Phycologia

Phycologia

ISSN: 0031-8884 (Print) 2330-2968 (Online) Journal homepage: www.tandfonline.com/journals/uphy20

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To cite this article: Vyacheslav Yu. Nikulin, Rezeda Z. Sushchenko, Arthur Yu. Nikulin, Veronika B. Bagmet, Shamil R. Abdullin & Andrey A. Gontcharov (20 Oct 2026): *Coelastrella iturupiensis sp. nov*. (Chlorophyta, Sphaeropleales) and new records of *C. polaris* and *C. affinis* from the Russian Far East, Phycologia, DOI: 10.1080/00318884.2026.2739890

To link to this article: https://doi.org/10.1080/00318884.2026.2739890

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https://doi.org/10.1080/00318884.2026.2739890

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Coelastrella iturupiensis sp. nov. (Chlorophyta, Sphaeropleales) and new records of *C. polaris* and *C. affinis* from the Russian Far East

Vyacheslav Yu. Nikulin, Rezeda Z. Sushchenko, Arthur Yu. Nikulin, Veronika B. Bagmet, Shamil R. Abdullin and Andrey A. Gontcharov

Laboratory of Botany, Federal Scientific Center of the East Asia Terrestrial Biodiversity Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia

ABSTRACT

Four strains of the coccoid green algal genus *Coelastrella* (Scenedesmaceae, Sphaeropleales) were isolated from moss-covered rocks and soils in the southern Russian Far East (Primorsky Territory and Iturup Island). An integrative taxonomic approach combining light and scanning electron microscopy, confocal laser microscopy, multi-locus phylogeny (nuclear SSU+ITS rDNA region and chloroplast *tuf*A), ITS2 secondary-structure analysis, and three molecular species-delimitation methods (PTP, ASAP, GMYC) were applied. Two strains (VCA-307 and VCA-308) formed a robust lineage within the 'core *Coelastrella*' clade, being sister to *C. striolata, C. multistriata*, and *C. corcontica*. Sequence divergences (4.06–13.13% in the ITS rDNA region) and a unique compensatory base change in the conserved barcode region of ITS2 helix III (A–U pair) clearly separated them from all described congeners. All delimitation algorithms recognized these strains as a distinct species-level cluster. Based on morphology (16–24 meridional rows of rounded warts, small lemon-shaped to ellipsoid cells 4.9–12.6 × 3.7–10.7 µm, lobed chloroplast in mature cells) and molecular data, the strains are described here as *Coelastrella iturupiensis sp. nov*. The remaining two strains represent the first records of the Arctic species *C. polaris* (VCA-309) and *C. affinis* (VCA-310) outside Yakutia and the Moscow Region, respectively. These findings substantially expand the known diversity and distribution of *Coelastrella* in Northeast Asia.

ARTICLE HISTORY
Received 22-07-2026
Accepted 23-09-2026
Version of Record 20-10-2026

KEYWORDS
Green algae; integrative taxonomic approach; ITS rDNA; new species; *tuf*A

INTRODUCTION

The green algal genus *Coelastrella* (Scenedesmaceae, Sphaeropleales) comprises coccoid green algae that are widely distributed in terrestrial and subaerial habitats, including soils, rock surfaces, tree bark, and biological soil crusts (Chodat, 1922; Kalina & Punčochářová, 1987; Wang *et al*., 2019). Remarkable ecological flexibility of *Coelastrella* is reflected in its worldwide distribution, ranging from polar to equatorial latitudes (Boutarfa *et al*., 2022; Krivina *et al*., 2025; Nayana *et al*., 2022; Skrebovska & Kostikov, 2012). Members of this genus are characterized by a parietal chloroplast with a single pyrenoid, asexual reproduction via autospores, and, in most species, a sculptured cell wall bearing meridional ribs (Andreeva, 1998; Kaufnerová & Eliáš, 2013; Tschaikner *et al*., 2007). However, considerable morphological heterogeneity exists in this genus: some species possess numerous (up to 40) ribs, others have only a few ribs or even a smooth cell wall, with polar thickenings present or not (Kawasaki *et al*., 2020; Wang *et al*., 2021). Because of this phenotypic plasticity, several *Coelastrella* species were originally described as members of other genera, including *Cryptodesmus, Scotiella, Scotiellopsis, Scenedesmus, Graesiella*, and even *Chlorella* (Hegewald & Hanagata, 2002; Kalina & Punčochářová, 1987; Kaufnerová & Eliáš, 2013; Shihira & Krauss, 1965). The advent of molecular phylogenetics has greatly improved the circumscription of *Coelastrella*, although the phylogenetic relationship between species and morphological characteristics remains to be refined as new data accumulate. Analyses of nuclear SSU rDNA and ITS rDNA (ITS1-5.8S-ITS2) regions, chloroplast *tuf*A gene revealed the genus clade as a distinct member of the Scenedesmaceae, comprising a 'core *Coelastrella*' lineage (including the type species *C. striolata* Chodat) and several allied lineages (Kaufnerová & Eliáš, 2013; Krivina *et al*., 2025; Wang *et al*., 2019). However, species delimitation within *Coelastrella* remains challenging, as SSU rDNA is too conserved to resolve closely related taxa; only ITS rDNA and *tuf*A provided sufficient resolution (Wang *et al*., 2024). The use of ITS2 secondary structure and compensatory base changes (CBCs) has proven particularly valuable for distinguishing species (Krivina *et al*., 2024; Nikulin *et al*., 2025; Novakovskaya *et al*., 2021). In addition, integrative approaches combining molecular data with light and electron microscopy have led to a surge in the description of new *Coelastrella* species over the last decade (Ambrocio Paque *et al*., 2026; Kawasaki *et al*., 2020; Krivina *et al*., 2024, 2025; Nikulin *et al*., 2025; Novakovskaya *et al*., 2025; Saini *et al*., 2026; Suarez-Montes *et al*., 2022; Wang *et al*., 2019, 2021). This ongoing discovery highlights that species diversity of this genus is still far from being fully documented.

Beyond taxonomic interest, *Coelastrella* species have attracted biotechnological attention due to their ability to accumulate high-value carotenoids and polyunsaturated fatty

CONTACT Vyacheslav Yu. Nikulin nikulinvyacheslav@gmail.com
Supplemental data for this article can be accessed online at https://doi.org/10.1080/00318884.2026.2739890
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acids under stress conditions (Goecke *et al.*, 2020; Liu *et al.*, 2026; Maltsev *et al.*, 2021; Mansurova *et al.*, 2026; Minyuk *et al.*, 2017; Novakovskaya *et al.*, 2025). Some strains can grow heterotrophically on organic carbon sources, making them potential candidates for wastewater treatment and biofuel production (Krivina *et al.*, 2024; Luo *et al.*, 2016). Thus, exploring *Coelastrella* diversity may also uncover new strains with promising biotechnological traits.

The Russian Far East is a region of high terrestrial habitat diversity, ranging from temperate monsoon forests to volcanic landscapes and Arctic tundras. However, microalgal flora of this area, particularly in soil and epiphytic habitats, remains poorly studied.

In this study, we characterized four *Coelastrella* strains isolated from moss-covered rocks and soils in Vladivostok (Primorsky Territory) and Iturup Island (Sakhalin Region) using an integrative approach that included light microscopy, scanning electron microscopy, phylogenetic analyses of SSU +ITS rDNA and *tuf*A, ITS2 secondary structure comparisons, and molecular species delimitation (PTP, ASAP, and GMYC). Our aims were to determine the identity of these strains from the Russian Far East.

MATERIAL AND METHODS

Study site, culture conditions and microscopy

Soil samples were collected in Primorsky Territory and Sakhalin Region (Iturup Island; Table 1, Fig. S1). Sampling was carried out according to a standard protocol (Kuzyakhmetov & Dubovik, 2001; Vasser *et al.*, 1989). Strains of green algae were isolated by the micro-pipette method (Andersen, 2005) and cultured in liquid and agar Waris-H nutrient medium (McFadden & Melkonian, 1986) at 20–22°C with a photon fluence of 17.9–21.4 µmol photons $m^{-2}s^{-1}$ in a 16:8 h light/dark cycle. The strains are maintained in the culture collection of the Laboratory of Botany in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Russia (strain numbers VCA-307, VCA-308, VCA-309, and VCA-310). Morphology of vegetative and reproductive cells was examined using a light microscope equipped with Nomarski-DIC optics. The cultures were repeatedly examined throughout their life cycle stages (i.e. in cultures of different ages (3, 6, 12 months)).

Cell wall structures were visualized by scanning electron microscopy (SEM) following the protocol of Novakovskaya *et al.* (2021), with modifications. The samples used for SEM analysis were cultivated in liquid Waris-H medium for at least 3–4 weeks. Algal cells were applied onto Thermanox coverslips (Thermo Fisher Scientific, Roskilde, Denmark) and allowed to settle for 2–3 hours to promote adhesion. The gradual dehydration of the cells was achieved by dipping them into an ethanol series of 10, 30, 50, 70, 90, and 96% for 8 min each, followed by transferring them into an ethanol: acetone solution (3:1), ethanol:acetone solution (1:1), and subsequently into 100% acetone. Thereafter, the samples were critical point dried, sputtered with chromium at the Department of Electron Microscopy of the A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS, the samples were examined with a Merlin SEM (Carl Zeiss, Jena, Germany) at the Instrumental Center of Biotechnology and Gene Engineering of FSCEATB FEB RAS. The fluorescence of chloroplasts in living cells was examined with a CLSM 710 LIVE (Carl Zeiss, Oberkochen, Germany). Chloroplast autofluorescence was recorded in the additional emission channel after 600 nm, using a Plan-Apochromat 63×/1.40 Oil DIC M27 objective with digital zoom. Z-stack of cells was recorded and analysed with ZEN 2011 software.

DNA extraction, amplification, and sequencing

For DNA analysis, the cultures were harvested during the exponential growth phase and concentrated by centrifugation. Total genomic DNA was extracted, as described previously by Abdullin *et al.* (2021). For amplification of the SSU rDNA and ITS region, the following primers were used: 82F (López-García *et al.*, 2003) and ITS4R (White *et al.*, 1990). PCR parameters were described previously in Mikhailyuk *et al.* (2018). The PCR products were purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, CA, USA) and sequenced in both directions using an ABI 3500 genetic analyser (Applied Biosystems, Waltham, MA, USA) with a BigDye terminator v.3.1 sequencing kit, with the amplification primers, plus SSU528F-800 (Hoef-Emden & Melkonian, 2003), 920F (Marin *et al.*, 1998), n1400R (Marin *et al.*, 2003), and Bd18SF1 (Goka *et al.*, 2009). Partial *tuf*A gene sequence was amplified using the primer pair *tuf*AF and *tuf*AR (Famà *et al.*, 2002). Sequences were edited and assembled with the Staden Package v.1.4 (Bonfield *et al.*, 1995). Sequences covering a partial SSU rDNA region, the complete ITS region, and the *tuf*A gene were deposited in the GenBank (accession numbers in Table 1).

Table 1. Sample site locations, strain information, and habitat characterization.

No.	Strain	GenBank accession numbers (SSU+ITS / *tuf*A)	Sampling site	Soil type	Coll. Date
1	VCA-307	PZ316189 / PZ315793	Vladivostok, Sedanka area, on moss and soil on a rock; 43°12.733′N 131°58.600′E	–	22 Apr. 2018
2	VCA-308 (holotype)	PZ316190 / PZ315794	Iturup Island, 13 km from the village of Reidovo towards the Black Rocks. A hill along the seashore, on soil; 45°15.467′N 148°10.550′E	Umbric Andosols	15 Jul. 2022
3	VCA-309	PZ316192 / PZ315796	Vicinity of the villages of Roshchino and Nezametnoye (Primorsky Territory), on soil; 45° 57.017′N 134°57.350′E	Haplic Cambisol	15 Jul. 2021
4	VCA-310	PZ316191 / PZ315795	Vladivostok, Malaya Sedanka River valley, on a rock outcrop, dry moss and soil; 43° 12.550′N 131°59.783′E	–	22 Apr. 2018

Molecular analyses

To clarify the phylogenetic position of the new strains, three datasets were used (Table S1; Fig. S2). The datasets were assembled based on the analyses presented by Nikulin *et al.* (2025) and included *Asterarcys quadricellularis* (K.Behre) E. Hegewald & A.W.F.Schmidt and *Enallax costatus* (Schmidle) Pascher closely related to *Coelastrella*. Ribosomal DNA sequence alignment was conducted using the SeaView program (Galtier *et al.*, 1996) with manual correction based on the secondary structure information for the ITS rDNA dataset. Alignments were trimmed by hand to remove introns in SSU rDNA and ambiguously aligned ITS rDNA regions.

The UNAfold web server (Zuker, 2003) was used with the default settings to predict the ITS2 secondary structures, which were then visualized using 4SALE program (Seibel *et al.*, 2008). ITS2 model was constructed based on the consensus secondary structure model proposed by Kaufnerová & Eliáš (2013) for *Coelastrella*. The ITS2 sequence alignment was translated into the barcode alignment by replacing each base-pair by a number (A–U = 1; U–A = 2, G–C = 3, C–G = 4, G•U = 5, U•G = 6, mismatch = 7, deletion or unpaired or single bases = 8) as described in Novakovskaya *et al.* (2021).

Phylogenetic analysis

Phylogenetic trees were constructed using maximum likelihood (ML) in IQTREE 2 (Minh *et al.*, 2020) and Bayesian inference (BI) in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Akaike information criterion (Akaike, 1974) implemented in jModelTest 2.1.1 (Darriba *et al.*, 2012) was used to determine the most appropriate DNA substitution model for the datasets (Table S1). In BI, four parallel Markov Chain Monte Carlo (MCMC) runs were carried out for two (SSU and ITS rDNA), five (ITS rDNA), and one (*tuf*A) million generations. Sampling was carried out every 100 generations. Convergence of the chains was assessed, and stationarity was determined according to the 'sump' plot with the first 25% of samples discarded as burn-in; posterior probabilities were calculated from the remaining trees. Robustness of the ML trees was estimated using ultrafast bootstrap (1000 replicates (UFBP); Hoang *et al.*, 2018) and posterior probabilities (PP) in the BI. Pairwise distances (*p*-distances) were estimated using MEGA 11 (Tamura *et al.*, 2021).

Molecular delimitation of species

*Tuf*A dataset was used for species delineation using the Assemble Species by Automatic Partitioning (ASAP) method (Puillandre *et al.*, 2021), accessed via an online service (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html; on 10 March 2026). A ML phylogeny was employed to delineate putative species by a maximum likelihood Poisson tree processes (PTP) model (Zhang *et al.*, 2013). This analysis was conducted using an online service (https://species.h-its.org/; accessed on 10 March 2026). The generalized mixed Yule coalescent (GMYC) method (Fujisawa & Barraclough, 2013) with the online service (https://species.h-its.org/gmyc; accessed on 10 March 2026), was used to select clusters at the species level on the tree and to determine the species delimitation threshold. The ultrametric phylogenetic tree for the GMYC analysis was reconstructed by the Bayesian method in the BEAST v1.10.4 program (Drummond & Rambaut, 2007) with an uncorrelated relaxed lognormal molecular clock and ten million generations.

RESULTS

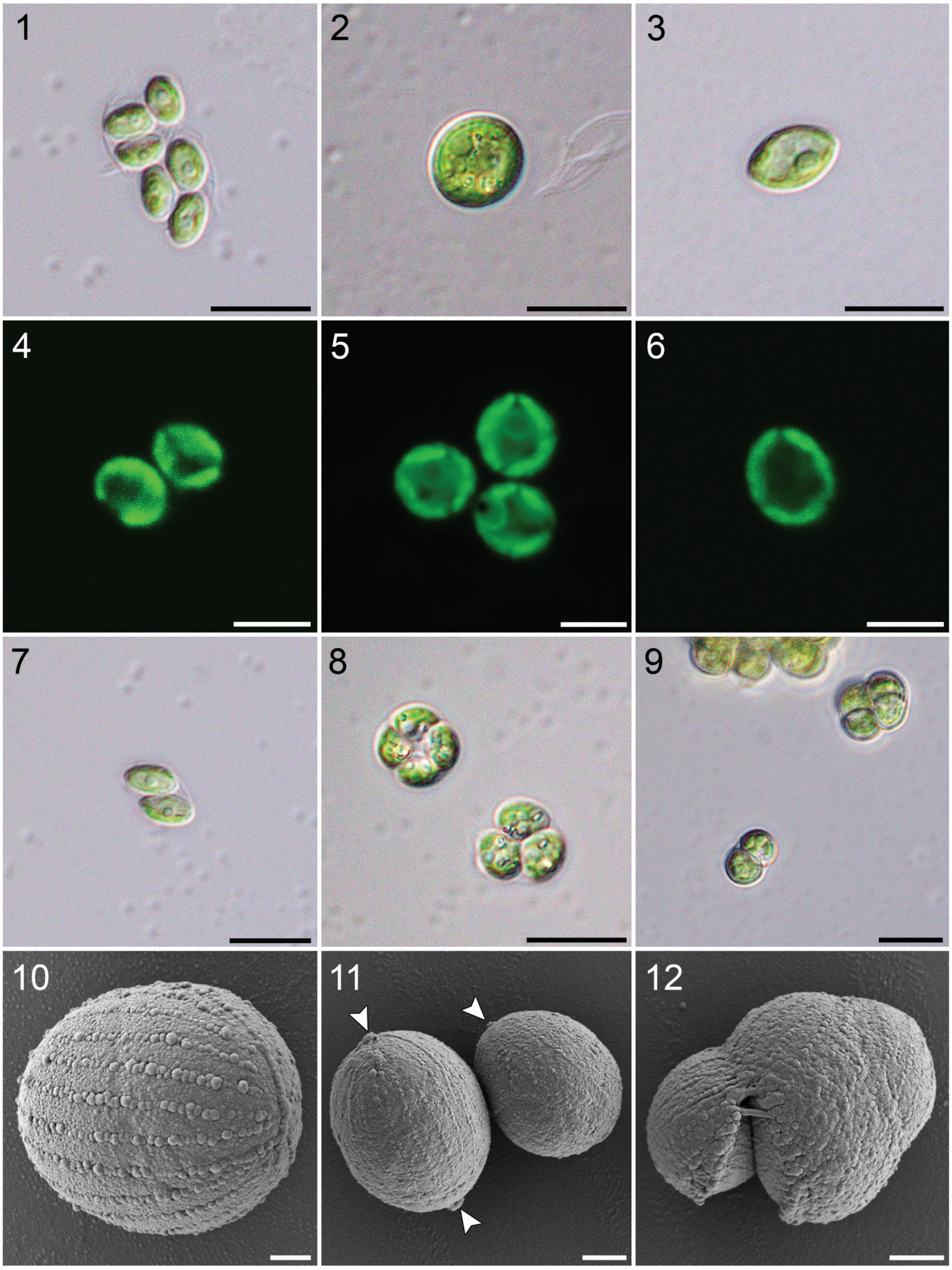
Morphological observations

All four *Coelastrella* strains examined shared diagnostic features of the genus: cells occurred solitary; each possessed a single parietal chloroplast with one pyrenoid surrounded by a starch envelope; asexual reproduction via autosporulation (Figs 1–24, Figs S3–S14; Table S2). No sexual stages, flagellated cells, or akinetes were observed under culture conditions.

Strains VCA-307 and VCA-308 were morphologically indistinguishable. Young cells were oval, lemon-shaped or ellipsoid (4.9–7.5 × 3.7–6.3 µm), rarely spherical, with several small vacuoles (Fig. 1, S3). The chloroplast was parietal, cup-shaped with a pyrenoid (Fig. 1, 4). Mature cells (range 7.6–10.9 (12.6) × 5.9–9.0 (10.7) µm) possessed a lobed parietal chloroplast with a pyrenoid (Fig. 5, 6, S6). Asexual reproduction by 2–4–8 autospores was observed (Fig. 7, 8, S7–S9). Scanning electron microscopy revealed that the cell wall is ornamented with 16–24 meridional rows of rounded warts (warts *sensu* Hegewald, 1997; Fig. 10–11, S12–14). These structures were not discernible under light microscopy and required SEM for visualization. Cells predominantly lacked polar thickenings, although small thickenings were occasionally observed only by SEM (Fig. 11, S13). Neither coenobia formation nor sexual reproduction was observed. Prolonged cultivation resulted in an overall olive-green to pale orange colouration of the culture; accumulation of coloured globules (possibly containing lipid droplets and carotenoids) in old cells was barely visible under the light microscopy (Fig. 9, S10–11). Cells from agar culture were more elongated (4.6–8.7 × 7.3–12.6 µm) and had pronounced pointed ends, but their size and structure (chloroplasts and vacuoles) did not differ from those in liquid culture (Fig. 1, 3, S5).

Young cells of strain VCA-309 were oval to broadly oval (Fig. 13), rarely spherical (8.1–9.7 × 5.4–7.5 µm). Mature cells were lemon-shaped, oval or spherical (Fig. 14; 10.8–19.6 × 7.8–17.2 µm). The chloroplast was parietal with a pyrenoid, transitioning from cup-shaped to lobed in young cells and becoming discoid in large mature cells; the surface showed small incisions. No polar thickenings were observed. Asexual reproduction produced 2–4–8 autospores (Fig. 15–16). Old cells contained numerous small orange-brown droplets (Fig. 18). Zoospores and sexual reproduction were not observed.

Young cells of strain VCA-310 possessed pointed ends that became less pronounced with age (Fig. 19). Older cells were spherical or lemon-shaped (Fig. 20; 9.1–14.6 × 6.4–13.0 µm). The chloroplast with a pyrenoid surrounded by a starch envelope was parietal and cup-shaped in young cells (Fig. 19),


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becoming discoid in mature cells (Fig. 20). Each autosporangium released 2–4–8 lemon-shaped or spherical autospores (Fig. 21–23; 7.6–8.7 × 5.8–8.5 µm). Cells in cultures older than 20 months remained spherical, reaching 7.8–13.2 µm in diameter (Fig. 24). Accumulation of coloured droplets was not visible.

Molecular phylogenetic analyses

To determine the phylogenetic relationships of the four strains we conducted molecular phylogenetic analyses. Nearly complete sequences of the nuclear SSU rDNA gene and ITS region (ITS1-5.8S-ITS2; 2868–3204 bp), as well as with the chloroplast *tuf*A gene (817–862 bp), were obtained for all four strains.

An analysis of the concatenated SSU+ITS rDNA dataset placed strains VCA-307 (Vladivostok) and VCA-308 (Iturup Island), having identical sequences, in a highly supported clade within the 'core *Coelastrella*' lineage (Fig. 25; 98 UFBS/1.00 PP). Their sister group comprised *C. striolata* (the type species of the genus), *C. multistriata* (Trenkwalder) Kalina & Punčochářová, and *C. corcontica* (Kalina & Punčochářová) Hegewald & N.Hanagata (–/0.99). Pairwise sequence divergence between the new lineage and other species ranged from 0.04–7.71% in SSU rDNA (Table S3) to 4.06–13.13% in the ITS rDNA region (Table S4).

The remaining two strains showed strong affinity to the type material of previously described species. Strain VCA-309 showed 99.4% sequence identity to *C. polaris* Krivina, Sinetova, Anissimova & Temraleeva in SSU rDNA and 99.8% in ITS rDNA, while VCA-310 exhibited 98.7% and 99.1% identity to *C. affinis* Krivina, Sinetova, Zadneprovskaya, Shibzukhova, Lobakova & Temraleeva, respectively.

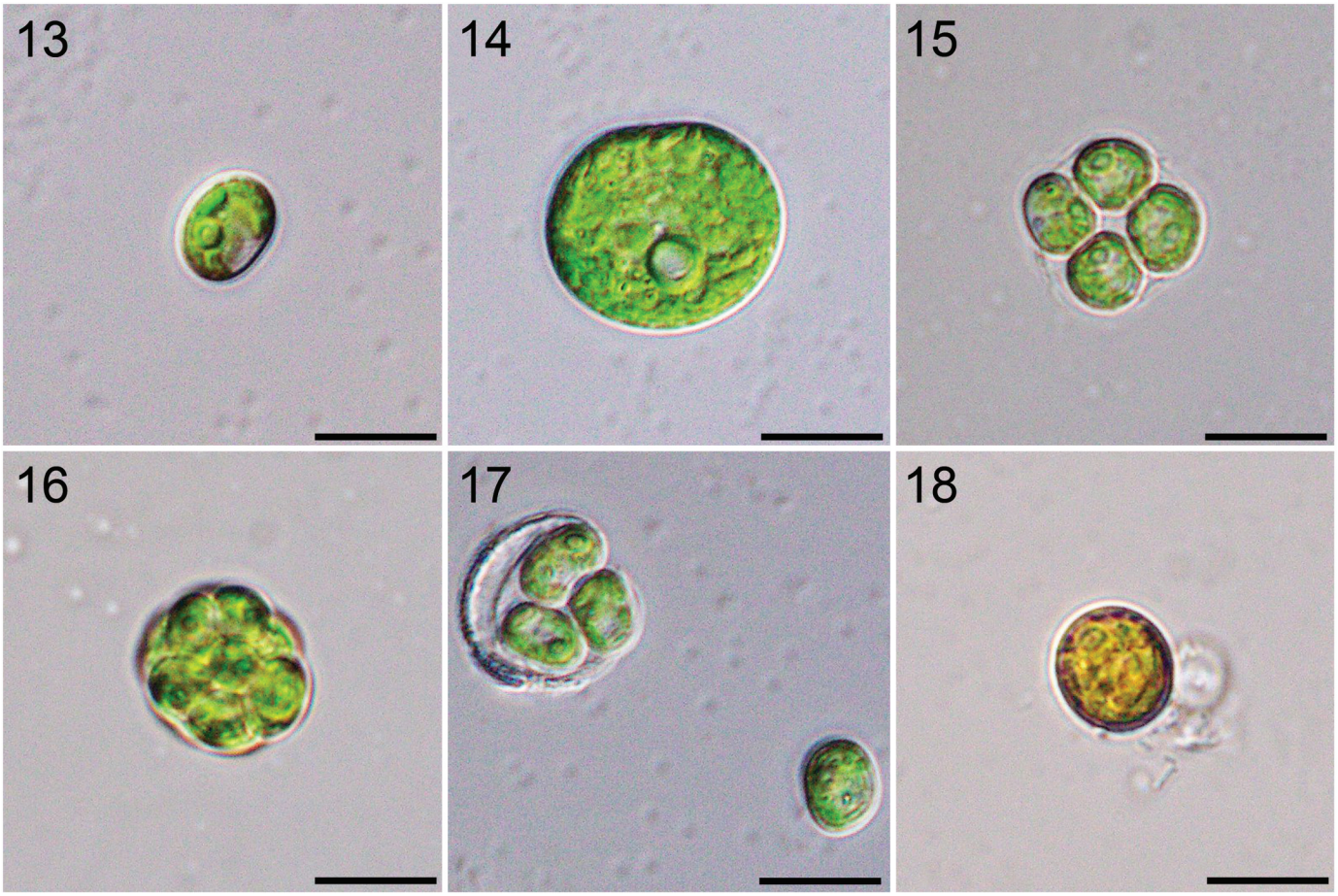
Phylogenetic analyses of the ITS rDNA region (Fig. S15) revealed most species-level clades with high to moderate support, although the backbone topology remained largely unresolved. Strains VCA-307 and VCA-308 again formed a distinct, fully supported clade (100/1.00) being a sister to the clade containing *C. striolata, C. multistriata*, and *C. corcontica*. Strains VCA-309 and VCA-310 grouped with the type strains of *C. polaris* and *C. affinis*, respectively. Two recently deposited to the GenBank sequences of *Coelastrella sp*. (VKM Al-71 and VKM Al-75) also fell within the *C. affinis* clade. A large, well-supported clade (100/1.00) of 26 unidentified *Coelastrella* sequences was recovered, most likely representing one or more undescribed species. Interestingly, a recently described species from India, *C. fusifera* A.Saini, J. Singh, R.Kant & V.Malik, falls within this same clade, further supporting the presence of undescribed diversity in the genus.

To evaluate diagnostic molecular features in the ITS2 barcode regions, secondary structure models were constructed following the conservative barcoding approach of Novakovskaya *et al.* (2021). The predicted structures displayed four helices and five single-stranded domains typical for the genus *Coelastrella*, with a branched helix I shared by some members of the genus (Fig. S16). Helix I exhibited one compensatory base change (CBC) and one hemi-compensatory base change (hCBC), attributed to the first base pair (Fig. 26). Three variable positions were identified in the helix II (one CBC, two hCBCs and two mismatches). Helix III was the most variable, exhibiting 15 variable positions, including three CBCs, 11 hCBCs, and five positions with indels or mismatches. The new strains (VCA-307 and VCA-308) were distinguished by unique substitution in helix III (A–U pair instead of a mismatch present in most congeners, except *C. vacuolata* (I.Shihira & R.W.Krauss) Hegewald & N.Hanagata, which possesses a U–G pair at this position; Fig. 26). The barcode sequence of VCA-309 was identical to that of *C. polaris* type strain, differing by only one substitution (hCBC: U–A vs. U–G) located outside the barcode region in the upper part of helix II. Strain VCA-310 differed from *C. affinis* by three substitutions and one 1-bp indel: one hCBC in helix I (U–G vs. U–A; outside the barcode), two changes in helix III (hCBC: C–G vs. U–G; C vs. A in an internal loop) and deletion of a single G.

Phylogenetic analyses of *tuf*A gene (Fig. 27) corroborated the distinctiveness of VCA-307 and VCA-308 (100/1.00). The two strains possessed identical *tuf*A gene sequences and were resolved as a part of the 'core *Coelastrella*' lineage. The closest sequences were *C. rubescens* (Vinatzer) Kaufnerová & Eliáš, *C. rubescens* var. *oocystiformis* Q.Wang, H.Song, X.Liu, G.Liu & Z.Hu, *C. ferroedaphica* Novakovskaya, Patova & Shadrin, and strain VCA-310 (98/1.00). Genetic distances between the new lineage and other species ranged from 1.41–5.33% (Table S5), while intraspecific variation within *Coelastrella* was low (0–0.21%). Strain VCA-309 showed no affinity to any species, whereas VCA-310 was sister to *C. ferroedaphica* though unsupported.

Three methods of molecular species delimitation (PTP, ASAP, and GMYC) were applied to the *tuf*A dataset (Fig. 27). The analyses recovered from 15 (PTP) to 22 (ASAP and GMYC) species-level clusters. In most cases, individual clades or divergent accessions corresponded to single species. Only in the *C. thermophila* Q.Wang, H.song, X.Liu, G.Liu & Z.Hu clade one to three clusters were resolved and

Figs 1–12. Micrographs of general morphology of *Coelastrella iturupiensis sp. nov.* (strain VCA-308).
Fig. 1. Young cells on solid medium. Scale bar = 10 µm.
Figs 2–3. Mature cell in liquid and solid medium, respectively. Scale bar = 10 µm.
Fig. 4. Confocal laser scanning microscopy (CLSM) image showing chloroplast autofluorescence (green) in young cells. Scale bar = 5 µm.
Figs 5–6. Confocal laser scanning microscopy (CLSM) images showing chloroplast autofluorescence (green) in mature cells. Scale bar = 5 µm.
Fig. 7. Autosporangium with two autospores on solid medium. Scale bar = 10 µm.
Fig. 8. Autosporangium with four autospores. Scale bar = 10 µm.
Fig. 9. Old cells showing slight yellow colour. Scale bar = 10 µm.
Fig. 10. Scanning electron micrograph showing vegetative cell with meridional rows of rounded warts. Scale bar = 1 µm.
Fig. 11. Scanning electron micrograph showing vegetative cells with meridional rows of rounded warts and small polar thickenings (arrowheads). Scale bar = 2 µm.
Fig. 12. Liberation of cells from autosporangium. Scale bar = 2 µm.



Figs 13–18. Micrographs showing morphology of *C. polaris* (strain VCA-309). Scale bar = 10 µm.
Fig. 13. Young cell.
Fig. 14. Mature cell with parietal chloroplast and pyrenoid clearly visible.
Fig. 15. Autosporangium with four autospores.
Fig. 16. Autosporangium with eight autospores.
Fig. 17. Liberation of four autospores from autosporangium.
Fig. 18. Old cell with orange colouration.

two to three clusters in the clade *C. saipanensis* N.Hanagata. The PTP method lumped *C. rubescens* (including var. *oocystiformis)*, *C. oocystiformis* (J.W.G.Lund) Hegewald & Hanagata, *C. affinis* including VCA-310), and *C. ferroedaphica* into a single cluster, while ASAP and GMYC clearly separated them. Almost all *C. yingshanensis* Q.Wang, H.Song, X.Liu, G.Liu & Z.Hu sequences (except one) together with *C. subsphaerica* Ambrocio Paque, M.W. Fawley & K.P.Fawley formed a single cluster by ASAP but split into two clusters under PTP and GMYC. Importantly, strains VCA-307/VCA-308, VCA-309, and VCA-310 were recovered as three species-level clusters by all three methods.

Thus, the phylogenetic analyses conclusively demonstrate that strains VCA-307 and VCA-308 represent a new species of *Coelastrella*, while VCA-309 and VCA-310 are conspecific with *C. polaris* and *C. affinis*, respectively.

Description of the new species

Based on the integrative morphological and molecular evidence presented above, strains VCA-307 and VCA-308 are here described as a new species of *Coelastrella*.

Coelastrella iturupiensis V.Yu.Nikulin, R.Z.Sushchenko, A. Yu.Nikulin, V.B.Bagmet, Sh.R.Abdullin & A.A.Gontcharov *sp. nov.*

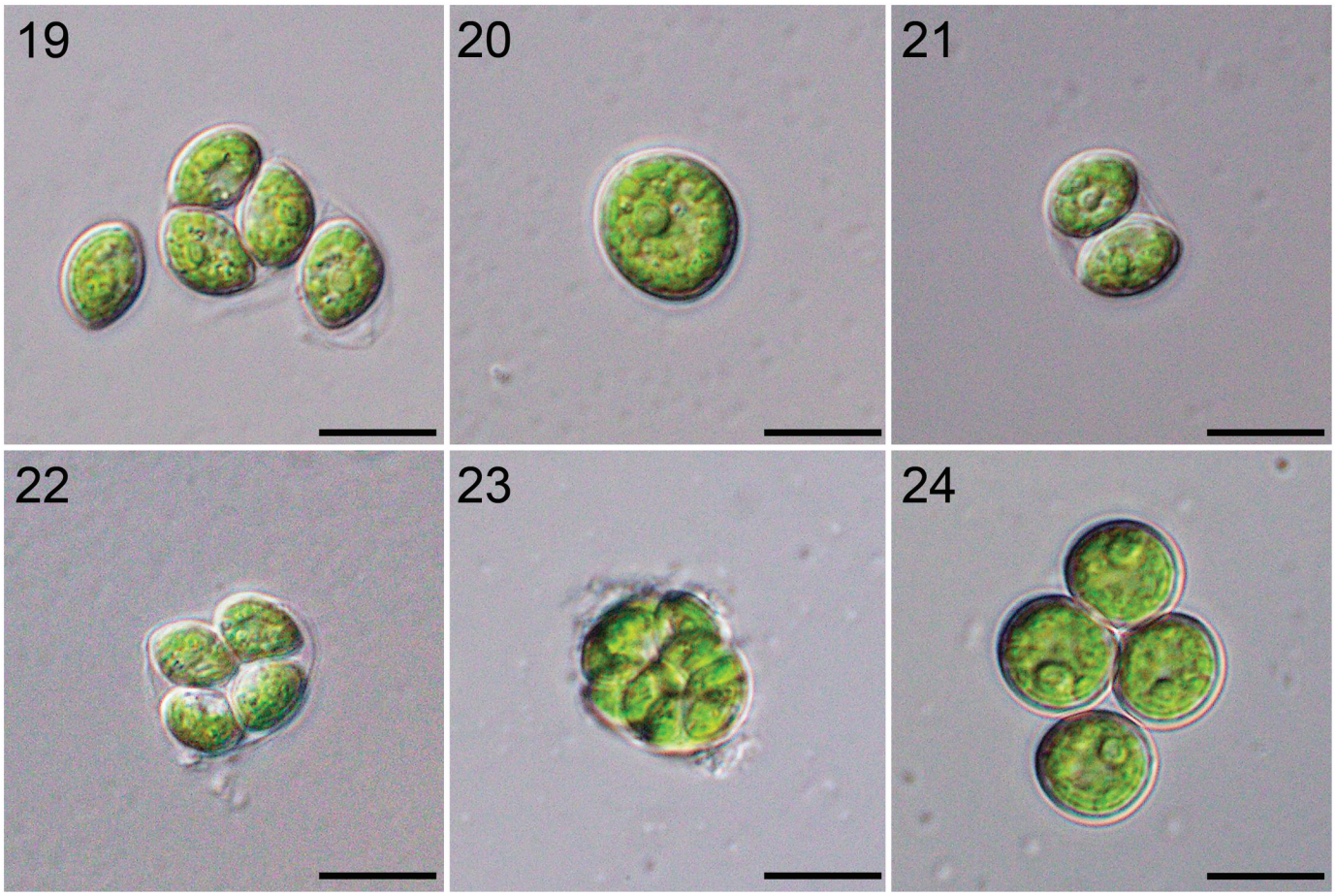
Figs. 1–12

DESCRIPTION: Cells solitary, lemon-shaped to ellipsoid (4.9–7.5 × 3.7–6.3 µm in young cells), rarely spherical, with several small vacuoles and rarely with polar thickenings. Mature cells (7.6–10.9 × 5.9–9.0 µm) with a parietal lobed chloroplast, cup-shaped in young cells having a pyrenoid surrounded by a starch envelope. Cell wall ornamented with 16–24 longitudinal rows of low, rounded warts. Asexual reproduction by 2–4–8 autospores. Sexual reproduction and flagellated cells not known. Akinetes not observed under culture conditions. GenBank accession numbers (holotype strain): SSU+ITS, PZ316190; *tuf*A, PZ315794.

HOLOTYPE: strain VCA-308 (type strain; voucher number VLA-CA -1722), dried biomass of a unialgal population deposited in the Herbarium of the Federal Scientific Center of the East Asia Terrestrial Biodiversity (VLA), Vladivostok, Russia.

TYPE LOCALITY: Russia, Sakhalin Region, Iturup Island, 13 km from the village of Reidovo towards the Black Rocks (45o15.4667'N, 148o10.550.'E), soil; collected by A.Yu. Nikulin, Sh.R. Abdullin, and A.A. Gontcharov, 15 July 2022.

ETYMOLOGY: The specific epithet refers to Iturup Island (Kuril Islands, Russia), the type locality.



Figs 19–24. Micrographs showing the morphology of *C. affinis* (strain VCA-310). Scale bars = 10 µm.
Fig. 19. Young cells with pointed ends.
Fig. 20. Mature cell.
Fig. 21. Autosporangium with two autospores.
Fig. 22. Autosporangium with four autospores.
Fig. 23. Autosporangium with eight autospores.
Fig. 24. Old cells without any visible orange colouration.

HABITAT AND DISTRIBUTION: Known only from terrestrial habitats on moss and soil in southern part of the Russian Far East (Kuril Islands and Primorsky Territory).

DISCUSSION

Our integrative analyses support the recognition of three taxa among the four *Coelastrella* strains examined: *C. iturupiensis sp. nov.*, and the previously described *C. polaris* and *C. affinis*, both recorded here from new geographical regions. The following discussion focuses on the morphological features and biogeographic implications of these taxa.

Both SSU+ITS and *tuf*A phylogenies placed *C. iturupiensis* within the 'core *Coelastrella*' lineage (Kaufnerová & Eliáš, 2013; Wang *et al.*, 2019) with high support. Within this clade, the new species was resolved as a distinct sister to a clade comprising *C. striolata, C. multistriata*, and *C. corcontica* based on SSU and ITS data. Interspecific genetic distances for *C. iturupiensis* (4.06–13.13% in ITS, 1.41–5.33% in *tuf*A) were well above the typical intraspecific variation observed in this genus (Krivina *et al.*, 2024), and all three molecular species-delimitation methods (PTP, ASAP, and GMYC) recovered the two strains as a distinct species-level cluster. Collectively, molecular data unequivocally support description of *C. iturupiensis*.

Coelastrella iturupiensis shares typical features of the 'core *Coelastrella*' group – solitary cells, parietal chloroplast with a single pyrenoid, and reproduction by autospores (Table S2). Cell wall ornamentation has long been regarded as one of the principal morphological characters in *Coelastrella*. The genus was originally established for species possessing numerous longitudinal ribs on the cell wall (Chodat, 1922). Subsequent ultrastructural studies confirmed ribbed ornamentation in many representatives of the genus, including *C. thermophila, C. corcontica*, and *C. striolata* (Kalina & Punčochářová, 1987; Wang *et al.*, 2019). Hanagata (2001) described net-like (reticulate) rib ornamentation in *C. saipanensis*. Several species, including *C. chongqingensis* Q.Wang, H.Song & X.Liu, *C. laevis* V.Yu.Nikulin, R.Z.Sushchenko, Sh.R. Abdullin, A. Yu.Nikulin & A.A. Gontcharov, *C. polaris*, and *C. tenuitheca* Q.Wang, H.Song, X.Liu, G.Liu & Z.Hu have been shown to possess smooth cell walls lacking any distinct sculpturing (Krivina *et al.*, 2025; Nikulin *et al.*, 2025; Wang *et al.*, 2019,

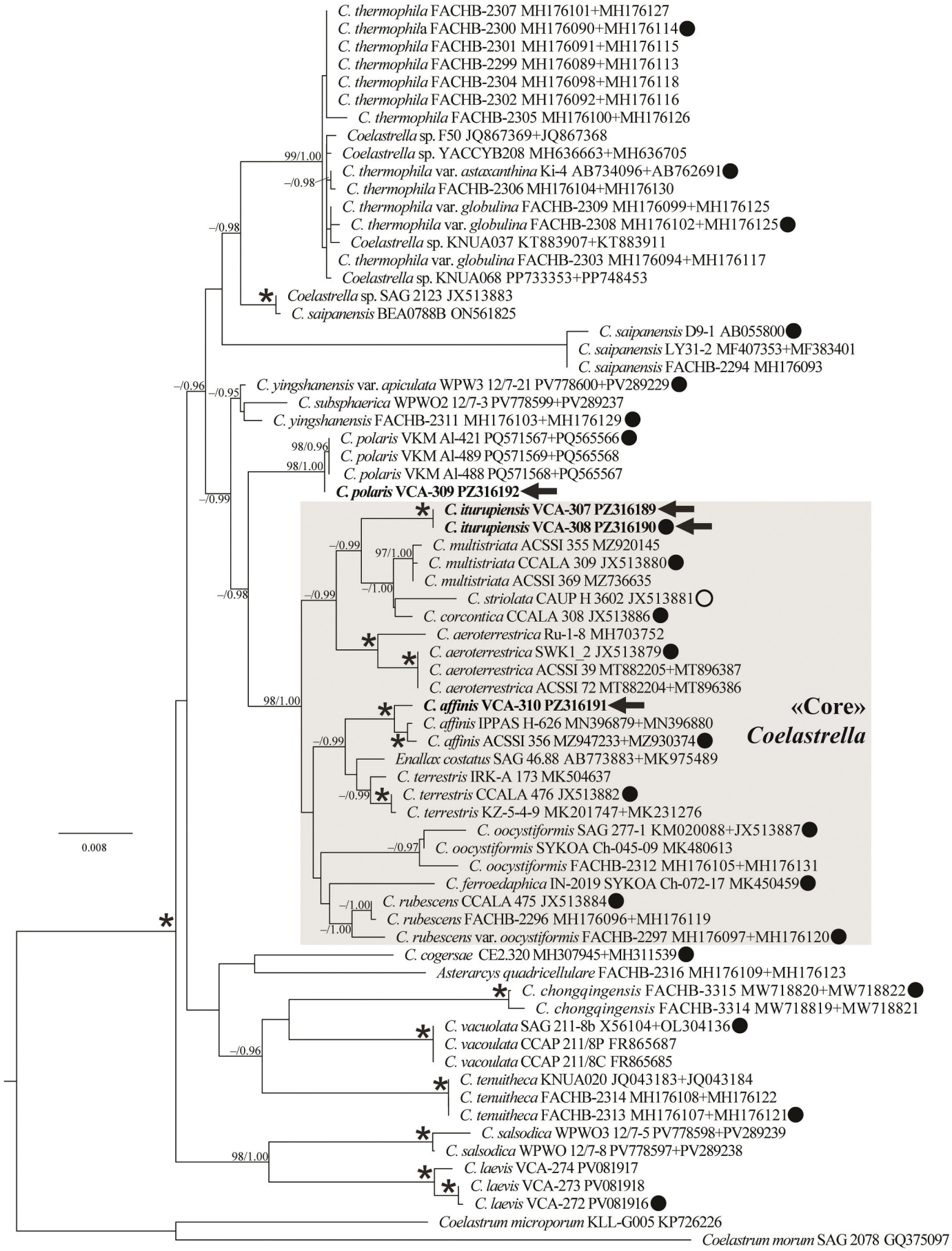


Fig. 25. Maximum likelihood phylogeny of *Coelastrella* showing position of the new strains (arrows and bold) based on the combined SSU and ITS rDNA sequence data (70 sequences, 2396 aligned positions). Black circles mark type strains. Unfilled circle = the type species of the genus. Support values [(UFBP) ≥ 95% and (PP) ≥ 0.95: ML/BI] are provided at branches. Nodes with 100% UFBP and 1.00 PP are marked with asterisk.

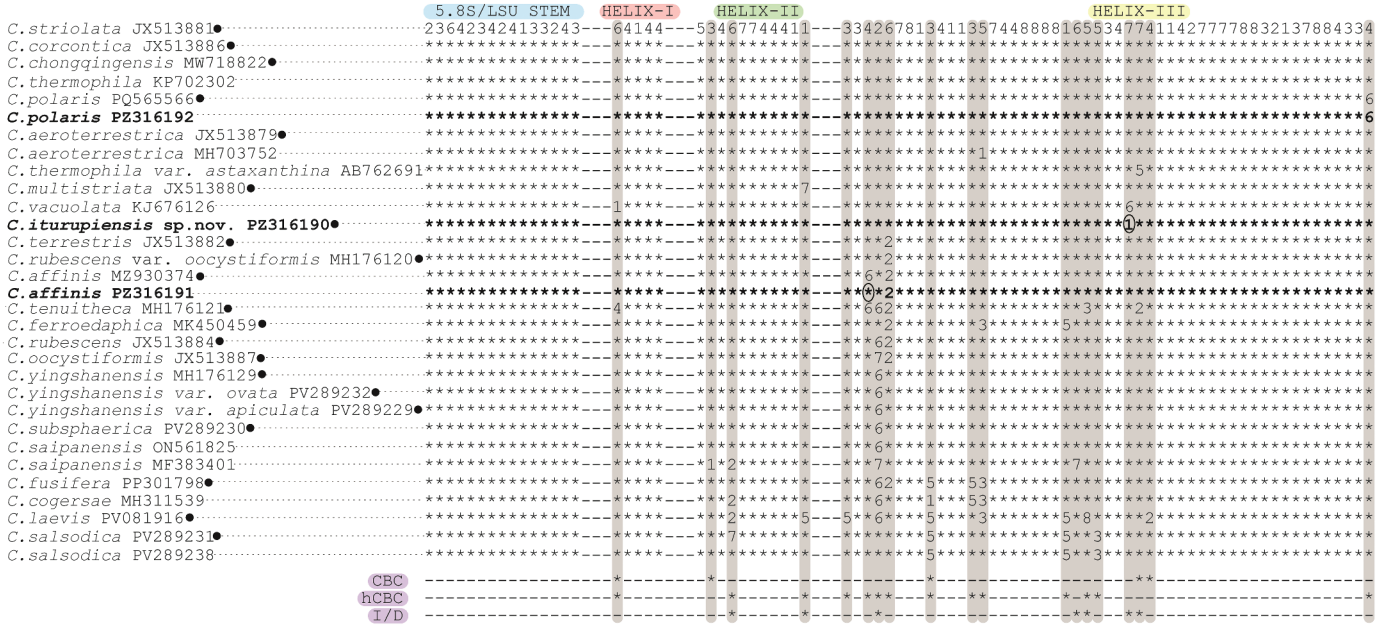


Fig. 26. Comparison of the conserved region of ITS2 among the species of *Coelastrella*. Number code for each base pair: 1 = A-U; 2 = U-A; 3 = G-C; 4 = C-G; 5 = G•U; 6 = U•G; 7 =mismatch; 8 = deletion, single or unpaired bases. The sequence of *C. striolata* is taken as a reference. Equal base-pairs are marked with asterisks. The barcode of new strains is shown in boldface. Grey shading indicates variable positions. Unique CBCs and hCBCs discussed in the text are circled. Black circles indicate type strains.

2021). In *C. iturupiensis*, SEM observations reveal yet another type of wall ornamentation. Instead of continuous ribs, each longitudinal element consists of a linear series of discrete, rounded warts. Such a pattern, warts organized into meridional rows, has been observed in some species of the allied genus *Desmodesmus*, where it is considered a type of 'warty' ornamentation (Hegewald, 1997; Staehelin & Pickett-Heaps, 1975). In *Desmodesmus*, warts can be arranged either randomly or in rows, and the size and shape of warts vary among species (Fawley *et al.*, 2011). Presence of row-forming warts in *C. iturupiensis* thus represents an interesting parallel and may reflect a shared developmental mechanism for cell wall patterning within the Scenedesmaceae.

A number of the cell-wall decorating rows in *C. iturupiensis* (16–24) overlaps with the ranges of *C. thermophila* (10–22 ribs), *C. levicostata* Korshikov (up to 20 ribs), and *C. corcontica/C. striolata/C. multistriata* (16–40 ribs). The new species can be distinguished from similar species by a combination of cell dimensions, autospore number, cell-wall ornamentation and other morphological characters. It differs from *C. thermophila* by the absence of prominent polar thickenings, a maximum of eight autospores rather than 16. From *C. levicostata* it differs by its smaller vegetative cells (4.9–12.6 × 3.7–10.7 µm vs. 12–17 µm). It differs from *C. corcontica* by the absence of cell aggregates and from *C. striolata* and *C. multistriata* by the lower maximum number of autospores (8 vs. 16).

Pigmentation should nevertheless be interpreted with caution as a diagnostic character. *Coelastrella* species are known to undergo secondary carotenogenesis in response to physiological and environmental conditions, including changes in light intensity, nutrient availability and other stress factors (Abe *et al.*, 2007; Goecke *et al.*, 2020; Liu *et al.*, 2026; Minyuk *et al.*, 2017). In the present study, strains VCA-307 and VCA-308 were maintained at relatively low irradiance (17.9–21.4 µmol photons m^{-2} s^{-1}) and 20–22°C, and prolonged cultivation resulted in an olive-green to pale-orange culture rather than the intense brick-red colouration reported for some congeners. Thus, the absence of brick-red pigmentation under our cultivation conditions should not be regarded as an absolute species-level character. Comparative cultivation of VCA-307 and VCA-308 under standardized temperature, irradiance and nutrient-stress regimes would be necessary to determine whether the observed pigmentation pattern represents a stable species characteristic or a response to cultivation conditions.

Occurrence of the new species in two distant localities approximately 1300 km apart (Primorsky Territory and Iturup Island) suggests that *C. iturupiensis* may be a widespread terrestrial alga in the southern part of the Russian Far East, possibly adapted to the region's temperate monsoon climate.

Strain VCA-309 from Primorsky Territory was nested within the clade of *C. polaris*, showing 99.4–99.8% sequence identity with the type strain from Yakutia (Krivina *et al.*, 2025). Its ITS2 secondary structure was nearly identical to that of the holotype, differing only by a single hemi-CBC in helix II outside the conservative barcode region. Previously, *C. polaris* was known only from the Sakha Republic (Pleistocene Park) and the New Siberian Islands, high-latitude Arctic permafrost soils. Our finding in the temperate monsoon climate of Primorsky Territory represents a remarkable southward range extension of more than 2500 km. Moreover, two recently deposited GenBank sequences (PX549900, PX549903) from a lichen thallus in Kostroma Oblast (central European Russia) suggest that

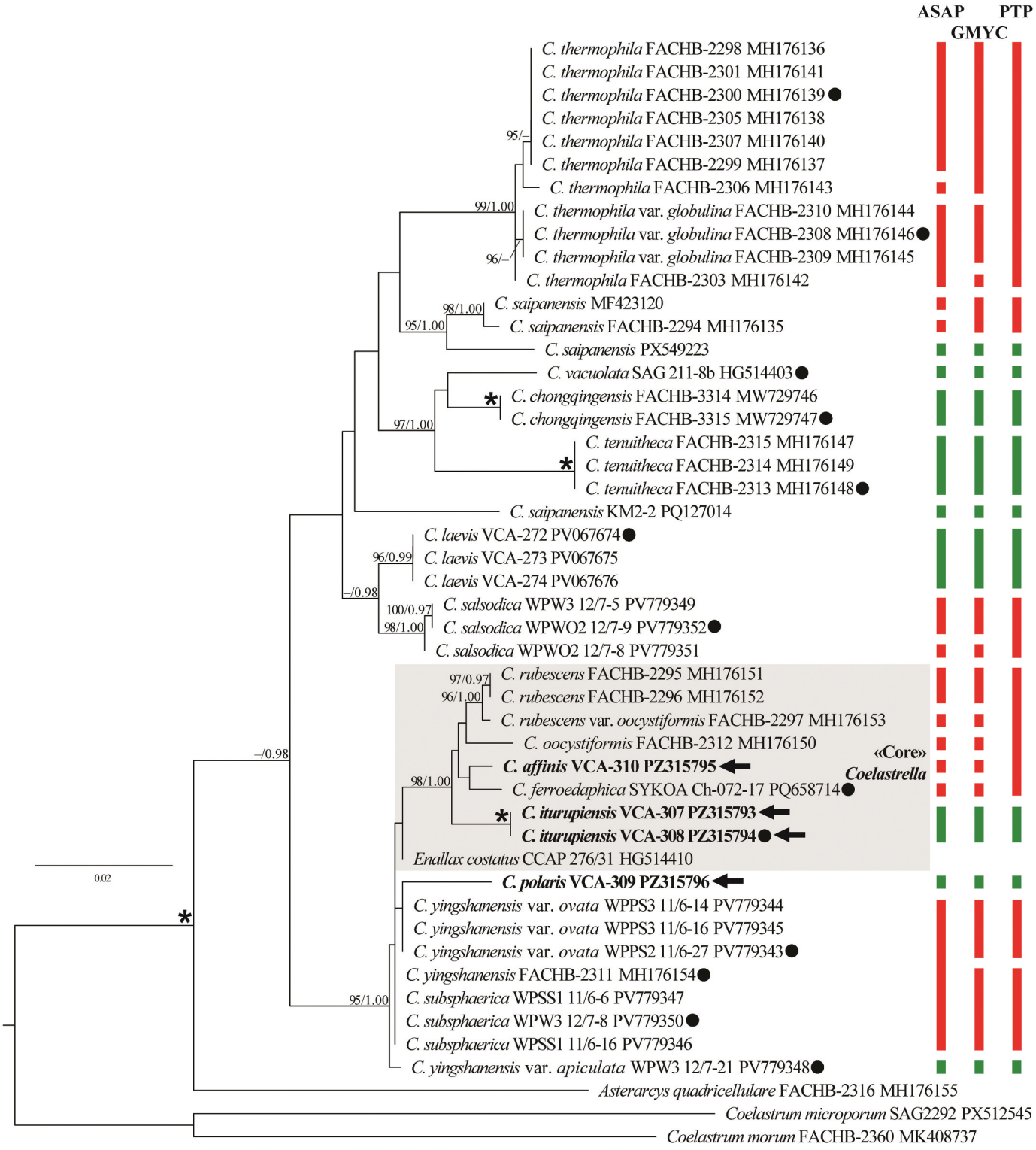


Fig. 27. Maximum likelihood phylogeny of *Coelastrella* based on *tuf*A gene sequence data (900 aligned positions, 48 sequences) showing the position of the new strains. See Fig. 25 legend for details. Rectangles indicate results of species delimitation using the ASAP, GMYC, and PTP methods (clusters of species-level sequences). Green rectangles represent identical clusters across all three methods, while red rectangles indicate disagreement in delimitation results.

C. polaris may be widely distributed across northern Eurasia, occurring not only in cryogenic soils but also as a lichen epiphyte or symbiote. This suggests a broader species ecological plasticity than it was assumed initially.

Strain VCA-310 clustered with the type of *C. affinis* (Krivina *et al.*, 2024). Morphologically, it matched the original description except for minor differences in cell dimensions and lack of visible pigment accumulation in old cultures, features likely influenced by cultivation conditions (Goecke *et al.*, 2020; Krivina *et al.*, 2024). Known distribution of *C. affinis* included Yakutia (soil) and the Moscow region (lichen epiphyte). Our record from Vladivostok extends its

range to the southern Russian Far East, demonstrating that this species can also inhabit moss-covered rock surfaces in a temperate monsoon climate.

SSU distances between accepted species can be very low (Table S3), whereas the ITS region provides a much higher sequence divergence, with interspecific distances rarely overlapping intraspecific variation (Krivina *et al.*, 2024; Wang *et al.*, 2019). Analysis of ITS2 secondary structure and detection of CBCs/hCBCs further strengthen species boundaries, as demonstrated for *C. iturupiensis* (unique barcode in helix III) and *C. polaris* (hCBC at the apex of helix III). Chloroplast *tuf*A gene, although less variable than ITS, still offers sufficient signal to separate many species. Combination of ITS rDNA and *tuf*A, together with morphological and ecological data, remains the most robust basis for description of new taxa in this genus (Wang *et al.*, 2019). Analysis of the ITS rDNA dataset revealed a well supported divergent clade containing 26 *Coelastrella* spp. accessions related to *Asterarcys quadricellularis*, suggesting existence of one or more undescribed species within the genus. One of these lineages was recently formally described as *C. fusifera* from freshwater habitats in India (Saini *et al.*, 2026), suggesting that this clade is a reservoir of overlooked species. However, a morphological comparison of these *Coelastrella* and *Asterarcys* strains is necessary for further taxonomic conclusions.

Several studies demonstrated ability of SSU rDNA and ITS rDNA regions to distinguish *Coelastrella* species in combination with GMYC, ASAP, and PTP methods (Krivina *et al.*, 2024, 2025). Krivina *et al.* (2025) showed that among all species discrimination algorithms, GMYC is the best for variable barcodes. Our analyses identified 15–22 species-level clusters in the *tuf*A dataset (Fig. 27). In most cases, lineages identified by the species delimitation algorithms were congruent with the morphology-based species. In contrast to that, sequences of *C. yingshanensis* and *C. subsphaerica* were identical and belonged to the same cluster despite their separate taxonomic assignments. Indeed, close relationship of these morphologically similar species, both in nuclear (ITS distance ca. 2%) and chloroplast markers, suggests that their divergence lies at the threshold values for intra-/interspecific variability for *tuf*A. We also noted a high intraspecific distance in *C. saipanensis* (1.51%) with one recently deposited sequence from Vietnam (PX549223), differing substantially from two other sequences of the same species. All three delimitation methods split this clade into two–three species clusters. Another sequence assigned to *C. saipanensis* (KM2-2, PQ127014) formed a separate branch in the *tuf*A tree with a divergence of 3.88% and was consistently recognized as a distinct species by all methods. Overall, species delimitation using GMYC, ASAP, and PTP did not fully match morphological species boundaries, with GMYC performing best among the three, although it still splits *C. thermophila, C. saipanensis*, and *C. salsodica* Ambrocio Paque, M.W. Fawley & K.P.Fawley into two to three groups each.

ACKNOWLEDGEMENTS

We are grateful to T.Yu. Gorpenchenko (Federal Scientific Center of the East Asia Terrestrial Biodiversity, FEB RAS, Vladivostok, Russia) for assistance with confocal laser scanning microscopy and to E.A. Zharikova and I.V. Kiseleva (same center) for assistance with soil classification. We are deeply indebted to D.V. Fomin for the helpful assistance during observations with the scanning electron microscope in the Department of Electron Microscopy of the A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Vladivostok, Russia). We sincerely thank E.E. Vekhova (same center) for her valuable guidance in SEM sample preparation. We also thank V.M. Kazarin (Federal Scientific Center of the East Asia Terrestrial Biodiversity, FEB RAS) for assistance with SEM observations at the Instrumental center of biotechnology and gene engineering.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

FUNDING

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 124012400285-7).

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