Fottea, 26(1), 107–118, 2026
doi: 10.5507/fot.2025.019

Czech Phycological Society

New taxa of the genus *Coelastrella* (Scenedesmaceae, Sphaeropleales) from Warren Prairie Natural Area, Arkansas, USA

Sara Ambrocio Paque, Marvin W. Fawley and Karen P. Fawley

Abstract

Coelastrella Chodat is a green alga frequently found in subaerial habitats that is typically characterized by ribbed cell walls. During our research on the soil algal community of sodic-saline slicks in Warren Prairie Natural Area, Arkansas, USA, we found several algal strains with the features of *Coelastrella*. We also expanded our study to include soil samples from sites in the barrens grassland soil around the sodic-saline slicks, which also yielded *Coelastrella* strains. There were also other strains that lacked ribbed cell walls but could be placed in the genus *Coelastrella* using nuclear ribosomal 18S and ITS1–5.8S–ITS2 DNA sequences. Analysis of ribosomal ITS sequences, plastid *tufA* sequences, and morphology revealed four distinct taxa among these strains, including two new species, *C. salsodica* and *C. subsphaerica*, and two new varieties of *C. yingshanensis*, *C. yingshanensis* var. *apiculata* and *C. yingshanensis* var. *ovata*. *C. salsodica*, *C. subsphaerica*, and *C. yingshanensis* var. *apiculata* were only found in the sodic-saline slicks, whereas *C. yingshanensis* var. *ovata* was only found in the barrens grassland soil. To date, no other *Coelastrella* species have been found in these sites.

Keywords

Barrens grassland soil; Chlorophyta; *Coelastrella*; New species; Phylogeny; Sodic-saline soil; Chlorophyceae

Natural Sciences and Mathematics, University of the Ozarks, Clarksville, Arkansas, USA

Corresponding author
Karen P. Fawley
kpfawley@gmail.com

Article History
Received July 16, 2025
Revised December 9, 2025
Accepted December 10, 2025
Published online April 1, 2026

Supplementary Material
Available online

How to cite
Ambrocio Paque, S., Fawley, M. W., & Fawley, K. P. (2026). New taxa of the genus *Coelastrella* (Scenedesmaceae, Sphearopleales) from Warren Prairie Natural Area, Arkansas, USA. *Fottea, 26*(1), 107–118. https://doi.org/10.5507/fot.2025.019

Introduction

The genus *Coelastrella* (Scenedesmaceae) was established by Chodat (1922) and includes spherical or ellipsoidal unicellular coccoid green algae with ribbed cell walls. The chloroplast is light green with a single pyrenoid. Reproduction is by the production of four to eight autospores. Most *Coelastrella* species have been found in subaerial environments, such as soil, rock surfaces, road pavement, block dividers, solid floors, and tree bark (Abe *et al.*, 2004; Hanagata, 2001; Kawasaki *et al.*, 2020; Krivina *et al.*, 2024; Novakovskaya *et al.*, 2021; Tschaikner *et al.*, 2008). However, recent studies have also described *Coelastrella* species from freshwater systems (Suarez-Montes *et al.*, 2022; Wang *et al.*, 2019, 2021). *Coelastrella* has a worldwide distribution, ranging from the boreal zone to equatorial areas (Wang *et al.*, 2019) and in extreme environments (Boutarfa *et al.*, 2022; Goecke *et al.*, 2020). *Coelastrella* has been used for many applications in the following processes: biodiesel production (Angelaalincy *et al.*, 2023), polluted water treatment (Beaulier *et al.*, 2024; Reddy *et al.*, 2023), production of lipids and carotenoids (Lee *et al.*, 2021; Saito *et al.*, 2023), and the phyco-fabrication of nanoparticles (Choudhary *et al.*, 2023).

We isolated strains of *Coelastrella* from Warren Prairie Natural Area (WPNA) (Bradley and Drew Counties) in southeast Arkansas as part of a larger project to characterize the soil crust algal communities in WPNA as described in Thermozier *et al.* (2024). This natural area is a mosaic of sodic-saline slicks that form flat, crusty depressions in a central area with a zone of lichens and a few rare angiosperms, and an outer

© Czech Phycological Society (2026)

zone of large algal mats. These slicks are surrounded by prairie grasses which are flanked by pine flatwoods (*Pinus taeda* Linnaeus) with understory layers of dwarf palmetto (*Sabal minor* (Jacquin) Persoon). The edge of the central area of these sodic-saline slicks is home to the rare, diminutive vascular plant, *Geocarpon minimum* Mackenzie (Caryophyllaceae), which is a federally protected threatened species (Foti & Witsell, 2013).

For this study, we evaluated *Coelastrella* strains isolated from soil samples from the slicks and the surrounding barrens grassland soil in WPNA. We characterized the strains using light microscopy and DNA sequence analysis of the nuclear 18S ribosomal RNA gene, ribosomal RNA ITS regions, and the plastid *tufA* gene. Here we describe new taxa of *Coelastrella* from the collection of strains from these WPNA sites.

Material and Methods

Sample collection sites: Initial surface soil samples were collected from a sodic-saline slick in Warren Prairie Natural Area, Bradley County, Arkansas, USA on 21 February 2016 and 7 December 2017 as described in Thermozier *et al.* (2024). Additional samples were collected from different sites in WPNA on 6 November 2021. One new site was from the margin of a different sodic-saline slick (WPSS1; 33°34'25" N, 91°58'51" W), and two nearby sites were located within the surrounding barrens grassland (WPPS2; 33°34'27" N, 91°58'51" W, and WPPS3; 33°34'57" N, 91°59'0.1" W). Soil samples from WPSS1, WPPS2, and WPPS3 were collected for chemical analyses, which were performed by Ward Laboratories, Kearney, Nebraska, USA (see Supplementary Table S1). Soil chemistry analysis results from a soil collection on 7 December 2017 are reported in Thermozier *et al.* (2024).

Strain isolation: Algal strains were isolated by the procedure given in Thermozier *et al.* (2024). For each soil sample, 0.5 g of soil was mixed with 50 ml of Woods Hole modified (WH+) medium (Fawley *et al.*, 2013) in a graduated cylinder and shaken well. The mixture was allowed to settle for 10 s, and 100 µl of the supernatant was spread on an agar Petri plate of WH+ medium. The plates were incubated under cool-white fluorescent light at room temperature until colony growth appeared. Individual algal colonies were picked from the plates and maintained on agar slants.

Light microscopy: A Nikon E600 microscope equipped with 60× (NA 1.4) and 100× (NA 1.4) objectives and differential interference contrast (DIC) optics was used for light microscopy (Nikon, Melville, New York, USA). Digital images and measurements were acquired with an Olympus SC180 camera system with CellSens imaging software (Olympus America, Center Valley, Pennsylvania, USA). To clearly visualize ribs on the cell wall surface, pressure was applied to the cover glass on a wet mount of the cells until the walls were ruptured.

Strain identification and phylogenetic analysis: Strains were identified to genus level using light microscopy and 18S rDNA sequence analysis (Fawley & Fawley, 2020). Strains were grown in either liquid or agar WH+ medium at room temperature or in a 20 ºC chamber with fluorescent lighting. DNA was extracted from cultures using the technique of Fawley & Fawley (2004), which was modified by the addition of an initial step to disperse possible mucilage that might surround the cells. After harvesting the cells by centrifugation, 200 µl of extraction buffer was added and the tube was agitated in a MiniBeadBeater (BioSpec Products, Inc., Bartlesville, Oklahoma, USA) at high speed for 10 to 15 s. The cells were pelleted again by centrifugation and new buffer was added. DNA was then extracted by the normal method of Fawley & Fawley (2004). Polymerase Chain Reaction amplification of the 18S rDNA gene used the primers18L-X and NSI-X with PCR conditions described in Fawley & Fawley (2004). The 18S rDNA was sequenced from only four strains. BlastN (Ye *et al.*, 2006) was used to determine possible genus-level affiliation based on the 18S rDNA sequence in GenBank.

For identification at the species level, we PCR amplified the ribosomal RNA ITS region, including ITS1, 5.8S rDNA, and ITS2, using the primers ITS1 (White *et al.*, 1990) and ITS-BR (Johnson *et al.*, 2007) with annealing temperature at 61 ºC. The plastid *tuf*A gene was also amplified using the primers of Famà *et al.* (2002) with annealing at 56.5 ºC. With the aid of published plastome sequences from the Scenedesmaceae, we designed the new primers, *tufA* F-Sara (5'ATTGGTCAYGTAGACCATG3') and *tufA* R-Sara (5'CGNCCWCCTTCACGAATWGC3'), that produce a larger amplicon (1167 bp) from the *tufA* gene of *Coelastrella* (and likely other Scenedesmaceae) than the primers of Famà *et al.* (2002). The annealing temperature for the new primer pair was 56.5 ºC. Sequencing of the *tufA* locus employed the same primers used in PCR. All DNA sequencing was performed by Sequetech (Mountain View, California, USA) and sequence reads were joined and inspected using the Staden Package 2.0.0b8 (Bonfield *et al.*, 1995).

All new DNA sequences were entered in GenBank, with accession numbers PV778597–PV778600 for 18S rDNA, PV289229–PV289239 for ribosomal ITS1–5.8S–ITS2, and PV779343–PV779352 for *tufA* sequences (Supplementary Material Table S2).

Sequences of *tufA* and the ITS region from our strains and published sequences were aligned with sequences from the closely related genera *Pectinodesmus* E. Hegewald *et al.*, *Tetradesmus* G. M. Smith and *Asterarcys* Comas Gonzáles using MEGA X (Kumar *et al*., 2018). Maximum Likelihood Phylogenetic analyses were performed with IQ-TREE 2 (Minh *et al.,* 2020) using the GTR+F+R4 model and mixture models with MIX{SYM,TIM2e+FO}+I+G as the best fit models for the ITS region and MIX{TIM2+FO,K81u+FO,K81u+FO}+I for *tufA*, as selected by MixtureFinder (Ren *et al.*, 2025) incorporated in IQ-TREE v2.4.0. Bootstrap analysis with 500 iterations was used to evaluate the resulting tree structures. We employed the Maximum Likelihood method for phylogenetic analysis because bootstrap support is more conservative than Bayesian posterior probability (Holder & Lewis, 2003) and the mixture model is easy to apply in IQ-TREE v2.4.0. The *tufA* analysis was performed using the 876 base alignment that was generated with the Famà *et al*. (2002) primers. Phylogenetic trees were visualized with iTOL (Letunic & Bork, 2019) and finished using Inkscape (Inkscape.org) and Paint.NET (getpaint.net).

Secondary structure of ITS2: ITS2 secondary structures were generated using RNAfold (Gruber *et al.*, 2008) and visualized with Forna (Kerpedjiev *et al.,* 2015) incorporated in the ViennaRNA Web Services (Gruber *et al.*, 2008).

Results

Most of the WPNA *Coelastrella* strains (Figs 1–28) possess the features of the genus, with spherical or ellipsoidal unicellular coccoid cells with ribbed walls. Chloroplasts are parietal and cup-shaped. Reproduction is by the production of two to eight autospores. However, several strains lacked the ribs on the cell walls that are characteristic of the genus *Coelastrella*. The morphological features of the WPNA strains and *C. yingshanensis* Qinghua Wang *et al.* (a close relative to some of our strains) are presented in Supplementary Material (Table S3). Based on the morphological and habitat differences, as well as the phylogenetic analysis of ribosomal ITS1–5.8S–ITS2 and plastid *tufA* DNA sequences, we propose the following new taxa of *Coelastrella*.

Descriptions of new taxa

***Coelastrella yingshanensis* var. *apiculata* Ambrocio Paque, M. W. Fawley *et* K. P. Fawley *var. nov.* (Figs 1–3)**

Description: Cells apiculate, 8.4 × 4.9 to 18.5 × 15.5 µm in liquid culture (Fig. 1) and 10.6 × 6.6 to 18.5 × 15.5 µm on agar culture (Fig. 2). Reproduction by the production of 2–8 autospores (Figs 1, 2). Mature cells rounded to (mostly) apiculate, with distinctive thickenings at the poles (Figs 1, 2). Young cells and autospores much thinner than the mature cells (Fig. 1). Well-developed ribs present on the cell wall (Fig. 3).

Holotype: Freeze-dried sample STAR037517 placed in the Arkansas State University Herbarium (STAR), State University, Arkansas, USA.
Type locality: Warren Prairie Natural Area, Bradley County, Arkansas, USA; 33°34'57" N, 91°58'59" W.
Habitat: Soil surface; biotic crust at edge of sodic-saline slicks.
Etymology: Named for the apiculate ends of the cells.
Reference strain: Strain WPW3 12/7-21, deposited in the CCMP collection of the National Center for Marine Algae and Microbiota (NCMA), East Boothbay, Maine, USA as CCMP3762.
GenBank accession numbers: PV778600 (nuclear 18S rDNA); PV289229 (nuclear ribosomal ITS1–5.8S–ITS2); PV779348 (plastid *tufA*).

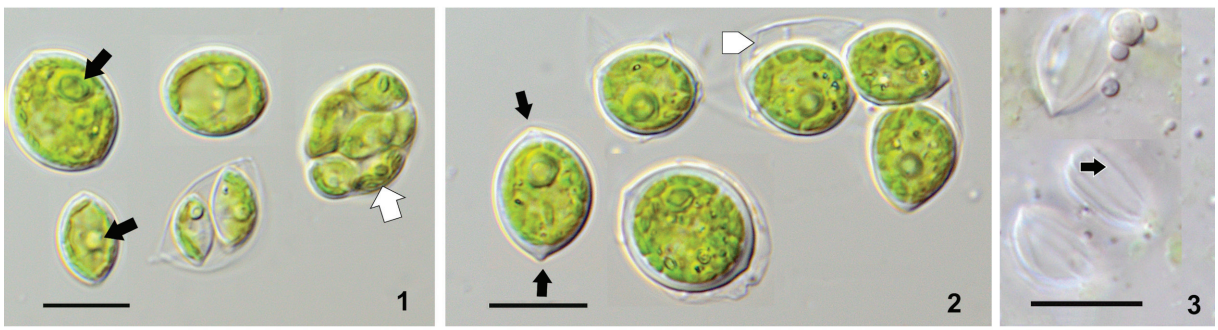
***Coelastrella yingshanensis* var. *ovata* Ambrocio Paque, M. W. Fawley *et* K. P. Fawley *var. nov.* (Figs 4–13)**

Description: Cells mostly ovate, 6.6 × 4.3 to 16.5 × 15.8 µm in liquid culture (Figs 4, 7, 11) and 6.2 × 4.9 to 16.7 × 12.3 µm on agar culture (Figs 5, 8, 12). Reproduction by the production of 2–4 (16) autospores (Figs 4, 5, 7, 8, 11, 12). Mature cells oval, sometimes with polar thickenings (Fig. 8). Younger cells and autospores are mostly oval (Figs 7, 8, 11). Some autospores and young cells have papilla-like ends (Fig. 5, 8). Cells typically have fine ribs coming from both poles in the cell wall (Figs 6, 9, 13).

Holotype: Freeze-dried sample STAR 037518 placed in the Arkansas State University Herbarium (STAR), State University, Arkansas, USA.
Type locality: Warren Prairie Natural Area, Bradley County, Arkansas, USA; 33°34'27" N, 91°58'51" W.
Habitat: Soil surfaces of barrens grassland that surround the sodic-saline slicks.
Etymology: Named from its shape, which is often slightly ellipsoidal.
Reference strain: Strain WPPS2 11/6-27 deposited in the NCMA at Bigelow Laboratories as CCMP3759.
Reference DNA sequences: ITS region: PV289232; *tufA*: PV779343.

***Coelastrella subsphaerica* Ambrocio Paque, M. W. Fawley *et* K. P. Fawley *sp. nov.* (Figs 14–19)**

Description: Cells are nearly spherical, measuring 6.0 × 10.8 to 16.5 × 15.8 µm in liquid medium (Figs 14, 17) and 6.2 × 4.9 to 16.7 × 12.3 µm in agar culture (Figs 15, 18). Cells have a prominent pyrenoid in both liquid and agar medium. Asexual reproduction is by

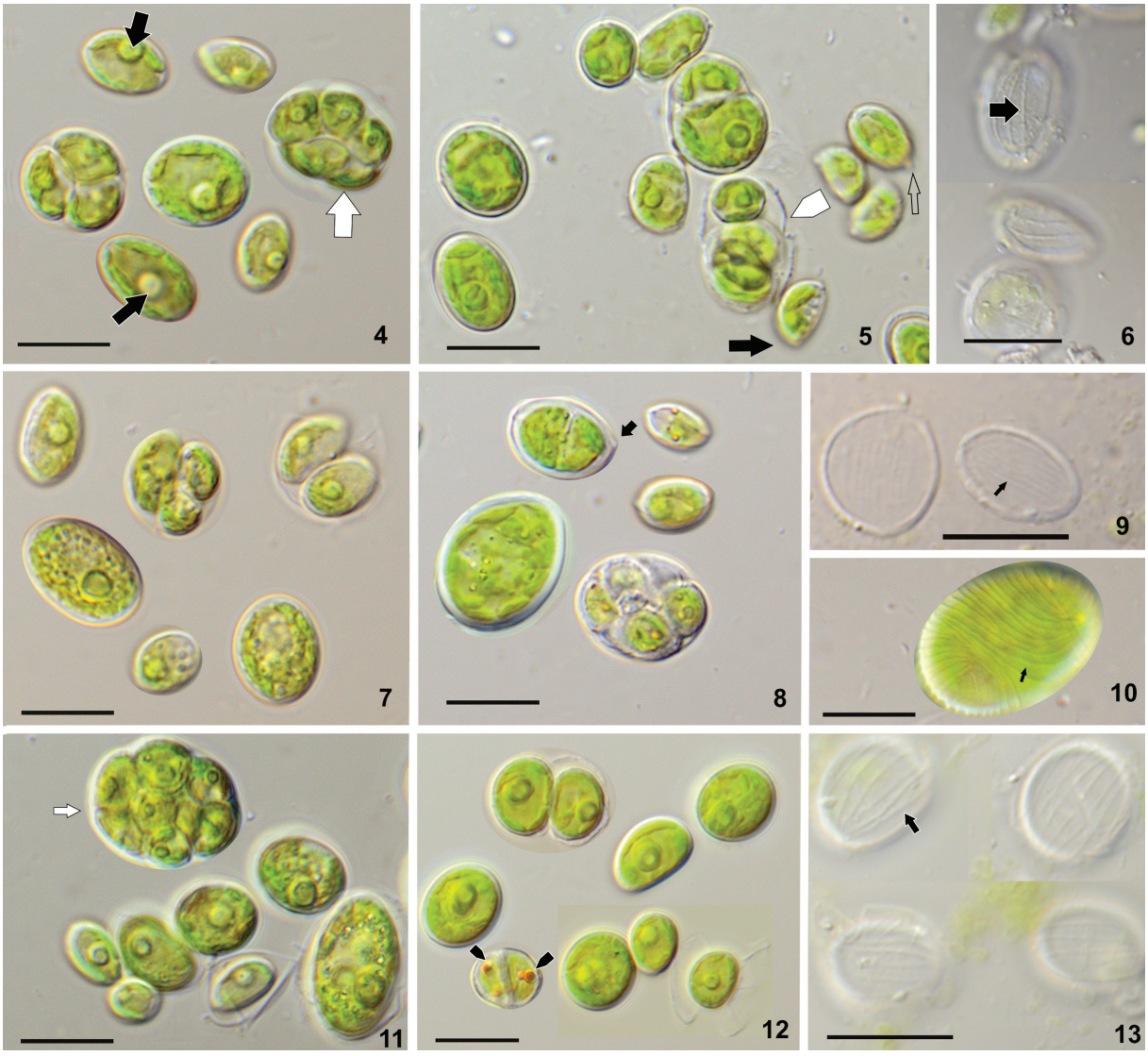


Figs 1–3. Light micrographs of *Coelastrella yingshanensis* var. *apiculata*, strain WPW3 12/7-21. **(1)** Cells grown in liquid medium; black arrows show the pyrenoid; an autosporangium with eight autospores is shown with a white arrow; the mother cell wall around two autospores is indicated by a white arrowhead. **(2)** Cells grown on agar medium; black arrows show polar thickenings; the mother cell wall is shown with a white arrowhead. **(3)** Presence of ribs (black arrow) and apiculate ends in the cell wall. Scale bars 10 µm.

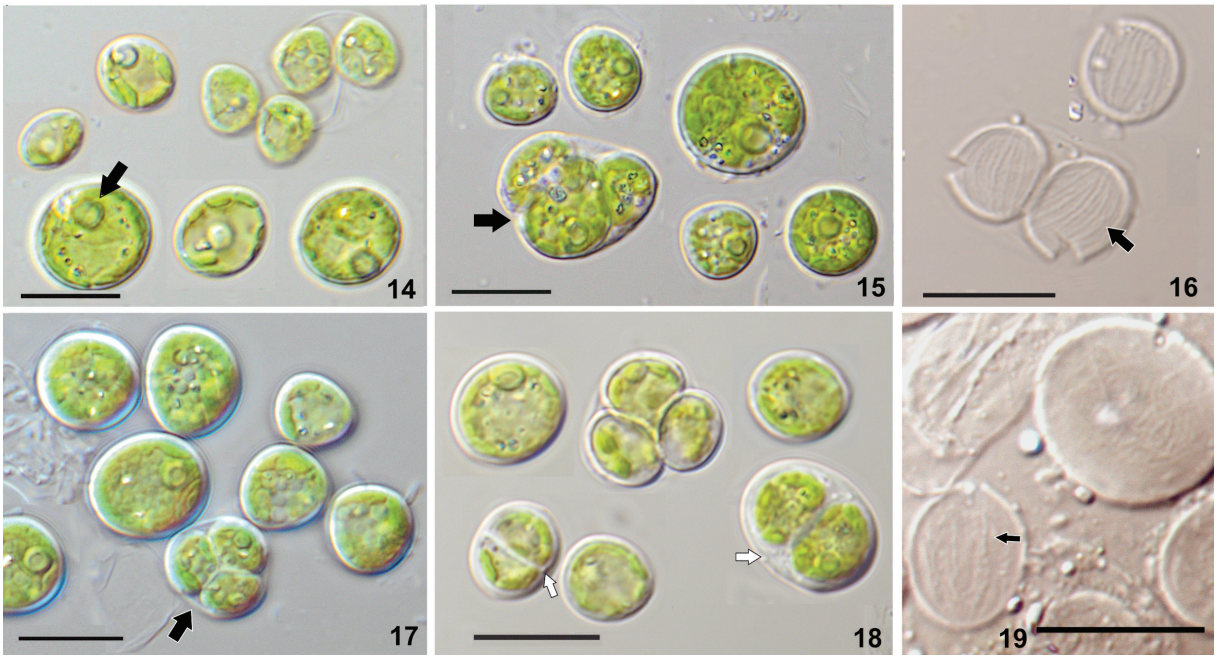
2–4 (8) autospores with angular to ovate shapes (Figs 14, 15, 17–18). Fine ribs were present in the cell wall (Figs 16, 19) of most cells; however, in at least one strain, the cell ribs are not easily visible with LM (Fig. 19).

Holotype: Freeze-dried sample STAR37514 placed in the Arkansas State University Herbarium (STAR), State University, Arkansas, USA.
Type locality: Warren Prairie Natural Area, Bradley County, Arkansas, USA; 33°34'57" N, 91°58'59" W.
Habitat: Soil surfaces of sodic-saline slicks.
Etymology: Named from its shape, which is often just slightly ellipsoidal.
Reference strain: Strain WPW3 12/7-8, deposited in the NCMA at Bigelow Laboratories as CCMP3761.
Reference DNA sequences: PV289230 (nuclear ribosomal ITS1–5.8S–ITS2 region); PV779350 (plastid *tufA*).

***Coelastrella salsodica* Ambrocio Paque, M. W. Fawley *et* K. P. Fawley *sp. nov.* (Figs 20–28)**
Description: Cells are nearly spherical, measuring 4.5 × 4.0 to 17.1 × 16.7 µm in liquid medium (Figs 20, 23, 26) and 4.3 × 3.1 to 14.2 × 13.7 µm on agar medium (Figs 21, 24, 27). Cells have a distinctive pyrenoid when grown in liquid medium (Figs 20, 23, 26), but when grown on agar medium, the pyrenoid is not visible (Figs 21, 24, 27). Asexual reproduction is by



Figs 4–13. Light micrographs of *Coelastrella yingshanensis* var. *ovata*, strains WPPS2 11/6-27 **(4–6)**, WPPS3 11/6-14 **(7–10)** and WPPS3 11/6-16 **(11–13)**. **(4)** Strain WPPS2 11/6-27 cells from liquid medium; black arrows show pyrenoids; the white arrow indicates an autosporangium with eight autospores. **(5)** Cells grown on agar medium; the white arrowhead indicates the mother cell wall broken by growth of autospores; black arrows indicate the papilla-like end of some autospores; the open arrow indicates a papilla. **(6)** Cells show fine ribs in the cell wall (black arrow). **(7)** Strain WPPS3 11/6-14 cells in liquid medium. **(8)** Cells of strain WPPS3 11/6-14 grown on agar medium; cells have an oval shape with thick cell walls and some polar thickening shown with a black arrow. **(9)** Thin ribs coming from both poles in the cell wall (small black arrows). **(10)** An unusually large cell with thin, curved ribs in the cell wall and visible without clearing the cells. **(11)** Strain WPPS3 11/6-16 grown in liquid medium; reproduction by 8–16 autospores is shown with a white arrow. **(12)** Orange pigments (likely in lipid bodies) are present in some cells grown on agar medium (black arrowheads). **(13)** Cells have fine ribs in the cell wall (black arrow). Scale bars 10 µm.



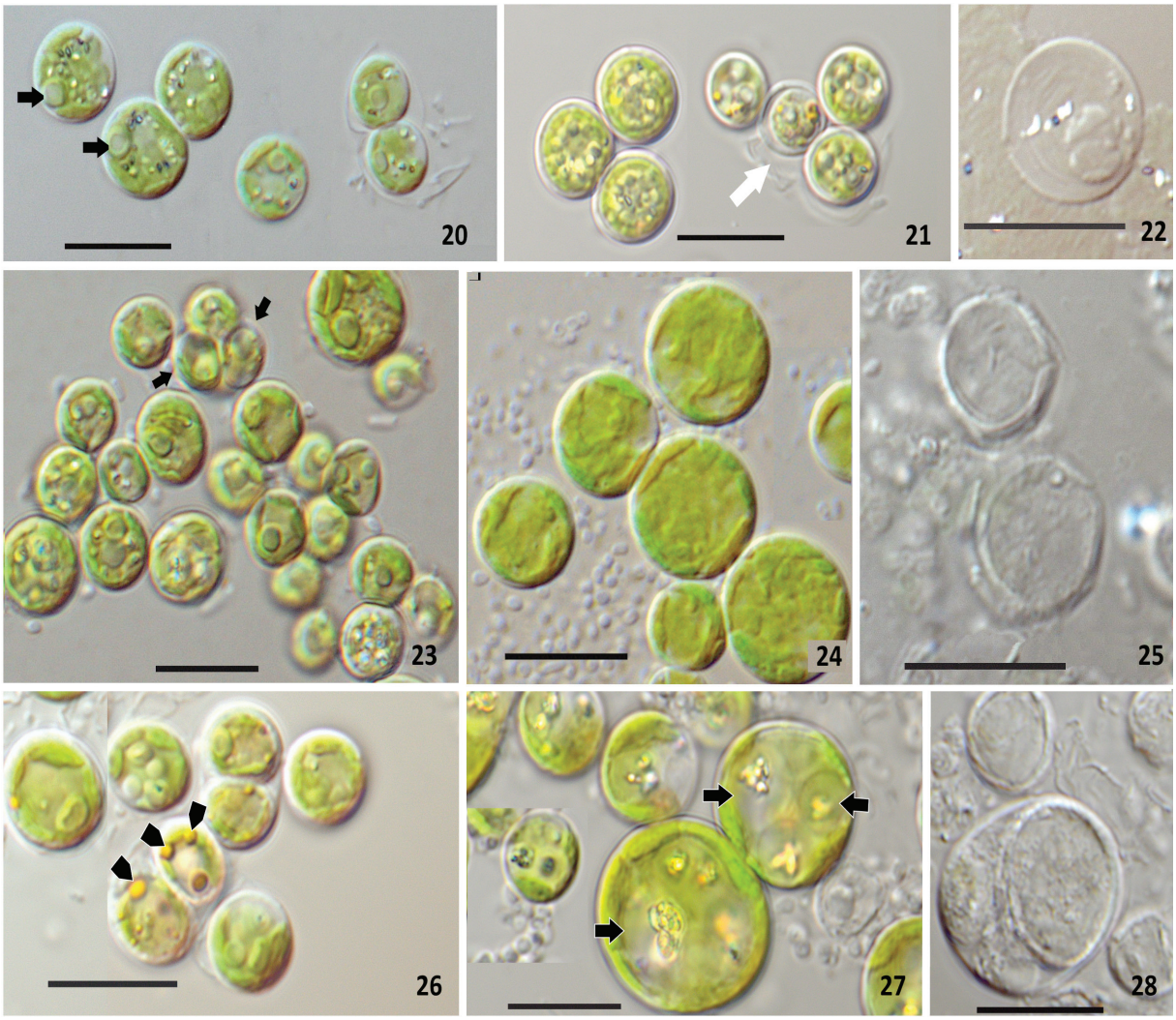
Figs 14–19. Light microscopy of *Coelastrella subsphaerica sp. nov.*, strains WPPS1 11/6-6 **(14–16)** and WPW3 12/7-8 **(17–19)**. **(14)** Strain WPPS1 11/6-6 cells from liquid medium; black arrows show pyrenoids. **(15)** Cells grown on agar medium; reproduction by autospores is shown with a black arrow. **(16)** Ribs in the cell wall shown with a black arrow. **(17)** Strain WPW3 12/7-8 from liquid medium; black arrow shows the autospores. **(18)** Strain grown on agar medium; white arrows show the thickened cell walls of autospore formation. **(19)** Thin ribs are shown with a black arrow. Scale bars 10 µm.

the production of 2–4 (8) autospores with ellipsoidal to nearly spherical shapes. No cell wall ribs were visible by light microscopy (Figs 22, 25, 28). When grown on agar medium, the cell walls are thick and some cells have orange structures that are likely lipid bodies (Figs 21, 27). Cells are often attached in packets or short chains (Figs 20, 23). Phylogenetic analyses of ITS1+5.8S+ITS2 and *tufA* both indicate that this species represents a new lineage within the genus *Coelastrella*.

Holotype: Freeze-dried sample STAR037516 placed in the Arkansas State University Herbarium (STAR), State University, Arkansas.
Type locality: Warren Prairie Natural Area, Bradley County, Arkansas, USA; 33°34'57"; N 91°59'00" W.
Habitat: Soil; sodic-saline slick.
Etymology: Named from its habitat of a sodic-saline slick.
Reference strain: Strain WPWO2 12/7-9, deposited in the NCMA at Bigelow Laboratories as CCMP3760.
Reference sequences: PV289231 (nuclear ribosomal ITS1–5.8S–ITS2 region); PV779352 (plastid *tufA*).

Soil analysis: Soil chemistry analysis of the soils in WPNA revealed large differences between the sodic--saline slick and the barrens grassland soil (Table 1, Supplementary Material Table S1). The pH of the sodic-saline slicks was 7.5 and 8.9, whereas the barrens grassland sites WPPS2 and WPPS3 had pH values of 5.9 and 5.5. The sodium ion concentration was also dramatically different, with very high levels in the slicks compared to the barrens. Calcium ions were only slightly higher in the slicks, whereas the metal ions Mn and especially Fe were higher in the barrens soil than they were in the slicks. Nitrate levels were much lower in the slicks. Values are all consistent with the characteristics of the sodic-saline slicks and surrounding soils of WPNA found in other studies (summarized in U.S. Fish and Wildlife Service, 1993). These results indicate that the soil of the sodic-saline slick sites and the barrens grassland sites are very different habitats for soil algae.

Phylogenetic analyses: Maximum Likelihood analyses of the ITS1–5.8S–ITS2 region (Fig. 29) produced nearly identical results with both the GTR+F+R4 model and the mixture model. The recently described species, *C. cogersae* Suarez-Montes, Borrell *et* J. M. Rico (Suarez-Montes *et al*., 2022), is resolved as a separate early-branching lineage of a well-supported group that includes both *Coelastrella* sensu Wang *et al.* (2019) and strains that have been placed in the genus *Asterarcys*. The genus *Coelastrella* sensu Wang *et al.* (2019) is resolved as a single lineage; however, this lineage is without bootstrap support. *Coelastrella* contains multiple lineages, including one that consists of the "core" group of the genus (Wang *et al.*, 2019) with the type species, *C. striolata.* Two groups of our WPNA strains were allied with *Coelastrella* senus Wang *et al.* (2019) at different positions in the phylogenetic analysis. One group was allied with *C. yingshanensis* and includes



Figs 20–28. Light micrographs of *Coelastrella salsodica sp. nov.*, strains WPWO2 12/7-8 **(20–22)**, WPWO2 12/7-9 **(23–25)**, and WPW3 12/7-5 **(26–28)**. **(20)** Strain WPWO2 12/7-8 cells from liquid medium; pyrenoids are shown with black arrows. **(21)** Strain WPWO2 12/7-8 cells grown on agar medium; white arrow shows the mother cell wall; no pyrenoids are visible in these cells. **(22)** Strain WPWO2 12/7-8 showing no ribs in the cell wall. **(23)** Strain WPWO2 12/7-9 cells grown in liquid medium; the black arrow shows the reproduction by autospores. **(24)** Cells of strain WPWO2 12/7-9 on agar medium; no pyrenoids are visible in these cells. **(25)** Cells of strain WPWO2 12/7-9 showing no visible ribs in the cell wall. **(26)** Strain WPW3 12/7-5 cells from liquid medium; black arrowheads show the presence of orange pigments in presumed lipid bodies. **(27)** Cells of strain WPW3 12/7-5 grown on agar medium with unusual, large vacuoles and refractile inclusions (black arrows); no pyrenoids are visible in these cells. **(28)** Strain WPW3 12/7-5 with ribs not visible in the cell wall. Scale bars 10 µm.

Table 1. Soil chemistry of samples for the Warren Prairie Natural Area sites used in this study. Only selected results are shown; for full results see Supplementary Material (Table S1). Fe includes both ions. Data for WPW and WPWO are from Thermozier *et al.* (2024).

Site Code(s)	pH	Na^{+}	Ca^{++}	Fe	Mn^{++}	NO_3^{-}	Notes
WPW and WPWO	7.3	326 ppm	314 ppm	6.4 ppm	7.1 ppm	2.2 ppm	Top soil from edge of sodic-saline slick
WPSS1	8.9	506 ppm	562 ppm	6.1 ppm	3.1 ppm	0.8 ppm	Top soil from edge of sodic-saline slick
WPPS2	5.9	44 ppm	278 ppm	56.9 ppm	14.5 ppm	6.0 ppm	Barrens grassland top soil
WPPS3	5.5	37 ppm	309 ppm	59.6 ppm	8.9 ppm	3.8 ppm	Barrens grassland top soil

the three new taxa, *C. yingshanensis* var. *apiculata*, *C. yingshanensis* var. *ovata*, and *C. subsphaerica*. The other group comprises the new species, *C. salsodica,* a well-supported lineage that is not clearly allied with any other *Coelastrella* strains. Analysis of ITS sequence data strongly supports the new species, *C. subsphaerica* and *C. salsodica*, and provides some evidence for the new varieties of *Coelastrella yingshanensis.* The ITS1–5.8S–ITS2 regions of the new varieties vary only slightly from *C. yingshanensis* (*C. yingshanensis* var. *ovata* with 5 substitutions and *C. yingshanensis* var. *apiculata* with 7 substitutions), whereas the new species, *C. subsphaerica,* differs from *C. yingshanensis* by 10–11 substitutions.

Monophyly of *Coelastrella* and strains that have been placed in *Asterarcys* is supported by analyses of *tufA* (Fig. 30). Unfortunately, a *tufA* sequence is not available for the new species, *C. cogersae*. The results of GTR+F+R4 and mixture models are once again very similar, with only somewhat different branching orders for some lineages, none of which have bootstrap support. Bootstrap support values from both analyses were all either identical or very similar. Both *tufA* analyses support the result from the ITS analyses that our WPNA strains are grouped into two separate lineages. Fig. 30 shows that *tufA* (at least this portion of *tufA*) is insufficiently variable to provide bootstrap support for any links between WPNA strains closely related to *C. yingshanensis*, but there are differences in *tufA* sequences that differentiate among some of these strains. The *tuf*A sequence does not differ from that of *C. subsphaerica*; however, there is one difference between *C. yingshanensis* and the variety *ovata* and two differences between *C. yingshanensis* and the new variety *apiculata*. The two new varieties have three differences between them. There are also minor differences in the *tufA* sequences of our WPNA strains in the new lineage, *C. salsodica*, which is well supported.

The ITS2 secondary structure analysis provides some results supporting the new taxa related to *C. yingshanensis.* Fig. 31 shows the comparison between the helix II of *C. yingshanensis* and those of *C. yingshanensis* var. *apiculata var. nov.*, *C. subpherica sp. nov.*, and *C. yingshanensis* var. *ovata var. nov.* In the stem of the helix II, there is a compensatory base change (CBC) between *Coelastrella yingshanensis* and *Coelastrella subspherica*, providing strong support for the new species. There were also hemi-CBCs (hCBCs) that were revealed by comparing *Coelastrella yingshanensis, Coelastrella yingshanensis* var. *apiculata*, *Coelastrella subspherica,* and *Coelastrella yingshanensis* var. *ovata*. These hCBCs are consistent with the establishment of the two new varieties.

The new taxa resulting from this study have some differences in habitats in WPNA. *Coelastrella salsodica, C. subsphaerica* and *C. yingshanensis* var. *apiculata* were only found in sodic-saline slicks, whereas *C. yingshanensis* var. *ovata* was found only in barrens grassland sites.

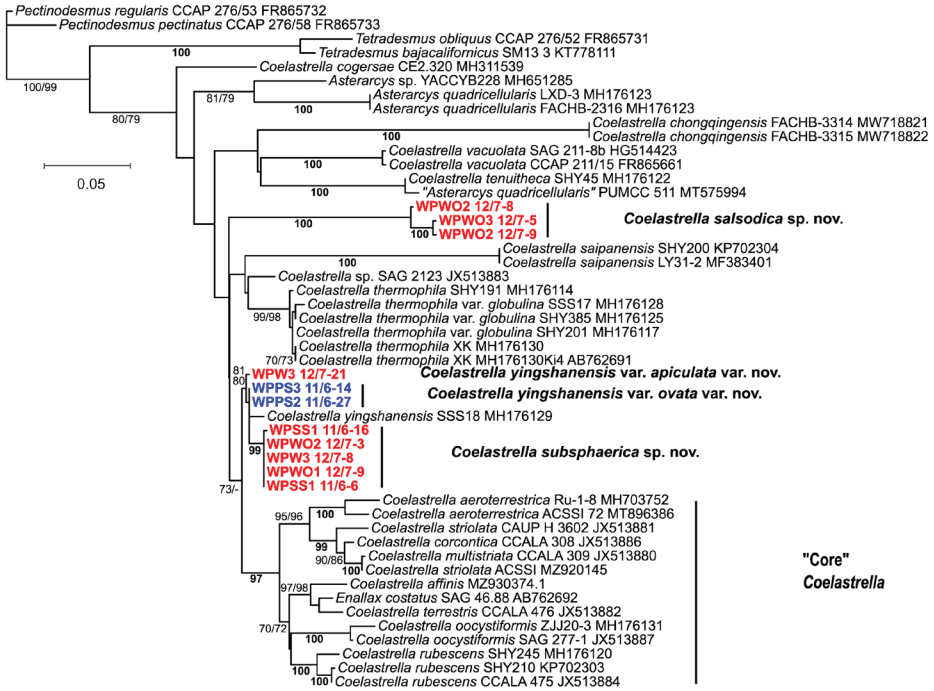


Fig. 29. Results of Maximum Likelihood phylogenetic analyses of nuclear ribosomal RNA ITS regions, including the 5.8S rDNA sequences. The tree produced using the mixture model is shown. Bootstrap values (70 or greater) are shown with the mixture model values first, followed by the GTR values; a number in bold font indicates that the branch had the same bootstrap value with both models. The strains in red text were isolated from sodic-saline slicks and those in blue text were from the surrounding barrens grassland soil.

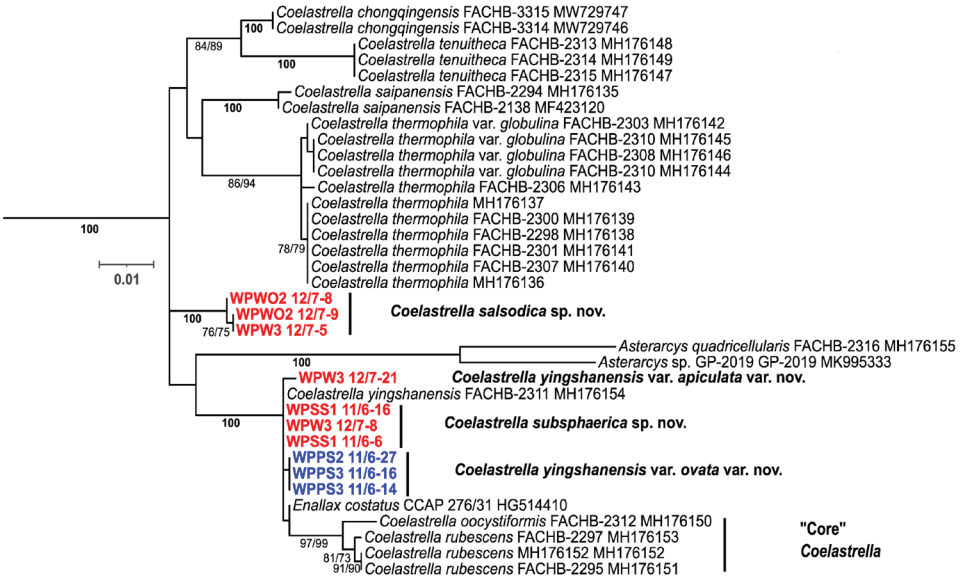


Fig. 30. Results of Maximum Likelihood phylogenetic analysis of plastid *tufA* DNA sequences. The tree produced using the mixture model is shown. Bootstraps and text colors as in Fig. 29.

Discussion

The taxonomy of the genus *Coelastrella* and related taxa in the Scenedesmaceae is fraught with confusion based on the use of simple morphological features to define new taxa. Kaufnerová & Eliáš (2013) provided a thorough overview of the taxonomy of *Coelastrella* in their study of the validity of the genus *Scotelliopsis* Vinatzer. Using phylogenetic analysis of 18S rDNA and ITS1–5.8S rDNA–ITS2 sequence data and ITS2 secondary structure, they determined that the genus *Scotelliopsis* should be abandoned and at least one species should be transferred to *Coelastrella*. Wang *et al.* (2019) provided a more comprehensive study of the genus *Coelastrella* using 18S, ITS region, and *tufA* DNA sequences, as well as morphology and habitat, to name new species and varieties of the genus. Wang *et al.* (2019) also discussed the possibility of including the genus *Asteracys* in the expanded genus *Coelastrella;* however, they concluded that the morphology of *Asteracys* differs significantly from that of *Coelastrella*. Retention of *Asterarcys* as a separate genus cannot be certain because no DNA sequence data are available for an authentic strain of the type species, *Asterarcys cubensis* Comas Gonzáles. Strains assigned to the genus *Enallax* Pascher also appear in various analyses of *Coelastrella*, and Wang *et al.* (2019) present an analysis of the possibility that *Enallax* should be merged into *Coelastrella*, but this is also hampered by a lack of type material.

Krivina *et al*. (2024) also present a thorough account of the taxonomic history of *Coelastrella* in their study of the new species, *C. affinis*. They evaluated the use of 18S rDNA and ITS2 sequence data and determined that 18S rDNA was not useful for species determination, but ITS2 can be used to identify all *Coelastrella* species.

In our view, Wang *et al*. (2019) provided a very good procedure for the description of new species and varieties in *Coelastrella*. Our philosophy for naming new species (Fawley *et al*., 2011) includes the use of multiple genes or DNA regions, preferably with some nuclear (e.g., ITS, including secondary structure) and some organellar (e.g., plastid *tufA*) data. Morphology and habitat can also be important. The more lines of evidence that support the hypothesis of a new species, the more likely that the new species represents an independent evolutionary unit (Fawley *et al.*, 2011).

Wang *et al*. (2019) used both ITS and *tufA* sequence data for their analysis, but they were unable to produce *tufA* sequences from some "Core" species using the Famà *et al.* (2002) primers. Our new *tufA* primers are likely to be useful for generating larger *tufA* sequences from throughout the Scenedesmaceae, including the "Core" *Coelastrella* species.

In this study, the results of phylogenetic analyses of sequence data from *tufA* and ITS are very similar as they pertain to our new species and varieties. The only major topological difference is the placement of the two *Asterarcys* strains that are at the base of the *Coelastrella* lineage in the ITS tree and immersed within *Coelastrella* in the *tufA* tree. Interestingly, Wang *et al*. (2019) found *Asterarcys* at the base of the lineage with *tufA*, but immersed within *Coelastrella* using combined 18S and ITS2. However, these differences are not significant, as there is no bootstrap support for either placement. In addition, the recently described species, *C. cogersae* Suarez-Montes, Borrell *et* J. M. Rico (Suarez-Montes *et al.*, 2022) is resolved in the ITS tree as a basal branch of the well-supported lineage

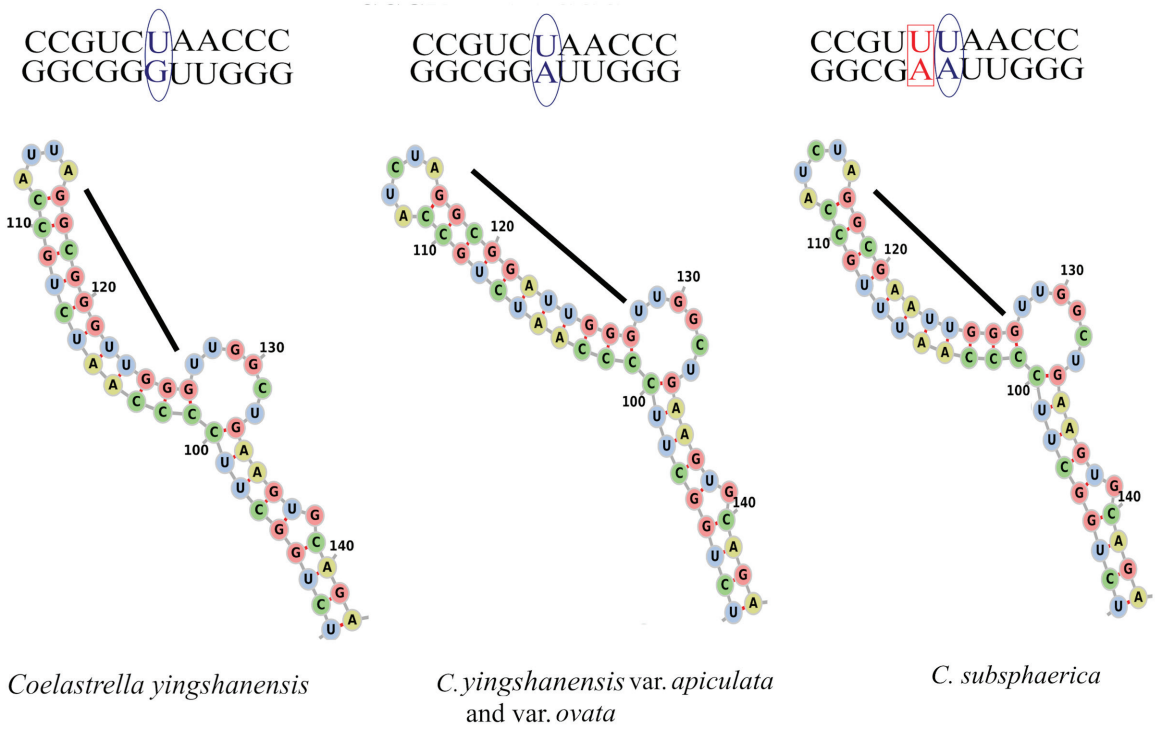


Fig. 31. Results of ITS2 secondary structure analysis. Comparison of helix II from ITS2 secondary structure of *Coelastrella yingshanensis*, to those of *C. yingshanensis* var. *apiculate var. nov.*, *C. yingshanensis* var. *ovata var. nov.* (the two varieties have identical helix II regions) and *C. subsphaerica sp. nov.* The site of a compensatory base changes (CBC) is in red font and a rectangle. Hemi-CBCs (hCBCs) are in blue font and in an oval. Complete secondary structures are presented in Supplementary Material (Fig. S1).

that includes both *Asterarcys* and *Coelastrella*. Unfortunately, Suarez-Montes *et al*. (2022) did not provide *tufA* data for *C. cogersae*. These results show that there are still uncertainties in the phylogeny of *Coelastrella* that probably need to be addressed by more thorough analyses of multiple loci or genomic data. The use of *rbcL* instead of, or in addition to, *tufA* would likely provide better resolution of the phylogeny as *rbcL* is usually more variable than *tufA* (Fawley & Fawley, 2020).

The secondary structure of ITS2 RNA is a common tool for delimiting species in green algae (Coleman, 2003). Compensatory base changes (CBCs) in the ITS2 secondary structure often correspond to different species, as do differences in the length of Helices I, II, and III. The secondary structure of ITS2 can also aid in the alignment for phylogenetic analysis. We feel that ITS2 secondary structure can provide excellent support for species delimitation, but that it is not required for separating species (Fawley *et al.*, 2011). In this study, the presence of a CBC in ITS2 when compared to the ITS2 of *C. yingshanensis* provides strong evidence for a new species (*C. subsphaerica*) that is closely related to *C. yingshanensis*, whereas only hCBCs were found to support two new varieties of *C. yingshanensis*. The new species *C. salsodica* is in a well-supported separate lineage, and the ITS2 secondary structure has several differences from other *Coelastrella* species.

One strain of *C. yingshanensis* var. *ovata*, WPPS2 11/6-27, has an ITS2 DNA sequence that differs from all other *Coelastrella* strains by the presence of a 22 bp tandem repeat. The morphology of this strain is not different from other strains of *C. yingshanensis* var. *ovata*; therefore, it is difficult to assess the importance of this tandem repeat. We have retained WPPS2 11/6-27 as the type specimen for *C. yingshanensis* var. *ovata* because of this unique feature, but there is the possibility that this feature could split *C. yingshanensis* var. *ovata* into two taxa. We are unwilling to do so because of the complete lack of additional evidence for two taxa.

Phylogenetic analysis of *tufA* sequences supports the species, *C. salsodica sp. nov.*, but provides little evidence for the other new taxa. *Coelastrella subsphaerica sp. nov.* has a *tufA* sequence identical to *C. yingshanensis*. The sequences of the two new varieties differ from *C. yingshanensis* by only one or two substitutions, although there are two differences between *C. yingshanensis* var. *ovata var. nov.* and *C. yingshanensis* var. *apiculata var. nov.* The *tufA* gene is usually not as variable as ITS2 or the plastid gene *rbcL* (Fawley & Fawley, 2020), so it is possible that *tufA* may not vary in closely related taxa. We feel that even these slight differences in *tufA* among the strains provide some support for the erection of the new varieties.

Although the morphological features of the Scenedesmaceae can be quite varied and plastic (e.g.,

Kaufnerová & Eliáš, 2013; Wang *et al.*, 2019), morphology has been used as one line of evidence to describe species in *Coelastrella* (e.g., Wang *et al.*, 2019). The original description of the genus *Coelastrella* (based on *C. striolata*) includes the presence of longitudinal cell wall striations or ribs, without mention of polar thickening or apiculate apices (Chodat, 1922). Recent studies have shown that *Coelastrella* sensu lato includes species that lack cell wall ribs and possess polar thickenings or apiculate apices (Kaufnerová & Eliáš, 2013; Wang *et al.*, 2019). These results indicate the morphology alone cannot be used to identify species of *Coelastrella* and it is essential to use DNA sequencing for identification.

The species *Coelastrella yingshanensis* was previously known only from one site in China (Wang *et al.*, 2019). Our results indicate that *C. yingshanensis* is also found in North America, but as new varieties of the species. *Coelastrella yingshanensis* was originally isolated from a river plankton sample, whereas our strains, representing two new varieties, were all from soil samples. The new variety *Coelastrella yingshanensis* var. *apiculata* was only found in sodic-saline slick soil in WPNA, whereas *C. yingshanensis* var. *ovata* was found in soil from the barrens grassland that surrounds the sodic-saline slicks. The differences in ITS sequence and secondary structure, as well as morphological and habitat differences, all support the need for new varieties of *C. yingshinensis*. The evidence that these two varieties are restricted to these very different habitats suggests recent adaptation. It would be interesting to see if these specialized varieties and the two new species we described are in similar habitats from other locations.

An additional new species of *Coelastrella, C. polaris* E. Krivina, M. Sinetova, O. Anissimova *et* A. Temraleeva (Krivina *et al*., 2025), was published after our analyses and manuscript were complete. However, the ITS1–5.8S–ITS2 sequence data from *C. polaris* (PQ565566) differ from all of our strains by 36 or more substitutions and additional indels and do not affect our conclusions. Another new species *C. laevis* V. Yu. Nikulin *et al.* (Nikulin *et al*., 2025) was published while we were preparing revisions. The sequence data for *Coelastrella laevis* were not yet available; however, the phylogenetic analyses in Nikulin *et al.* (2025) of both 18S+ITS and *tufA* strongly suggest that *C. laevis* is not closely related to any of our new taxa.

Acknowledgements

This work was supported by the Arkansas INBRE program through a grant (P20 GM103429) from the National Institute of General Medical Sciences of the National Institutes of Health, a 2016–2018 University of Arkansas at Monticello (UAM) Faculty Research Grant to KPF, and a Beta Beta Beta Biological Honor Society research grant to SAP. We happily thank Rachel Knight and Caleb Lamb from UAM for their work on this project. Brent Baker, formerly with the Arkansas Natural Heritage Commission, assisted with site selection in WPNA.

References

Abe, K., Takizawa, H., Kimura, S., & Hirano, M. (2004). Characteristics of chlorophyll formation of the aerial microalga *Coelastrella striolata* var. *multistriata* and its application for environmental biomonitoring. *Journal of Bioscience and Bioengineering*, *98*(1), 34–39. https://doi.org/10.1016/S1389-1723(04)70239-X

Angelaalincy, M., Nishtha, P., Ajithkumar, V., Ashokkumar, B., Moorthy, I. M. G., Brindhadevi, K., Chi, N. T. L., Pugazhendhi, A., & Varalakshmi, P. (2023). Phycoremediation of arsenic and biodiesel production using green microalgae *Coelastrella* sp. M60 – an integrated approach. *Fuel*, *333*(2), article 126427. https://doi.org/10.1016/j.fuel.2022.126427

Beaulier, C., Dannay, M., Devime, F., Galeone, A., Baggio, C., El Sakkout, N., Raillon, C., Courson, O., Bourguignon, J., Alban, C., & Ravanel, S. (2024). Characterization of a uranium-tolerant green microalga of the genus *Coelastrella* with high potential for the remediation of metal-polluted waters. *Science of The Total Environment*, *908*, article 168195. https://doi.org/10.1016/j.scitotenv.2023.168195

Boutarfa, S., Senoussi, M. M., Gonzalez-Silvera, D., López-Jiménez, J. Á., & Aboal, M. (2022). The green microalga *Coelastrella thermophila* var. *globulina* (Scenedesmaceae, Chlorophyta) isolated from an Algerian hot spring as a potential source of fatty acids. *Life*, *12*(4), article 560. https://doi.org/10.3390/life12040560

Chodat, R. H. (1922). Matérieux pour l'histoire des algues de la Suisse. *Bulletin de la Société Botanique de Genève, Série II, 13,* 66–114.

Choudhary, S., Kumawat, G., Khandelwal, M., Khangarot, R. K., Sangela, V., Kumar, M., Deora, S., Rai, N., Saharan, V., & Mangesh, H. (2023). Sustainable phyco-fabrication of silver nanoparticles using *Coelastrella terrestris* and their multiple downstream applications. *Biocatalysis and Agricultural Biotechnology*, *53*, article 102854. https://doi.org/10.1016/j.bcab.2023.102854

Coleman, A. W. (2003). ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics*, *19*(7), 370–375. https://doi.org/10.1016/S0168-9525(03)00118-5

Famà, P., Wysor, B., Kooistra, W. H., & Zuccarello, G. C. (2002). Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tufA* gene. *Journal of Phycology*, *38*(5), 1040–1050. https://doi.org/10.1046/j.1529-8817.2002.t01-1-01237.x

Fawley, M. W., & Fawley, K. P. (2004). A simple and rapid technique for the isolation of DNA from microalgae 1. *Journal of Phycology*, *40*(1), 223–225. https://doi.org/10.1111/j.0022-3646.2004.03-081.x

Fawley, M. W., & Fawley, K. P. (2020). Identification of eukaryotic microalgal strains. *Journal of Applied Phycology*, *32*(5), 2699–2709. https://doi.org/10.1007/s10811-020-02190-5

Fawley, M. W., Fawley, K. P., & Hegewald, E. (2011). Taxonomy of *Desmodesmus serratus* (Chlorophyceae, Chlorophyta) and related taxa on the basis of morphological and DNA sequence data. *Phycologia*, *50*(1), 23–56. https://doi.org/10.2216/10-16.1

Fawley, M. W., Fawley, K. P., & Hegewald, E. (2013). Desmodesmus baconii (Chlorophyta), a new species

with double rows of arcuate spines. *Phycologia*, *52*(6), 565–572. https://doi.org/10.2216/12-116.1

Foti, T., & Witsell, C. T. (2013). Effects of physical factors on the distribution of native flora and vegetation in the natural divisions of Arkansas. In J. L. Gentry, G. P. Johnson, B. T. Baker, C. T. Witsell, & J. D. Ogle (Eds.), *Atlas of the Vascular Plants of Arkansas* (pp. 35–36). University of Arkansas.

Goecke, F., Noda, J., Paliocha, M., & Gislerød, H. R. (2020). Revision of *Coelastrella* (Scenedesmaceae, Chlorophyta) and first register of this green coccoid microalga for continental Norway. *World Journal of Microbiology and Biotechnology*, *36*(10), article 149. https://doi.org/10.1007/s11274-020-02897-0

Gruber, A. R., Lorenz, R., Bernhart, S. H., Neuböck, R., & Hofacker, I. L. (2008). The Vienna RNA websuite. *Nucleic Acids Research*, *36*(suppl. 2), W70–W74. https://doi.org/10.1093/nar/gkn188

Hanagata, N. (2001). New species of *Coelastrella* and *Scenedesmus* (Chlorophyceae, Chlorophyta). *The Journal of Japanese Botany*, *76*(6), 129–136. https://doi.org/10.51033/jjapbot.76_3_9505

Holder, M., & Lewis, P. O. (2003). Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics*, *4*(4), 275–284. https://doi.org/10.1038/nrg1044

Kaufnerová, V., & Eliáš, M. (2013). The demise of the genus *Scotiellopsis* Vinatzer (Chlorophyta). *Nova Hedwigia*, *97*(3–4), 415–428. https://doi.org/10.1127/0029-5035/2013/0116

Kawasaki, S., Yoshida, R., Ohkoshi, K., & Toyoshima, H. (2020). *Coelastrella astaxanthina* sp. nov. (Sphaeropleales, Chlorophyceae), a novel microalga isolated from an asphalt surface in midsummer in Japan. *Phycological Research*, *68*(2), 107–114. https://doi.org/10.1111/pre.12412

Kerpedjiev, P., Hammer, S., & Hofacker, I. L. (2015). Forna (force-directed RNA): Simple and effective online RNA secondary structure diagrams. *Bioinformatics*, *31*(20), 3377–3379. https://doi.org/10.1093/bioinformatics/btv372

Krivina, E., Sinetova, M., Zadneprovskaya, E., Ivanova, M., Starikov, A., Shibzukhova, K., Lobakova, E., Budkin, Y., Portnov, A., & Temraleeva, A. (2024). The genus *Coelastrella* (Chlorophyceae, Chlorophyta): molecular species delimitation, biotechnological potential, and description of a new species *Coelastrella affinis* sp. nov., based on an integrative taxonomic approach. *Antonie Van Leeuwenhoek*, *117*(1), 113. https://doi.org/10.1007/s10482-024-02008-1

Krivina, E., Sinetova, M., Starikov, A., Anissimova, O., & Temraleeva, A. (2025). Testing the effectiveness of DNA barcoding markers and species delimitation methods within the genus *Coelastrella* (Sphaeropleales, Chlorophyta), with a description of *Coelastrella polaris* sp. nov. isolated from arctic soils. *Current Microbiology*, *82*(7), article 311. https://doi.org/10.1007/s00284-025-04293-z

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. http://doi:10.1093/molbev/msy096

Lee, S. A., Ko, S. R., Lee, N., Lee, J. W., Van Le, V., Oh, H. M., & Ahn, C. Y. (2021). Two-step microalgal (*Coelastrella* sp.) treatment of raw piggery wastewater resulting in higher lipid and triacylglycerol levels for possible production of higher-quality biodiesel. *Bioresource Technology*, *332*, article 125081. https://doi.org/10.1016/j.biortech.2021.125081

Letunic, I., & Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research*, *47*(W1), W256–W259. https://doi.org/10.1093/nar/gkz239

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. https://doi.org/10.1093/molbev/msaa015

Müller, T., Philippi, N., Dandekar, T., Schultz, J., & Wolf, M. (2007). Distinguishing species. *RNA*, *13*(9), 1469–1472. https://doi.org/10.1261/rna.617107

Nikulin, V. Y., Sushchenko, R. Z., Abdullin, S. R., Nikulin, A. Y., & Gontcharov, A. A. (2025). *Coelastrella laevis* sp. nov. (Chlorophyta, Sphaeropleales) from the soils of the Russian Far East. *Phycologia*, *64*(5), 420–428. https://doi.org/10.1080/00318884.2025.2562622

Novakovskaya, I. V., Egorova, I. N., Kulakova, N. V., Patova, E. N., Shadrin, D. M., & Anissimova, O. V. (2021). Morphological and phylogenetic relations of members of the genus *Coelastrella* (Scenedesmaceae, Chlorophyta) from the Ural and Khentii Mountains (Russia, Mongolia). *Phytotaxa*, *527*(1), 1–20. https://doi.org/10.11646/phytotaxa.527.1.1

Reddy, K., Renuka, N., Kumari, S., Ratha, S. K., Moodley, B., Pillay, K., & Bux, F. (2023). Assessing the potential for nevirapine removal and its ecotoxicological effects on *Coelastrella tenuitheca* and *Tetradesmus obliquus* in aqueous environment. *Environmental Pollution*, *317*, article 120736. https://doi.org/10.1016/j.envpol.2022.120736

Ren, H., Wong, T. K., Minh, B. Q., & Lanfear, R. (2025). MixtureFinder: Estimating DNA mixture models for phylogenetic analyses. *Molecular Biology and Evolution*, *42*(1), article msae264. https://doi.org/10.1093/molbev/msae264

Saito, M., Watanabe, H., Sasaki, M., Ookubo, M., Yarita, T., Shiraiwa, M., & Asayama, M. (2023). Coproduction of lipids and carotenoids by the novel green alga *Coelastrella* sp. depending on cultivation conditions. *Biotechnology Reports*, *37*, article e00769. https://doi.org/10.1016/j.btre.2022.e00769

Suarez-Montes, D., Borrell, Y. J., Gonzalez, J. M., & Rico, J. M. (2022). Isolation and identification of microalgal strains with potential as carotenoids producers from a municipal solid waste landfill. *Science of the Total Environment*, *802*, article 149755. https://doi.org/10.1016/j.scitotenv.2021.149755

Thermozier, K. N., Fawley, M. W., & Fawley, K. P. (2024). A new terrestrial species of *Diplosphaera* (Trebouxiophyceae), *Diplosphaera sundellii*, from a sodic-saline slick in Warren Prairie Natural Area, Arkansas, USA. *Fottea*, *24*(1), 109–117. https://doi.org/10.5507/fot.2023.013

Tschaikner, A. G., & Kofler, W. (2008). *Coelastrella aeroterrestrica* sp. nov.(Chlorophyta, Scenedesmoideae) a new, obviously often overlooked aeroterrestrial species. *Algological Studies*, 11–20. https://doi.org/10.1127/1864-1318/2008/0128-0011

U.S. Fish and Wildlife Service (1993). *Recovery Plan for Geocarpon minimum MacKenzie* [Recovery Plan].

Ye, J., McGinnis, S., & Madden, T. L. (2006). BLAST: improvements for better sequence analysis. *Nucleic Acids Research*, *34*(suppl. 2), W6–W9. https://doi.org/10.1093/nar/gkl164

Wang, Q., Song, H., Liu, X., Liu, B., Hu, Z., & Liu, G. (2019). Morphology and molecular phylogeny of coccoid green algae *Coelastrella* sensu lato (Scenedesmaceae, Sphaeropeales), including the description of three new species and two new varieties. *Journal of Phycology*, *55*(6), 1290–1305. https://doi.org/10.1111/jpy.12915

Wang, Q. H., Song, H. Y., & Liu, X. D. (2021). *Coelastrella chongqingensis* sp. nov., a novel species of the subaerial coccoid green algae within the *Coelastrella* (Scenedesmaceae, Sphaeropleales). *Acta Hydrobiologica Sinica*, *45*(6), 1351–1360.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press.