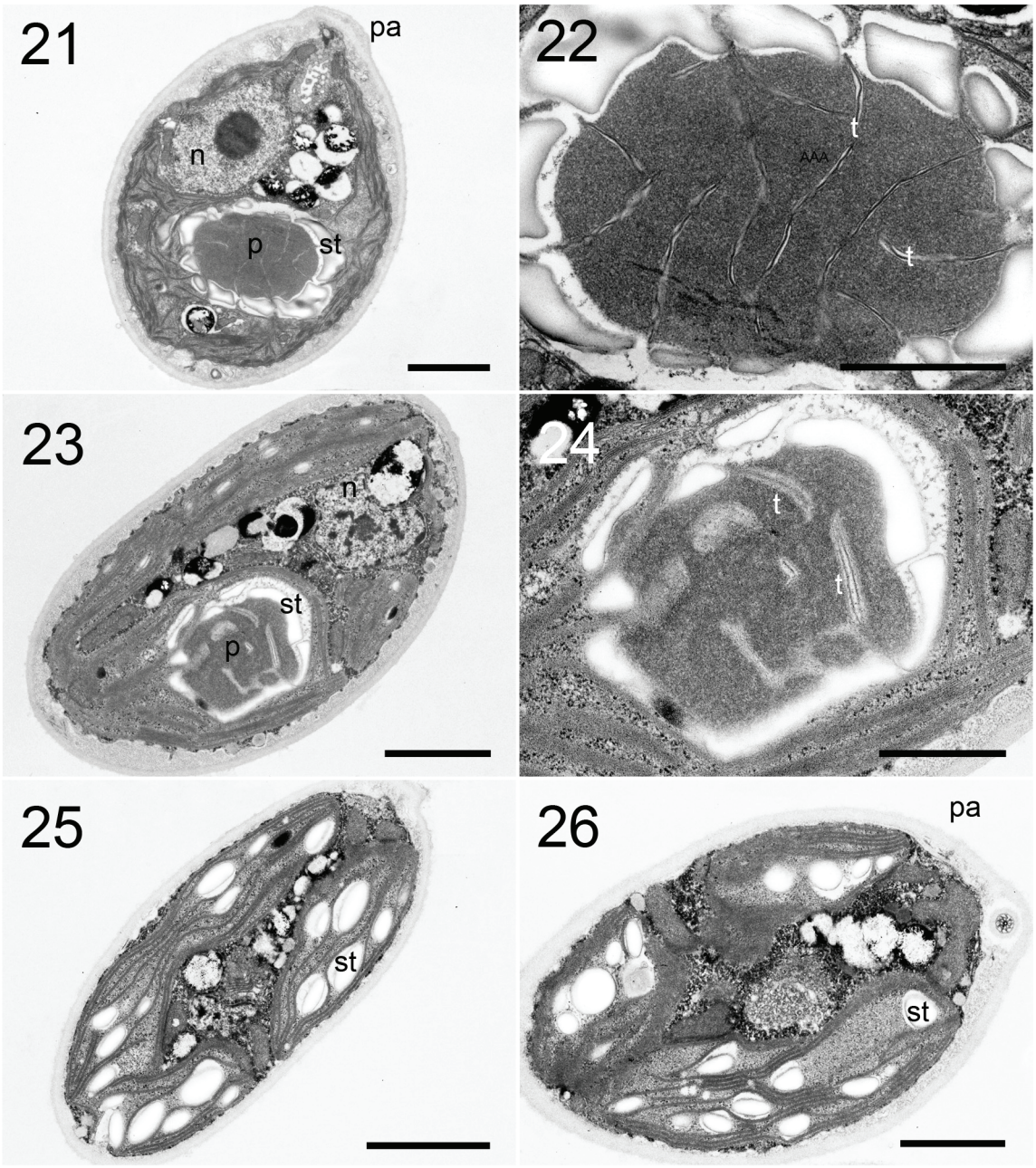


Figures 15–20. Morphology of *Microglena apyrenoides* FACHB-3856. **(15)** Optical section of a vegetative cell. **(16)** Surface view of a vegetative cell, showing a stigma. **(17)** A vegetative cell fixed with Lugol's solution, showing two flagella and a papilla. **(18, 19)** Morphology of chloroplast structure, Figure 19, fluorescent image. **(20)** Sporangium with four zoospores. cv, contractile vacuole; n, nucleus (not conspicuous); pa, papilla; s, stigma. Scale bars 10 µm.

majority of cases, one CBC is sufficient to distinguish closely related species (Müller *et al.*, 2007). Compared to 15 reported *Microglena* species, our three strains vary by at least two CBCs (Table S1), suggesting they are not the same as any previously described *Microglena* species. The three strains also displayed at least two CBCs among themselves, further demonstrating that they represent three independent species.

Our phylogenetic analyses also displayed that the strains FACHB-3854 and FACHB-3856 are closely related to *Tetracystis pampae* SAG 96.80 and *T. vinatzeri* SAG 22.95 (Figure 2). Both *T. pampae* and *T. vinatzeri* share some morphological similarities with *Microglena* species, such as having a parietal, hollow spherical chloroplast with incisions, an elliptical pyrenoid surrounded by starch grains and two contractile vacuoles (Brown & Bold, 1964; Ettl & Gärtner, 1987; Massalski *et al.*, 2001). However, the key point is that all described *Tetracystis* species are characterized by coccoid (i.e., immobile without flagella) vegetative cells. By contrast, our three strains have biflagellate vegetative cells. This fundamental difference means that FACHB-3854 and FACHB-3856 are not the same as *T. pampae* or *T. vinatzeri*. Besides, FACHB-3854 and FACHB-3856 differ from the two *Tetracystis* strains by at least one CBC, indicating that our two strains are not identical to SAG 96.80 or SAG 22.95. The type species of *Tetracystis*, *T. aeria*, was reassigned into *Chlorococcum*, and the genus *Tetracystis* has been abandoned (Watanabe & Lewis, 2017). Based on the phylogenetic positions of SAG 96.80 and SAG 22.95, the two species *T. pampae* and *T. vinatzeri* should perhaps be transferred into *Microglena*. This also means the generic concept of *Microglena* may need to be thoroughly redefined. However, during certain stages of their growth, flagellated species also become non-motile and highly resemble *Tetracystis* species in terms of cell shape, chloroplast and pyrenoid. Based on this, as well as the close phylogenetic relationship between these two strains and *Microglena* species, we cannot rule out the possibility that SAG 96.80 and SAG 22.95 actually represent two flagellated *Microglena* species, but they were misidentified as *Tetracystis* species. A detailed

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Figures 21–26. Transmission electron microscope micrographs of the four strains. **(21, 22)** *Microglena wushuiensis* FACHB-3857; Figure 21, longitudinal section of a vegetative cell; Figure 22, details of the pyrenoid structure. **(23, 24)** *Microglena weihensis* FACHB-3854; Figure 23, longitudinal section of a vegetative cell; Figure 24, details of the pyrenoid structure. **(25, 26)** *Microglena apyrenoides* FACHB-3856, both longitudinal sections of vegetative cells. n, nucleus; p, pyrenoid; pa, papilla; st, starch; t, thylakoid membrane. Scale bars 2 µm (a, c, e-h), 1 µm (b, d).

examination for the two strains is required before a conclusion can be drawn regarding this speculation.

The chloroplast morphology of all three strains is cup-shaped without a thick basal part, which is highly similar to the group *Chlamydella* of *Chlamydomonas sensu* Ettl (1983). However, in terms of overall morphological characteristics, none of these three algal strains fit any known species (see Diagnosis). For *Gloeomonas* which was characterized by the absence of pyrenoids, some species of this genus have been found to actually possess pyrenoid matrixes without associated starch grains (Nozaki *et al.*, 2010). However, no potential pyrenoid matrix protruding inward into the stroma regions of the chloroplast, similar to *Gloeomonas* species, was observed in FACHB-3856. This strain was recognized as a species lacking pyrenoids. Combined with morphological comparisons with those similar *Chlamydomonas* species as well as some species belonging to *Microglena* and *Chloromonas*, the three strains were confidently identified as three new species in this study.

Regarding the relationship between the three new species and other *Microglena* species, *M. weihensis* and *M. apyrenoides* are closer to *M. basinucleata*, as shown by the concatenation analysis (Figure 2), and the comparison

results of CBCs and *p*-distances also revealed smaller differences among these three *Microglena* species (Table S1). Furthermore, the feature of the nucleus located in the basal position of the cells is only shared by these three species within *Microglena*, which further implies the close relationship among the three species. The 18S rDNA phylogeny showed that *M. wushuiensis* is closely related to *M. redcarensis* and the Polar-clade (Figure 1), but this relationship was not significantly supported by the concatenated 18S+ITS rDNA phylogeny (Figure 2). Analyses of CBCs and *p*-distances displayed significant differences between *M. wushuiensis* and other *Microglena* species (Table S1). Therefore, *M. wushuiensis* may represent a distinct lineage within *Microglena*.

The previous study by Demchenko *et al.* (2012) considered that the most striking features of *Microglena* species are various chloroplast structures and their embedded pyrenoids, and three morphotypes were delineated within *Microglena* based on the two features. According to the chloroplast morphology, all three new *Microglena* species are similar to the morphotype II, characterized as having a cup-shaped chloroplast without a thick basal part, however, they differ from morphotype II in pyrenoids. In comparison to typical species belonging to morphotype II of *Microglena*, *M. wushuiensis* and *M. weihensis* have a spherical or ellipsoidal pyrenoid rather than a half-looped one (Figures 3, 6, 11, 14). Particularly noteworthy is that the pyrenoids are completely lost in *M. apyrenoides*, indicating this species may have undergone a unique evolutionary process compared to other *Microglena* species. The mechanism of CO_2-concentrating in *M. apyrenoides* is likely more similar to typical *Chloromonas* species without pyrenoids. Moreover, morphological variability in the position of the pyrenoid during ontogenesis was mentioned by Demchenko *et al.* (2012). In mature vegetative cells of *M. braunii* and *M. opisthopyren* (both species belonging to morphotype II), a pyrenoid is located on the lateral part of the chloroplast, whereas in young cells it is situated at the basal part of the chloroplast, which results in a thickening basal region of the chloroplast, with a morphology more closely resembling type I. However, no change of pyrenoid position was detected during observations for cultures of *M. wushuiensis* and *M. weihensis*. In summary, based on the discovery of these three new species, our findings demonstrate that the morphological diversity of chloroplasts and associated pyrenoids in the genus *Microglena* is evidently higher than previously recognized.

Microglena was considered as a genus harboring high ecological diversity. In addition to species inhabiting freshwater and marine environments, many *Chlamydomonas*-like strains isolated from snow or ice fields are phylogenetically close to *Microglena* species. Four strains collected from the Antarctic region, CCCryo309-06, ICE-L, ICE-L and IMA076A, are recognized as a new species *M. antarctica* by Trentin *et al.* (2022). As shown by phylogenetic analysis in this study (Figure 1) and Demchenko *et al.* (2012), two strains sampled from Svalbard CCCryo002b-99 and CCCryo038-99 (Leya, 2004) as well as the strain Antarctic 2E9 should also be identified as *M. antarctica*. In addition, *C. kuwadae* (strain NIES-968) and an unidentified *Chlamydomonas* sp. (strain ARC), also belonging to the Polar-clade, may represent new species or new combinations within *Microglena*, which needs further research.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Brown, R. M., & Bold, H. C. (1964). *Comparative studies of the algal genera Tetracystis and Chlorococcum*. University of Texas Publication 6417.

Buchheim, M. A., Michalopulos, E. A., & Buchheim, J. A. (2001). Phylogeny of the Chlorophyceae with special reference to the Sphaeropleales: A study of 18S and 26S rDNA data. *Journal of Phycology*, *37*(5), 819–835.

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, *25*(15), 1972–1973.

Demchenko, E., Mikhailyuk, T., Coleman, A. W., & Pröschold, T. (2012). Generic and species concepts in *Microglena* (previously the *Chlamydomonas monadina* group) revised using an integrative approach. *European Journal of Phycology*, *47*(3), 264–290.

Eddie, B., Krembs, C., & Neuer, S. (2008). Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. *Marine Ecology Progress Series*, *354*, 107–117.

Ehrenberg, C. G. (1832). Über die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlichen Akademie der Wissenschaften, Berlin*, *1831*, 1–154.

Ehrenberg, C. G. (1834). Dritter Beitrag zur Erkenntnis großer Organisation in der Richtung des kleinsten Raumes. *Abhandlungen der Königlichen Akademie der Wissenschaften, Berlin*, *1833*, 145–336.

Ettl, H. (1965). Beitrag zur Kenntnis der Morphologie der Gattung *Chlamydomonas* Ehrenberg. *Archiv für Protistenkunde*, *108*, 271–420.

Ettl, H. (1970). *Die Gattung Chloromonas Gobi emend. Wille* (Beihefte zur Nova Hedwigia, Vol. 34). Nova Hedwigia.

Ettl, H. (1976). *Die Gattung Chlamydomonas Ehrenberg* (Beihefte zur Nova Hedwigia, Vol. 49). Nova Hedwigia.

Ettl, H. (1978). Xanthophyceae, 1. Teil. In H. Ettl, J. Gerloff, & H. Heynig (Eds.), *Süßwasserflora von Mitteleuropa* (Vol. 3, No. 1). Gustav Fischer.

Ettl, H. (1983). Chlorophyta I. Phytomonadia. In H. Ettl, J. Gerloff, & D. Mollenhauer (Eds.), *Süßwasserflora von*

Fottea, 26(2), 156–169, 2026
doi: 10.5507/fot.2026.005

Mitteleuropa (Vol. 9). Gustav Fischer.

Ettl, H., & Gärtner, G. (1987). Taxonomic and nomenclatural changes and descriptions of new taxa of the Tetrasporales, Chlorococcales and Gloeodendrales (Chlorophyta, Chlamydophyceae and Chlorophyceae). *Nova Hedwigia*, *44*, 509–517.

Fritsch, F. E., & John, R. P. (1942). An ecological and taxonomic study of the algae of British soils. II. Consideration of the species observed. *Annals of Botany*, *6*(3), 371–395.

Fulnečková, J., Hasíková, T., Fajkus, J., Lukešová, A., Eliáš, M., & Sýkorová, E. (2012). Dynamic evolution of telomeric sequences in the green algal order Chlamydomonadales. *Genome Biology and Evolution*, *4*(3), 248–264.

Gerloff, J. (1940). Beiträge zur Kenntnis der Variabilität und Systematik der Gattung *Chlamydomonas*. *Archiv für Protistenkunde*, *94*, 311–502.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies, assessing the performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321.

Hoshaw, R. W., & Rosowski, J. R. (1973). Isolation and purification 3: Methods for microscopic algae. In J. R. Stein (Ed.), *Handbook of phycological methods. Culture methods and growth measurements* (pp. 53–67). Cambridge University Press.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*(6), 587–589.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780.

Klebs, G. A. (1896). *Ueber die Fortpflanzungs-Physiologie der niederen Organismen, der Protobionten. Specieller Theil. Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*. Gustav Fischer.

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetics Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, *41*(12), Article msae263.

Leya, T. (2004). *Feldstudien und genetische Untersuchungen zur Kryophilie der Schneealgen Nordwestspitzbergens*. Shaker.

Liu, C., Huang, X., Wang, X., Zhang, X., & Li, G. (2006). Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. *Phycologia*, *45*(2), 190–198.

Lund, J. W. G. (1947). Observations on soil algae III. Species of *Chlamydomonas* Ehr. in relation to variability within the genus. *New Phytologist*, *46*(2), 185–194.

Massalski, A., Mrozinska, T., & Olech, M. (2001). Ultrastructural observations on five pioneer soil algae from ice denuded areas (King George Island, West Antarctica). *Polar Bioscience*, *14*, 61–70.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*(5), 1530–1534.

Moewus, F. (1931). Neue Chlamydomonaden. *Archiv für Protistenkunde*, *75*, 284–296.

Müller, T., Philippi, N., Dandekar, T., Schultz, J., & Wolf, M. (2007). Distinguishing species. *RNA*, *13*(9), 1469–1472.

Nakada, T., Misawa, K., & Nozaki, H. (2008). Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18S rRNA phylogenetic analyses. *Molecular Phylogenetics and Evolution*, *48*(1), 281–291.

Nakada, T., & Tomita, M. (2014). Light microscopy and phylogenetic analyses of *Chlamydomonas* species (Volvocales, Chlorophyceae). II. Molecular phylogeny, secondary structure of ITS-2, cell morphology, and nomenclature of *Microglena opisthopyren* and *M. media* comb. nov. *Acta Phytotaxonomica et Geobotanica*, *65*(2), 67–73.

Nakada, T., Takahashi, S., & Tomita, M. (2018). *Microglena redcarensis* sp. nov. (Volvocales, Chlorophyceae), a brackish water chlamydomonad with contractile vacuoles. *Phycological Research*, *66*(4), 310–317.

Nakazawa, A., & Nozaki, H. (2004). Phylogenetic analysis of the tetrasporalean genus *Asterococcus* (Chlorophyceae) based on 18S ribosomal RNA gene sequences. *Journal of Japanese Botany*, *79*(4), 255–261.

Nozaki, H., Misumi, O., & Kuroiwa, T. (2003). Phylogeny of the quadriflagellate Volvocales (Chlorophyceae) based on chloroplast multigene sequences. *Molecular Phylogenetics and Evolution*, *29*(1), 58–66.

Nozaki, H., Nakada, T., & Watanabe, S. (2010). Evolutionary origin of *Gloeomonas* (Volvocales, Chlorophyceae), based on ultrastructure of chloroplasts and molecular phylogeny. *Journal of Phycology*, *46*(1), 195–200.

Pascher, A. (1927). *Die Süsswasser-Flora Deutschlands, Österreichs und der Schweiz. Heft 4: Volvocales = Phytomonadinae*. Gustav Fischer.

Pascher, A. (1930). Neue Volvocalen (Polyblepharidinen-Chlamydomonadinen). *Archiv für Protistenkunde*, *69*, 103–146.

Pawlowski, J., Holzmann, M., Fahrni, J. F., & Hallock, P. (2001). Molecular identification of algal endosymbionts in large miliolid foraminifera: 1. Chlorophytes. *Journal of Eukaryotic Microbiology*, *48*(3), 362–367.

Reuter, J. S., & Mathews, D. H. (2010). RNAstructure: Software for RNA secondary structure prediction and analysis. *BMC Bioinformatics*, *11*, Article 129.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542.

Siebert, S., & Backofen, R. (2005). MARNA: Multiple alignment and consensus structure prediction of RNAs based on sequence structure comparisons. *Bioinformatics*, *21*(16), 3352–3359.

Skuja, H. (1956). Taxonomische und biologische Studien über das Phytoplankton schwedischer Binnengewässer. *Nova Acta Regiae Societatis Scientiarum Upsaliensis*, *19*(3), 1–404.

Stein, F. (1878). *Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischere Reihenfolge bearbeitet. III. Abtheilung. Die Naturgeschichte der Flagellaten oder Geisselinfusorien*. Verlag von Wilhelm Engelmann.

Trentin, R., Negrisolo, E., Moschin, E., Veronese, D., Cecchetto, M., & Moro, I. (2022). *Microglena antarctica* sp. nov. a new antarctic green alga from Inexpressible Island (Terra Nova Bay, Ross Sea) revealed through an integrative approach. *Diversity*, *14*(5), Article 337.

Watanabe, M. M., & Hiroki, M. (1997). *NIES-collection. List*

of strains: Microalgae and protozoa (5th ed.). National Institute for Environmental Studies.

Watanabe, S., & Lewis, L. A. (2017). Phylogenetic interpretation of light and electron microscopic features of selected members of the phylogroup Moewusinia (Chlorophyceae), with new generic taxonomy. *Phycologia*, *56*(3), 329–353.

Wolf, M., Friedrich, J., Dandekar, T., & Müller, T. (2005). CBCAnalyzer: Inferring phylogenies based on compensatory base changes in RNA secondary structures. *In Silico Biology*, *5*(3), 291–294.