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The demise of the genus *Scotiellopsis* Vinatzer (Chlorophyta)

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With 8 figures and 2 tables

Abstract: The genus *Scotiellopsis* was established in 1975 by Vinatzer for a newly described species *Scotiellopsis rubescens*, but after revision by Punčochářová & Kalina in 1981 it included five species. The genus comprises coccoid green algae typically with apical thickenings and meridional ribs. Its similarity to another genus, *Coelastrella* Chodat, was noted, and indeed, two *Scotiellopsis* species were formally transferred to the genus *Coelastrella* on the basis of molecular evidence. Here we studied authentic strains of two more *Scotiellopsis* species, including the type species *S. rubescens*, to resolve the taxonomic status of the genus. Both 18S rDNA and ITS2 sequences indicate that *S. rubescens* cannot be separated from *Coelastrella* species on the generic level. We therefore propose a new combination *Coelastrella rubescens*, rendering *Scotiellopsis* a junior synonym of *Coelastrella*. The second species studied, *Scotiellopsis reticulata*, is shown to be closely related, if not conspecific, with *Scenedesmus rubescens* or *Scenedesmus dissociatus* representing the (sub) genus *Acutodesmus*. The position of the remaining *Scotiellopsis* species, *Scotiellopsis levicostata*, remains uncertain due to a lack of a culture.

Key words: *Acutodesmus*; *Coelastrella*; ITS2; Scenedesmaceae; *Scotiellopsis.*

Introduction

The taxonomic history of the green algal genus *Scotiellopsis* Vinatzer is somewhat convoluted. Vinatzer (1975) observed a soil alga with citriform (lemon-shaped) cells with an apical thickening and a smooth cell wall changing with age to a wide oval-to-spherical shape without thickenings. To accommodate this organism he established a new genus *Scotiellopsis* with *Scotiellopsis rubescens* Vinatzer as a sole species, and he incorporated it into the family Oocystaceae (Chlorophyta). Vinatzer pointed

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out a morphological similarity with *Scotiella terrestris* Reisigl, but he stated that the difference between both species was in the existence of longitudinal ribs on a cell wall surface of *S. terrestris*.

One year later, Fott (1976) established a new green algal genus also using the name *Scotiellopsis*. This new genus, characterised by fusiform or citriform solitary cells with the cell wall raised to form longitudinal ribs and polar papillae, was proposed for those species previously classified in the genus *Scotiella* Fritsch that reproduce by autospores, including *Scotiella levicostata* Gollerbach (a type species of the genus *Scotiellopsis* sensu Fott), *Scotiella oocystiformis* Lund, and *Scotiella terrestris* Reisigl. However, while the paper was in proofs, Fott realized the homonymy of his genus *Scotiellopsis* with that described one year earlier by Vinatzer, and in an appendix to his paper (Fott 1976) he proposed an alternative name *Scotiellocystis* Fott (with *Scotiellocystis levicostata* (Gollerbach) Fott as the type species). Fott further established a new subfamily Scotiellocystoideae in the family Oocystaceae to accommodate his new genus.

Punčochářová & Kalina (1981) compared the cell wall structure of *Scotiellopsis rubescens* and *Scotiellocystis* species and found the same general pattern – the presence of longitudinal ribs and apical thickenings. Hence, these authors revised the description of *S. rubescens* by Vinatzer, who had claimed that the cell wall is smooth. Following this observation, Punčochářová & Kalina formally transferred all three *Scotiellocystis* species into the genus *Scotiellopsis* (sensu Vinatzer) as new combinations and they furthermore described a new species, *Scotiellopsis reticulata* Punčochářová & Kalina, based on a morphological analysis of a strain isolated by Hindák. Punčochářová & Kalina (1981) also noted a close relationship of *Scotiellopsis* with *Coelastrella* Chodat. They mentioned that main differences between both genera are the cell shape and the absence of polar thickenings in *Coelastrella*.

Several years later, Kalina & Punčochářová (1987) moved the subfamily Scotiellocystoideae into the family Chlorellaceae and expanded it by including the genera *Coelastrella*, *Graesiella* Kalina & Punčochářová, *Kermatia* Kalina & Punčochářová, *Halochlorella* Dangeard, *Mychonastes* Simpson & Van Valkenburg, and *Auxenochlorella* (Shihira & Krauss) Kalina & Punčochářová. However, by sequencing the 18S rDNA region of several representatives of the Scotiellocystoideae, Hanagata (1998) revealed a polyphyletic nature of this subfamily and a distant relationship of most its members to true Chlorellaceae (including the genus *Chlorella*) placed in the class Trebouxiophyceae. Instead, a majority of species investigated turned out to be closely related to the genus *Scenedesmus* Meyen in the class Chlorophyceae. This group included also the species *Scotiellopsis oocystiformis* (Lund) Punčochářová & Kalina, *Scotiellopsis terrestris* (Reisigl) Punčochářová & Kalina, and *Coelastrella multistriata* (Trenkwalder) Kalina & Punčochářová, which were for this reason reclassified into the genus *Scenedesmus* (Hanagata 1998). The opinion of this author changed only two years later with a broader study of 18S rDNA sequences of Scenedesmaceae that led Hegewald & Hanagata (2000, 2002) to reinstate the combination *Coelastrella multistriata* and to propose new combinations *Coelastrella oocystiformis* (Lund) Hegewald & Hanagata and *Coelastrella terrestris* (Reisigl) Hegewald & Hanagata.

At present, three species remain classified in the genus *Scotiellopsis* – *Scotiellopsis rubescens* (the type species), *Scotiellopsis reticulata*, and *Scotiellopsis levicostata* (Gollerbach) Punčochářová & Kalina. Since no molecular data have been reported from these species, their phylogenetic position and taxonomic status are uncertain. In this report we provide molecular evidence that the "residual" genus *Scotiellopsis* is polyphyletic and that its type species, *S. rubescens*, should be reclassified as a species of the genus *Coelastrella*, rendering the former genus a junior synonym of the latter.

Material and methods

A total of 10 algal strains representing the genera *Scotiellopsis* and *Coelastrella* were obtained from the culture collections CCALA (Culture Collection of Autotrophic Organism, Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic), CAUP (Culture Collection of Algae of the Charles University in Prague, Czech Republic), and SAG (Culture Collection of Algae at the University of Göttingen, Germany) (Table 1). The strain SWK1:2 of *Coelastrella aeroterrestrica* Tschaikner, Gärtner & Kofler (maintained in the ASIB collection, Culture Collection of Algae at the Botanical Institute, University of Innsbruck, Austria) was kindly provided by Prof. Georg Gärtner, University of Innsbruck. Strains for microscopic and molecular analyses were cultivated in liquid and/or agar-solidified BBM medium (Bischoff & Bold 1963).

For scanning electron microscopy (SEM), the CCALA 474, CCALA 475, and CCALA 476 strains were grown in liquid and agar-solidified BBM medium for 3–4 weeks. Glass coverslips were coated with three plies of poly-L-lysine solution (1:10 in distilled water) to aid adhesion of the cells. Gradual dehydration of the cells was achieved by transferring into an acetone series of the 30, 50, 70, 90, 95, 99 and 100% concentration. Subsequently, cells were critical-point dried with CO_2 and finally sputter-coated with gold. A Phenom Desktop scanning electron microscope was used to visualise the fixed cells.

For molecular analyses, genomic DNA was extracted using the Invisorb® Spin Plant Mini Kit (Invitek). Amplification of the 18S rDNA region was achieved using the forward (F) primer according to Katana et al. (2001) and the reverse primers 18L (Hamby et al. 1988) or 1650Rvivi (Kipp 2004). The ITS1-5.8S-ITS2 rDNA region was amplified using the 1500af primer (Helms et al. 2001) and the ITS4 primer (White et al. 1990). PCR products were purified using the JetQuick PCR Product Purification Kit (Genomed). Sequencing of the purified PCR products was done using an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730xl) at Macrogen Corp. in Seoul, Korea; sequencing primers were those used for PCR plus additional internal primers according to Katana et al. (2001) for sequencing the long 18S rDNA region. Sequencing reads were assembled and the contigs were manually edited using the SeqAssem 09/2004 DNA sequence assembly software (Hepperle 2004). The newly determined sequences were deposited at GenBank with accession numbers indicated in Table 1.

Mega 4.0 (Tamura et al. 2007) was used to build and manually refine multiple alignments of 18S rDNA and ITS2 rDNA sequences. The 18S rDNA alignment comprised seven new sequences of *Scotiellopsis* and *Coelastrella*, 115 other sequences of the family Scenedesmaceae (for accession numbers see Fig. 1) were selected from the GenBank database on the basis of extensive searches by blastn at National Centre for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/). For phylogenetic analyses, positions with deletions in most sequences were removed from the alignment, yielding 1748 unambiguously aligned positions (the alignment is available at http://www1.osu.cz/~elias/data/Scotiellopsis_paper.html). A maximum likelihood (ML) tree was inferred from the 18S rDNA sequence alignment using RAxML 7.0.4 (Stamatakis 2006). The substitution model employed was GTR+Γ4 and the search procedure included rapid bootstrapping on 100 replicates followed by a thorough ML search on the original dataset. A tree was also inferred using MrBayes 3.1 (Huelsenbeck & Ronquist 2001). Two parallel Markov chain Monte Carlo runs were carried out for 1,850,000 generations, each with one cold and three heated chains employing the GTR+Γ+I evolutionary model. Trees were sampled every 100 generations, the initial 5,000 trees from each

Table 1. Algal strains investigated in this study. Abbreviations of culture collections: CAUP – Culture Collection of Algae at Charles University in Prague, Czech Republic, CCALA – Culture Collection of Autotrophic Organism at Institute of Botany, Academy of Science in Třeboň, Czech Republic, SAG – Culture Collection of Algae at the University of Göttingen, Germany, ASIB – Culture Collection of Algae at Botanical Institute of University at Innsbruck, Austria. *C. aeroterrestrica* has been described recently by Tschaikner et al. (2008), but it is unclear from the paper who is the actual isolator of the strain. Since the ITS1-5.8S-ITS2 region of the two independent strains of *C. terrestris* proved identical, we did not sequence the 18S rDNA from *C. terrestris* CAUP H 4403. Strains indicated as "authentic" are the strains derived from the original culture on which the respective species was described; authentic strains are no longer available for *C. striolata* and *C. terrestris*. The GenBank accession numbers printed in bold were newly determined in this study.

Original name	Strain	Strain origin	18S rDNA GenBank accession number	ITS1-5.8S-ITS2 rDNA GenBank accession number
Coelastrella aeroterrestrica	ASIB SWK 1:2	Tschaikner et al., 2008, authentic	**JX513879**	**JX513879**
Coelastrella corcontica	CCALA 308	Kalina 1967/9, authentic	AB037082	**JX513886**
Coelastrella multistriata	CCALA 309	Trenkwalder 1975, authentic	**JX513880**	**JX513880**
Coelastrella oocystiformis	SAG 277-1	Fogg (before 1957), authentic	AB012848	**JX513887**
Coelastrella striolata	CAUP H 3602	Kalina 1969/1	**JX513881**	**JX513881**
Coelastrella terrestris	CCALA 476	Hindák 1963/14	**JX513882**	**JX513882**
Coelastrella terrestris	CAUP H 4403	Trenkwalder 1975	-	**JX513888**
Coelastrella sp.	SAG 2123	Lang, D3a	**JX513883**	**JX513883**
Scotiellopsis rubescens	CCALA 475	Vinatzer, authentic	**JX513884**	**JX513884**
Scotiellopsis reticulata	CCALA 474	Hindák 1967/40, authentic	**JX513885**	**JX513885**

run were discarded as "burn-in" and posterior probabilities of tree bipartitions were calculated on the basis of the consensus of the remaining trees.

The multiple alignment of ITS2 rDNA sequences was manually corrected using 4SALE 1.5 (Seibel et al. 2006, 2008) simultaneously with constructing secondary structure models of the ITS2 region aided by previously published secondary structure models for the ITS2 region in the Scenedesmaceae (Fawley et al. 2011). Final editing and visualisation of the ITS2 model of *C. striolata* Chodat (Fig. 2) was done using VARNA 3.7 (Darty et al. 2009).

Results

We complemented the available set of 18S rRNA gene sequences from *Scotiellopsis* and *Coelastrella* species by sequencing the remaining species of both genera represented by strains in culture collections (Table 1). Some of the strains harboured one (*C. striolata* CAUP H 3602, *C. multistriata* CCALA 309, *S. rubescens* CCALA 475, *C. terrestris* CCALA 476) or two (*C. aeroterrestrica* ASIB SWK 1:2) putative group I introns in the 18S rDNA region sequenced.

Table 2. Hemi-CBCs between species of the "core" *Coelastrella* clade. The hemi-CBCs between the species compared and their locations (with respect to the reference ITS2 structure of *C. striolata* CAUP H 3602, see Fig. 2) are listed above the diagonal; Roman numerals denote helices of the ITS2 structure, Arabic numerals mean the position of the substitution resulting in hemi-CBC. The number of hemi-CBCs between each species pair is shown below the diagonal. There were no hemi-CBCs in helix IV and no CBCs in either helix I, II, III, or IV.

	Coelastrella striolata CAUP H 3602	*Coelastrella multistriata* CCALA 309	*Coelastrella corcontica* CCALA 308	*Coelastrella terrestris* CAUP H 4403	*Coelastrella oocystiformis* SAG 277-1	*Coelastrella aeroterrestrica* ASIB SWK1:2	*Coelastrella rubescens* CCALA 475
Coelastrella striolata CAUP H 3602		I:38 C-G x U-G II:103 U-G x U-A	I:38 C-G x U-G II:103 U-G x U-A	I:38 C-G x U-G II:102 G-C x G-U III:219 U-G x U-A	III:219 U-G x U-A	I:38 C-G x U-G II:105 U-A x U-G	III:219 U-G x U-A, 220 U-A x U-G
Coelastrella multistriata CCALA 309	2		–	II:102 G-C x G-U III:219 U-G x U-A	I:38 C-G x U-G III:219 U-G x U-A	II:105 U-A x U-G	I:38 C-G x U-G III: 219 U-G x U-A; 220 U-A x U-G
Coelastrella corcontica CCALA 308	2	0		II:102 G-C x G-U III:219 U-G x U-A	I:38 C-G x U-G III:219 U-G x U-A	II:105 U-A x U-G	III:219 U-G x U-A, 220 U-A x U-G
Coelastrella terrestris CAUP H 4403	3	2	2		I:38 C-G x U-G II:102 G-C x G-U	II:102 G-C x G-U; 105 U-A x U-G III:219 U-G x U-A	I:38 C-G x U-G II:102 G-C x G-U III:220 U-A x U-G
Coelastrella oocystiformis SAG 277-1	1	2	2	2		I:38 C-G x U-G II:105 U-A x U-G III:219 U-G x U-A	–
Coelastrella aeroterrestrica ASIB SWK1:2	2	1	1	3	3		I:38 C-G x U-G II:105 U-A x U-G III:219 U-G x U-A; 220 U-A x U-G
Coelastrella rubescens CCALA 475	2	3	2	3	0	4	

Fig. 1. Maximum likelihood phylogenetic tree inferred from 18S rDNA sequences of the family Scenedesmaceae. Numbers at branches are branch support values from a bootstrap analysis and posterior probabilities from a Bayesian analysis (only values >50 and 0.9, respectively, are shown). Following a previous analysis employing a non-senedesmacean outgroup (Eliáš et al. 2010), the tree is rooted with the sequence of the strain Scenedesmaceae sp. Mary 9/21 BT-16w, but note that there in no resolution among the deepest branches of the tree, so the actual position of the root remains unknown. Sequences newly determined in this study are highlighted in bold. Leaves are labelled with GenBank accession numbers of the sequences followed by the taxon name; in several cases the taxon name differs from that indicated in the respective GenBank record, as we followed recent

Based on blastn searches and preliminary phylogenetic analyses, all the newly determined sequences were robustly nested within sequences of the family Scenedesmaceae. We therefore performed a more detailed phylogenetic analysis focusing on available scenedesmacean 18S rDNA sequences (Fig. 1). The resulting tree shows distant relationship between the two *Scotiellopsis* species investigated. *S. rubescens* belongs to a cluster dominated by sequences of the genus *Coelastrella*, specifically to a subgroup here designated as the "core" *Coelastrella* clade. The differences between the 18S rDNA sequence of *S. rubescens* and some of the *Coelastrella* species were very small, e.g. only a single substitution as compared to the *C. terestris* CCALA 476 or two substitutions as compared to *C. multistriata* CCALA 309. In contrast, *S. reticulata* is closely related to *Scenedesmus* (=*Dactylococcus*) *dissociatus* (Verses & Trainor) Hegewald & Hanagata and *Scenedesmus rubescens* (Dangeard) Kessler, Schafer, Hummer, Kloboucek & Huss within a more inclusive clade corresponding to the (sub) genus *Acutodesmus* Hegewald. The 18S rDNA sequence of *S. reticulata* is actually identical to that of *S. rubescens* CCAP 232/1 (an authentic strain of the species), while the 18S rDNA sequence of *S. dissociatus* UTEX 1537 (also an authentic strain of the species) differs from that of *S. reticulata* by two deletions.

To gain more insights into the taxonomic status of *S. rubescens*, we sequenced the ITS1-5.8S-ITS2 rDNA region of this species as well as of other nominal species of the "core" *Coelastrella* clade (using the respective authentic strains, if available) and performed a comparative analysis of the ITS2 sequences employing a model of its secondary structure, paying particular attention to compensatory base changes (CBCs) and hemi-compensatory base changes (hemi-CBCs) as defined by Coleman (2003). Differences in the sequences were mapped onto a predicted secondary structure of the ITS2 region of the type species of the genus *Coelastrella*, *C. striolata* (strain CAUP H 3602; Fig. 2). Apart from variability in the loop regions and in the terminal parts of individual arms, substitutions and even deletions were recorded also in the helical regions of the ITS2 structure. Some of them resulted in hemi-CBCs in helices I, II and III (positions 38, 102, 103, 105, 219, 220 of the reference structure of *C. striolata*), whereas no CBC was observed for any species pair (Table 2). Considerable differences between some species were visible in the terminal part of the first branch of helix I and of helix II (Fig. 2).

The sequence of the ITS1-5.8S-ITS2 rDNA region of *S. reticulata* proved to be very similar to that of *S. dissociatus* UTEX 1537, with only one indel and two substitutions in the ITS1 region and a single substitution in the ITS2 region resulting in a hemi-CBC in the second branch of helix I of the ITS2 structure (not shown). Since the morphological documentation available for *S. reticulata* was insufficient (see Discussion), we reinvestigated its morphology by SEM (Figs 3–4). The ornamentation of the cell surface was mainly by a dense net of ribs barely raised above the surface. The morphology

taxonomic revisions not reflected by the database records. Authentic strains of interest are marked with black dots. Identical sequences attributed to the same nominal taxon are indicated in parentheses with the specification of the respective accession number and strain or isolate name. Important well-supported clades are annotated on the right. For the sake of clarity, the part of the tree representing sequences of the genus *Desmodesmus* is shown only schematically.

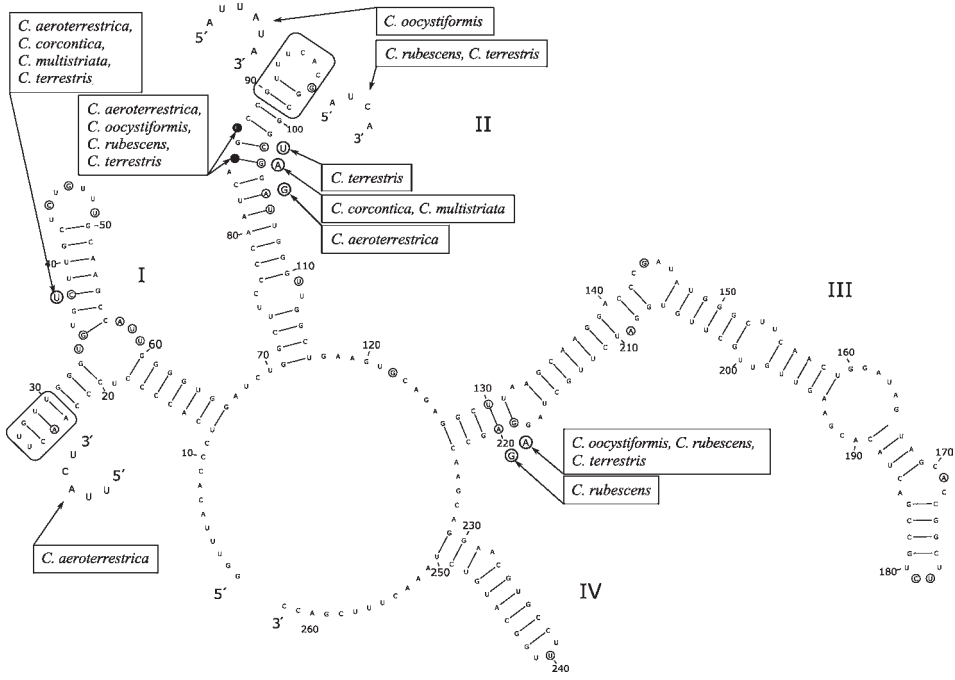
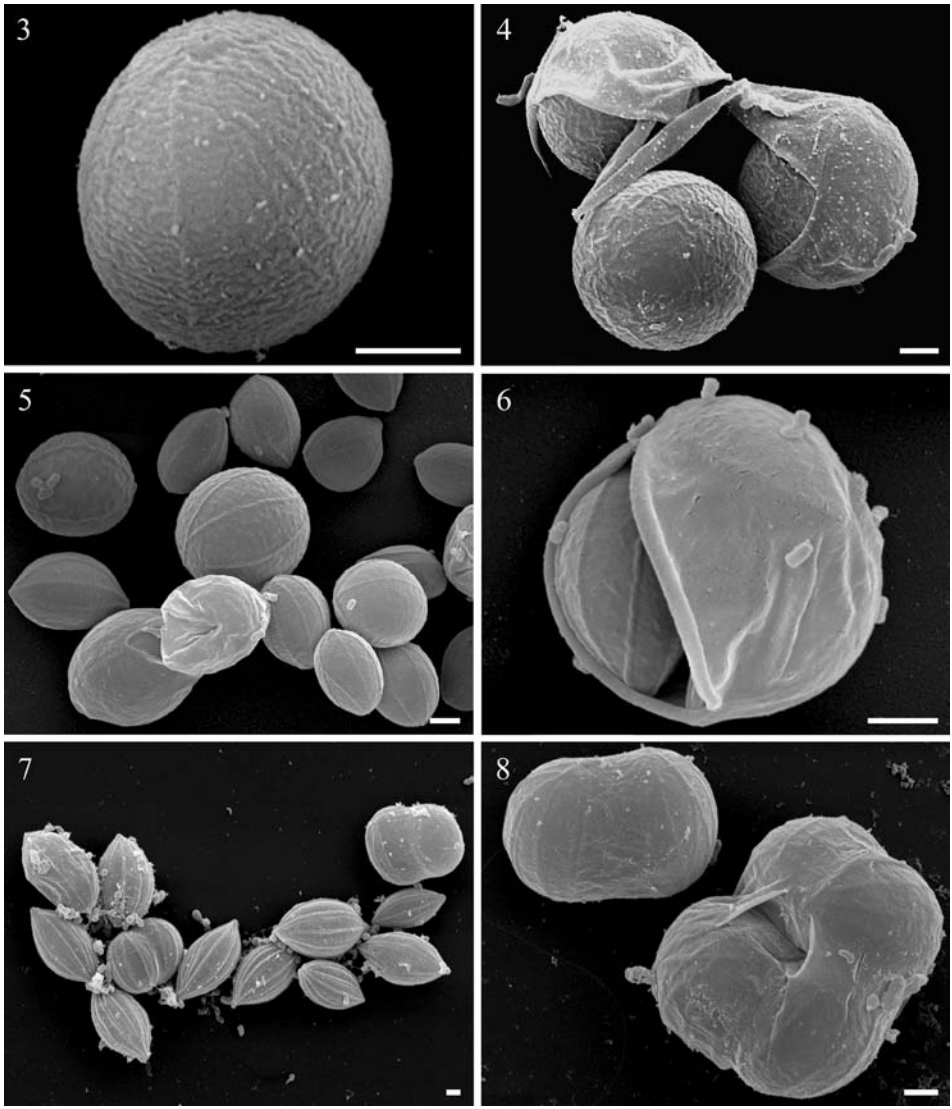


Fig. 2. The variability in the ITS2 region among the „core“ *Coelastrella* species. Differences in ITS2 sequences are mapped onto a secondary structure model of the ITS2 region of *Coelastrella striolata* (strain CAUP H 3602). Positions with substitutions are indicated by circles. For substitutions representing hemi-CBCs the substituted nucleotide is shown together with the species name(s) bearing the respective substitution. Deletions are indicated by black filled circles. Substantially different termini in some strains of the first branch of helix I and of helix II (boxed) are shown in full. ITS2 sequences of the following strains were compared with that of *C. striolata* CAUP H 3602: *C. multistriata* CCALA 309, *C. corcontica* CCALA 308, *C. terrestris* CAUP H 4403, *C. oocystiformis* SAG 277-1, *C. aeroterrestrica* ASIB SWK1:2, *C. rubescens* CCALA 475.

of *S. reticulata* grown on an agar-solidified medium did not differ from that grown in a liquid medium (data not shown). The morphology of *S. rubescens* was markedly different, with the cells bearing apical thickenings and prominent meridional ribs, as reported previously (Punčochářová & Kalina 1981; Figs 5–6).

Discussion

A probable close relationship between the genera *Scotiellopsis* and *Coelastrella* was previously noted on several occasions (Punčochářová & Kalina 1981; Hegewald & Hanagata 2000), but the shape of adult cells and autospores with (in *Scotiellopsis*) or without (in *Coelastrella*) polar thickenings and the formation of 4–12(20) (in *Scotiellopsis*) or 16–40 (in *Coelastrella*) meridional ribs were viewed as features substantial enough to distinguish the two genera (Kalina & Punčochářová 1987).



Figs 3–8. SEM of *Scotiellopsis reticulata* (strain CCALA 474), *Coelastrella* (=*Scotiellopsis*) *rubescens* (strain CCALA 475), and *Coelastrella terrestris* (strain CCALA 476). Fig. 3. *S. reticulata*, vegetative cell. Fig. 4. *S. reticulata*, release of autospores. Fig. 5. *C. rubescens* – vegetative cells. Fig. 6. *C. rubescens* – autosporangium with autospores. Fig. 7. *C. terrestris* – vegetative cells. Fig. 8. *C. terrestris* – autosporangia. Scale bar = 2 μm.

However, by analyzing sequences of the 18S rDNA region from *S. terrestris*, *S. oocystiformis* and several *Coelastrella* species, Hegewald & Hanagata (2000) demonstrated a close phylogenetic relationship of these two *Scotiellopsis* species to the genus

Coelastrella and recombined them into the latter genus. The key point of our study is extending the previous analysis by data from an authentic strain of *S. rubescens*, the type species of *Scotiellopsis*, which thus enabled us to finally resolve the question about the mutual status of *Scotiellopsis* and *Coelastrella*.

Indeed, our results unambiguously show that the status of *Scotiellopsis* as a separate genus is untenable. First, the 18S rDNA sequence of *S. rubescens* is very similar to the sequences of *Coelastrella* species, particularly to a group including *C. striolata*, *C. multistriata*, *C. corcontica* (Kalina & Punčochářová) Hegewald & Hanagata, *C. aeroterrestrica*, *C. terrestris*, and *C. oocystiformis*. In the tree inferred from the 18S rDNA sequences, *S. rubescens* and these species constitute a tight cluster ("core" *Coelastrella*) excluding some nominal *Coelastrella* species (*C. saipanensis* Hanagata, *C. vacuolata* (Shihira & Krauss) Hegewald & Hanagata, *Coelastrella* sp. SAG 217.5, and *Coelastrella* sp. SAG 2123; Fig. 1). Second, the affinity of *S. rubescens* to *Coelastrella* is evident also on the ITS2 sequences, as no CBC and only 0–4 hemi-CBCs separate *S. rubescens* from the *Coelastrella* species investigated. Third, the morphological features of *S. rubescens* fit well the general morphological habitus of the genus *Coelastrella*, here exemplified by SEM photos of *C. terrestris* CCALA 476 (Figs 7–8). Thus, the very close phylogenetic relationship between *S. rubescens* and the species of the genus *Coelastrella*, including the type species *C. striolata*, combined with the highly similar morphology of these organisms, implies that they are best classified in the same genus. Since *Coelastrella* was described by Chodat in 1922 and *Scotiellopsis* by Vinatzer in 1975, the former name has priority; we therefore propose reclassification of *S. rubescens* into the genus *Coelastrella* as a new combination (see Taxonomic conclusion below). Given the fact that *S. rubescens* is the type species of *Scotiellopsis* Vinatzer, this taxonomic act renders the genus *Scotiellopsis* a junior synonym of the genus *Coelastrella*.

Two more species remain presently classified in the genus *Scotiellopsis* – *S. reticulata* and *S. levicostata*. We discuss their taxonomic fate in turn.

Punčochářová & Kalina (1981), while describing *Scotiellopsis reticulata*, indicated its similarity to *Scenedesmus rubescens* (in the overall morphology as visible in the light microscope). However, their observation of the cell wall structure by transmission electron microscopy of empty cell walls led them to conclude that apart a dense net of anastomosing ribs, the cell wall surface was sometimes ornamented with 3–4 more prominent longitudinal ribs running to the poles, reminiscent of those found in *Scotiellopsis* and *Coelastrella* species. Based on this, Punčochářová & Kalina assigned their new species into the genus *Scotiellopsis*. They also reported a SEM photo of a fixed cell of *S. reticulata* that shows a net of highly projecting anastomosing ribs. Our reinvestigation by SEM of the *S. reticulata* morphology provides a different picture with the cell surface covered by a network barely raised above the surface (Figs 3–4), suggesting that the appearance of the cell in Punčochářová & Kalina is artificial resulting from its collapse due to poor fixation. We actually cannot exclude the possibility that the pattern observed on the cell surface of *S. reticulata* in our experiments is also due to the cell wall being artificially wrinkled during preparation of the sample. Regardless, the surface pattern of *S. reticulata* is different from that of typical *Scotiellopsis* species characterised by prominent meridional ribs (Figs 5–6).

Furthermore, the 18S rDNA sequence of *S. reticulata* (the same strain as studied by Punčochářová & Kalina) determined in this study is identical to that of *S. rubescens*, indicating that the hints from light microscopy pointing towards an affinity to *S. rubescens* were more significant that the alleged similarity of the cell wall pattern to that of *Scotiellopsis*.

An additional taxon closely related to *S. reticulata* is *S. dissociatus*. The 18S rDNA sequence as determined by Hegewald & Hanagata for *S. dissociatus* differs from that of *S. reticulata* (and from that of *S. rubescens*) only by two deletions, which appear unusual with respect to the general consensus of the scenedesmacean 18S rDNA sequences and need to be confirmed by resequencing the respective strain. The ITS1-5.8S-ITS2 region of *S. dissociatus* differs from that of *S. reticulata* only in one indel and three substitutions, suggesting again a very close relationship, if not conspecificity. However, some morphological differences between *S. reticulata* on the one side and *S. rubescens* and *S. dissociatus* on the other were reported; particularly, *S. rubescens* and *S. dissociatus* form small colonies of cells interconnected by appendages emanating from the cell poles (Verses & Trainor 1966, Kalina & Punčochářová 1987) that were not observed in *S. reticulata*. Hence, we refrain from formally proposing a taxonomic revision of this group of taxa at this point, as additional studies are apparently needed, specifically sequencing the ITS2 region from *S. rubescens* and possibly other highly variable markers from all three species complemented by a rigorous morphological comparison. If the three species were eventually shown to be synonymous, *S. rubescens* (with the basionym *Halochlorella rubescens* Dangeard 1966) would have priority. An uncertainty surrounds also the generic assignment of these species. Phylogenetically they belong to a clade of *Scenedesmus* species that is well separated in the 18S rDNA tree from a clade comprising the type species of the genus *Scenedesmus*, *S. obtusus* Meyen (*Scenedesmus* sensu stricto in Fig. 1), and which might be equated with the subgenus *Acutodesmus*, raised by Tsarenko (in Tsarenko & Petlevanny 2001) to the level of a separate genus. Recently, Hegewald et al. (2010) proposed that *Acutodesmus* be kept as a subgenus of a broadly defined genus *Scenedesmus* based on their phylogenetic analysis of ITS2 sequences and structures. The apparent discordance of the 18S rDNA and ITS2 phylogenies calls for additional analyses with different methods, markers and taxon sampling.

The status of the remaining *Scotiellopsis* species, *S. levicostata*, also remains uncertain, since there is no authentic strain for this species and even our more extensive survey of strains with *Scotiellopsis* or *Coelastrella* morphology have not so far revealed any strain that could be unambiguously determined as *S. levicostata* (data not shown). As previously discussed by Punčochářová & Kalina (1981), it is possible that the Gollerbach's *S. levicostata* is the same species as described later by Koshikov (1953) under the name *Coelastrella levicostata*. Korshikov differentiated *C. levicostata* from *S. levicostata* by the presence of only one branched chloroplast in the former species contrasting with multiple discoid chloroplasts observed by Gollerbach in the latter species, but this difference is questionable given the limits of light microscopy to accurately describe the chloroplast shape in these small organisms (Punčochářová & Kalina 1981). Cultured strains of *C. levicostata* are likewise not available, leaving the question of the position of this species open. If future investigations are able to define the actual phylogenetic position of *S. levicostata* and *C. levicostata*, several

alternative taxonomic situations may happen with respect to *S. levicostata*: (1) it will be recombined to the genus *Coelastrella* as *C. levicostata*; (2) it will be recombined into the genus *Coelastrella* with a new species epithet in case it is found to represent a species different from Korshikov's *C. levicostata*; (3) it is recombined into another existing genus; (4) it will be called *Scotiellocystis levicostata* (Gollerbach) Fott in case it is found to represent a genus of its own.

In addition to conclusions directed towards the genus *Scotiellopsis*, our study provides some new insights into the taxonomy of the genus *Coelastrella*. First, our analysis of the ITS2 sequences of seven "core" *Coelastrella* species, including the new combination *C. rubescens*, revealed no CBC and only 0–4 hemi-CBCs between the species. The absence of CBCs does not imply that the whole group should be automatically treated as a single species (Müller et al. 2007), but the species concept in *Coelastrella* needs more attention. Indeed, the absence even of hemi-CBCs between some species pairs should be addressed in the future, but at least the species of the pair "*Coelastrella* (=*Scotiellopsis*) *rubescens* – *C. oocystiformis*" exhibit distinctly different morphologies (regarding the cell size and the number of meridional ribs; Punčochářová & Kalina 1981) supporting their separate species status. A much higher molecular diversity in the genus *Coelastrella* than reflected by the current classification scheme for this taxon is indicated by the existing 18S rDNA sequences (Fig. 1). For example, the sequences of the strains CCMP 1625 (*Scenedesmus* sp.) or CCAP 11/64A ("*Chlamydomonas moewusii*", apparently a misidentified or contaminated culture) differ from the sequences of the named *Coelastrella* species to the degree suggesting that they may represent separate species; possible additional species might be a source of several sequences of environmental clones different from but branching together with the named *Coelastrella* species. It is possible that some of these potential extra species may correspond to *Coelastrella* species currently lacking molecular data, namely *C. levicostata* (see above) or *C. compacta* Skuja.

The phylogeny of the 18S rDNA sequences reported here raises many additional questions concerning the taxonomy of scenedesmacean algae. Most relevant to the subject of this study, the actual delimitation of the genus *Coelastrella* remains to be resolved, as species currently attributed to genera *Asterarcys* A.Comas Gonzales, *Graesiella*, *Scenedesmus*, or *Ettlia* Komárek are nested among taxa nominally representing *Coelastrella* (Fig. 1). Furthermore, the topology of the tree is incompatible with the subdivision of the family Scenedesmaceae advocated in a recent study by Hegewald et al. (2010) based on an analysis of the ITS2 sequences and structures, as their subfamilies Scenedesmoideae and Coelastroideae are polyphyletic in the 18S rDNA tree. It is obvious that further studies based on additional molecular markers (such as *rbc*L) and a wider taxon sampling are required for bringing a better order into the classification of these interesting organisms.

Taxonomic conclusion

Coelastrella rubescens (Vinatzer) Kaufnerová & Eliáš COMB. NOV.

BASIONYM: *Scotiellopsis rubescens* Vinatzer, 1975, Plant Syst. Evol. 123: 216

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