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Elliptochloris bilobata var. *corticola* var. nov. (Trebouxiophyceae, Chlorophyta), a novel subaerial coccal green alga*

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Abstract: We investigated a previously unidentified subaerial corticolous strain of the genus *Elliptochloris* Tschermak-Woess. The alga shares the generic morphological characters with *Elliptochloris bilobata*, the type species of the genus, but it has a thicker cell wall of adult globular cells, different chloroplast structure and it also differs in shape of elliptical autospores. The differences of the autospore shape between both species were evaluated using landmark-based geometric morphometrics. The 18S rDNA gene sequence of the new alga forms a monophyletic clade with the authentic strain of *E. bilobata* within the green algal class Trebouxiophyceae close to representatives of the genus *Coccomyxa*. We describe the new alga as *Elliptochloris bilobata* var. *corticola* var. nov.

Key words: subaerial algae; Trebouxiophyceae; *Elliptochloris*; Chlorophyta; taxonomy; geometric morphometrics

Introduction

The genus *Elliptochloris* was described by Tschermak-Woess (1980) with a single species *E. bilobata* Tschermak-Woess as a phycobiont of the terricolous lichen *Catolechia wahlenbergii* collected in an altitude 2200 m a.s.l. in Kärnten, Austria. The genus was characterized by coccal, irregularly globular cells with a single, pyrenoid-less lobate chloroplast. As a conspicuous character, Tschermak-Woess (1980) mentioned the presence of two types of asexual spores; two to four large, elliptical and 16 to 32 small, elongated autospores.

Later, Ettl & Gärtner (1995) transferred two additional species and one variety into the genus *Elliptochloris* based on the presence of these two types (small and large) of autospores. *Elliptochloris reniformis* (S. Watanabe) Ettl & Gärtner was originally described as *Chlorella reniformis* by Watanabe (1977) from a Japanese soil sample. This species produces both elongated and irregularly spherical autospores and most adult cells have an irregularly elliptical shape with conspicuously lobed chloroplasts. Hanagata et al. (1998) investigated the SAG 211-9b strain isolated originally by Pringsheim in 1939 from a garden basin in England, which was originally designated as *Chlorella saccharophila* (Krüger) Migula. They concluded, on the basis of morphological comparison, that this strain is probably conspecific with *Elliptochloris reniformis*. However, they established a new genus *Watanabea* for their strain on the basis of cell wall ultrastructural characteristics. They described *Watanabea reniformis* as a new species with the type strain SAG 211-9b. *Elliptochloris subsphaerica* (Reisigl) Ettl & Gärtner was originally described as *Pseudochlorella subsphaerica* by Reisigl (1964) from a soil sample collected in an altitude 3100 m a.s.l. in Austria. This species was differentiated from other members of the genus *Elliptochloris* by the presence of a pyrenoid, but it shared some other *Elliptochloris*-like morphological characters, notably the two types of autospores and a lobed chloroplast. Broady (1982) described *Pseudochlorella subsphaerica* var. *antarctica* from an Antarctic soil sample. It differed from the type variety of the species mainly by having smaller and shorter cells. Ettl & Gärtner (1995) transferred this variety into the *Elliptochloris* as *E. subsphaerica* var. *antarctica* (Broady) Ettl & Gärtner. Recently, Hoffmann et al. (2007) investigated morphological diversity and variability of *Elliptochloris*-like morphotypes and they added two additional *Elliptochloris* species occuring in Belgian soil micro-communities. In this exquisite morphological study several populations with *E. bilobata* features were investigated. The authors noted occurrence of two morphotypes differing by shape of elliptical spores, as well as by several other intracellular morphological features (cytoplasm vacuolisation, oil droplets, plastid shape). Two new species – *Elliptochloris incisiformis* Hoffmann & Kostikov and *E. perforata* Hoffmann & Kostikov – were defined primarily by their plastid features. Hoffmann et al. (2007)

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note the probable relationship of the genus *Elliptochloris* with the similar trebouxiophycean genus *Myrmecia* based on presence of *Myrmecia*-like cells among mature globular cells of several *Elliptochloris* populations.

In this study, we present a new *Elliptochloris* variety based on the investigation of CAUP H7103 strain isolated originally from a bark sample from tropical mountain rainforest. The new alga is reminiscent of *E. bilobata*, but light microscopical observations and sequencing of the 18S rRNA gene revealed important differences. We also conducted a quantitative geometric morphometric analysis of the shape of elliptical autospores in both species to test for their morphological differences. In this analysis, we used the shape of cellular outlines as they were described by landmark-based generalised Procrustes analysis (GPA). This method is based on standardizing the size of the objects and optimising their rotation and position so that the distances between corresponding landmarks are minimized (Dryden & Mardia 1998; Zelditch et al. 2004). The method has been recently widely used in morphological and taxonomic studies of different organisms (see e.g. Adams et al. 2004), including diatoms (Beszteri et al. 2005; Potapova & Hamilton 2007) or green microalgae (Neustupa 2005a; Neustupa & Hodač 2005; Neustupa & Nemjová 2008).

Material and methods

The CAUP H7103 strain originates from a bark sample of *Cleistocalyx operculata* (Roxb.) Merr. & Perry collected in Cibodas Botanical Garden (geographical coordinates 6°45′30″S and 107°00′10″E) in an altitude of 1300 meters a.s.l. The alga was cultivated on BBM (Bischoff & Bold 1963) agar medium in temperature 25 °C and illumination 40 μmol m^{-2} s^{-1} provided by 18W cool fluorescent tubes (Philips TLD 18W/33). Microphotographs were taken by Olympus BX51 light microscope and Olympus Z5060 digital equipment using Nomarski differential contrast. For morphological comparison, we investigated the original strain of *Elliptochloris bilobata* that was obtained from Culture Collection of Algae SAG (strain No. 245.80). For the morphological comparison and the geometric morphometric analysis we cultivated both strains for three weeks in identical conditions on agar-solidified BBM (Bischoff & Bold 1963) at a temperature of 25 °C and illumination 40 μmol m^{-2} s^{-1} provided by 18W cool fluorescent tubes.

For amplification of the 18S rRNA gene, cells were scraped from agar plates with a clean spatula, transferred to an Eppendorf tube, resuspended in distilled water and harvested by centrifugation. Total DNA was extracted using the Invisorb®Spin Plant Mini Kit (Invitek) following the instructions of the manufacturer. A part of the 18S rDNA gene was amplified by PCR using universal forward and reverse primers according to Katana et al. (2001). The PCR product was resolved by electrophoresis on a vertical agarose gel, a band of the expected size was dissected from the gel and the DNA was purified using the QIAquick Gel Extraction Kit (Qiagen) following the instruction provided by the manufacturer. The purified product was sequenced from both ends by a set of primers designed according to Katana et al. (2001). Sequencing reads were assembled with the CAP3 assembler server and manually edited by visual inspection of sequencing chromatograms. The newly obtained sequence was deposited in GenBank with the accession number EF688289.

The newly determined sequence was added to an alignment of a representative set of trebouxiophycean sequences and three outgroups sequences representing Chlorophyceae, Ulvophyceae and Chlorodendrales (80 taxa altogether). The alignment was produced by ClustalX (Thompson et al. 1997) and manually edited in GeneDoc (K.B. Nicholas & H.B. Nicholas, http://www.psc.edu\biomed\genedoc) according to the secondary structure model for the *Chlamydomonas reinhardtii* 18S rRNA molecule available from the European Ribosomal RNA Database (http://www.psb.ugent.be/rRNA/secmodel/Crei_SSU.html; Wuyts et al. 2000). Ambiguously aligned regions, introns, and positions with deletions in most sequences were removed from the alignment, resulting in an alignment comprising 1675 positions (the alignment is available from authors upon request). A maximum likelihood (ML) phylogenetic tree was constructed using PhyML-aLRT 1.0 (Anisimova & Gascuel 2006), using a BioNJ input tree and a GTR model of nucleotide evolution with nucleotide frequencies and rate parameters estimated from the data. Γ correction with 8 rate categories plus invariant positions and the alpha parameter estimated from the data were employed to cope with a site-to-site rate variation. ML bootstrap analysis was performed on 100 replicates created with SEQBOOT in the PHYLIP 3.62 software package (J. Felsenstein, University of Washington) using PhyML-aLRT and GTR+Γ+I model with parameters estimated separately for each dataset. Bootstrapping was performed also with neighbour joining (NJ) on 1000 replicates using the PHYLIP package. Distance matrices were computed using DNADIST with F84 model of nucleotide evolution, transition/transversion ratio 2.0, one rate category and no Γ correction. Replicate trees were reconstructed using the NEIGHBOR programme; the majority rule consensus tree was obtained using CONSENSE.

For the geometric morphometric analysis, we randomly selected 50 elliptical autospores in an intensively reproducing culture of each species. In each cell, we delimited 14 structurally corresponding landmarks, so that two of them were located on the poles of a cell and six sliding landmarks delimited outlines on each of the longitudinal sides. The landmarks were superimposed by generalized Procrustes analysis (GPA) (Zelditch et al. 2004). The Procrustes coordinates resulting from GPA that removes size, position and rotation differences in landmark configurations were further used as the shape descriptors of individual objects. As the apical and basal parts of the cells could not always be recognized, we averaged the landmark data in all cells along their equatorial axis following the standard formula of Klingenberg et al. (2002). Thus, further analyses of these symmetrized configurations omitted the asymmetric component of the shape variation (Klingenberg et al. 2002; Neustupa & Škaloud 2007). We then conducted the principal component analysis of Procrustes coordinates (Zelditch et al. 2004). The first eight PC axes described more than 99% of the total variation and we used the scores of the objects on these axes to test for the group shape differences using the permutation test on Mahalanobis distance with 10 000 permutations (Hammer et al. 2001). The shape characteristics of autospores in individual species were illustrated as deformation grids of group means from the overall consensus. The morphometric and the subsequent statistical analyses were conducted in TPS software pack-

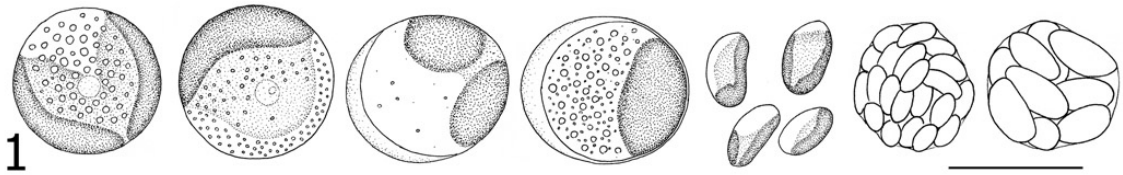
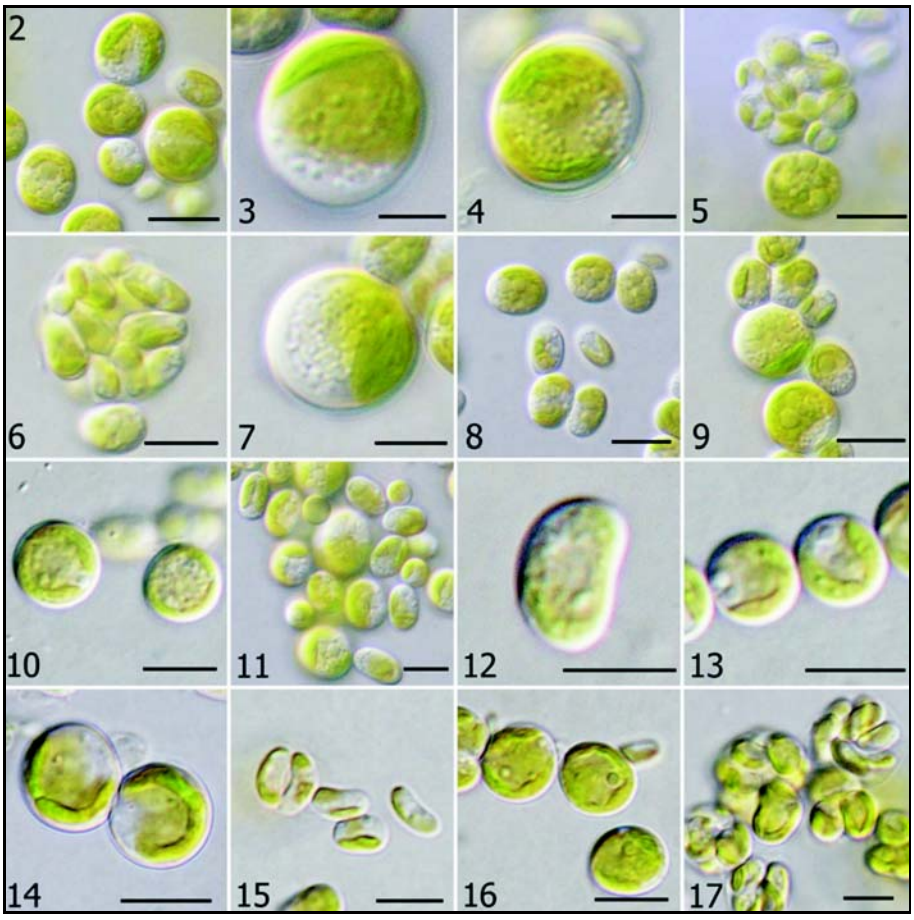


Fig. 1. *Elliptochloris bilobata* var. *corticola*. Drawings of vegetative cells, elliptical autospores and sporangia; scale 10 μm.



Figs 2–17. 2–4, 7 – *Elliptochloris bilobata* var. *corticola*, vegetative cells; 5, 6 – *E. bilobata* var. *corticola*, sporangia; 8–13 – *E. bilobata* var. *corticola* young vegetative cells; 14–17 – *Elliptochloris bilobata* var. *bilobata*, strain SAG 245.80, vegetative cells, elliptical spores and sporangia; scale 5 μm (Figs 3, 4, 6, 8, 11, 12, 15, 17) and 10 μm (Figs 2, 5, 7, 9, 10, 13, 14, 16).

age (Rohlf 2006) and in PAST, ver. 1.75 (Hammer et al. 2001).

Results and discussion

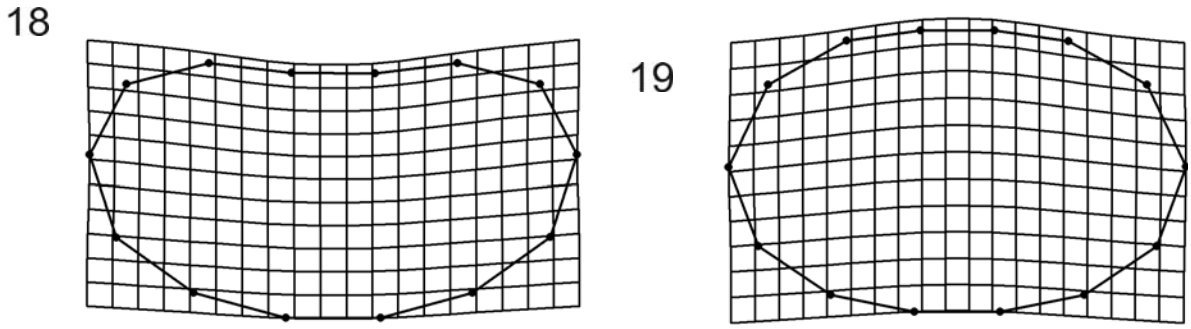
Elliptochloris bilobata var. corticola Eliáš, Neustupa & Škaloud *var. nov.*

Descriptio: *Cellulae sphaericae usque (7.5–) 8–13.5 (–15.5) μm diametro. Chloroplastus parietalis, bilobatae, sine pyrenoide. Propagatio per 2 aut 4 autosporis grandis vel 16 aut 32 minutae sporis ellipticae. Autosporae sphaericae (4.5–) 5–7.5 (–8.5) μm diametro. Sporae ellipticae iuvenes (2–) 2.5–3 (–3.5) × (4.5–) 5–6 (–6.5) μm. Cytoplasma plerumque alveolatum. Reproductio sexualis non observata.*

Holotypus: Lyophilised material is deposited in the Culture Collection of Algae of the Charles University of Prague, Czech Republic (CAUP) as specimen No. LYO-H7103. The living culture (ex-holotype, authentic strain) is deposited as CAUP H 7103 in the Culture Collection of Algae of the Charles University of Prague, Czech Republic (Škaloud & Neustupa 2007).

Type locality: Indonesia, West Java, Cibodas. On the bark of *Cleistocalyx operculata* in a mountain rainforest, 1300 m a.s.l.

The alga is composed of individual coccal cells of irregularly elliptical to globular outline. The mature cells are mostly globular (7.5–) 8–13.5 (–15.5) μm in diameter (Figs 1, 2–4, 7, 9, 10). Two types of autospores are produced. The globular autospores are produced in sporangia containing 2–4(–8) daughter cells. They are (4.5–) 5–7.5 (–8.5) μm in diameter (Fig. 13). The elliptical autospores are produced in sporangia containing 16–32 cells (Fig. 6). The shape of these spores varies from typical regularly elliptical to ovoid, planoconvex or fabaceous (Figs. 1, 6, 8, 11–12). Their dimensions are



Figs 18, 19. The deformation grids illustrating differences in shape of elliptical autospores of *E. bilobata* var. *bilobata* and *E. bilobata* var. *corticola*. The splines of the consensus configuration to the means of individual species were two-times magnified in order to enhance the discriminative shape characters. 18 – *E. bilobata* var. *bilobata*; 19 – *E. bilobata* var. *corticola*.

(2–) 2.5–3 (–3.5) × (4.5–) 5–6 (–6.5) μm. The cell wall of mature globular cells is typically irregularly thickened up to about 1.5–2 μm (Figs. 4, 7). These conspicuous thickenings involve about 25 to 40% of the cell outline. They are formed by colour-less cell wall material, with no visible inner layers. The pyrenoid-less chloroplast is, parietal, lump-formed to lobate, mostly with two equal parietal lobes. Cytoplasm is usually filled up by numerous small vacuoles.

Some morphological characteristics of *E. bilobata* var. *corticola* remind to other *Elliptochloris* taxa, but there are several differences that distinguish these organisms. In *E. bilobata* var. *bilobata*, the cell wall is regularly thin, even in mature cells before sporogenesis (Tschermak-Woess 1980; Figs 14–17). In addition, chloroplast of *E. bilobata* var. *bilobata* is regularly bilobed, contrary to the more variable plastid structure in *E. bilobata* var. *corticola*. The elliptical spores of *E. bilobata* var. *bilobata* mostly have more pronounced fabaceous inflection. This feature was further tested by a geometric morphometric analysis.

The mean shape of the autospores was clearly different in *E. bilobata* var. *bilobata* and *E. bilobata* var. *corticola* (two-group permutation test on Mahalanobis distance = 0.206, permutation p-value = 0.0002). The deformation grids of group means from the overall mean configuration illustrated that the difference in shape of the autospores and young cells relied mostly in the degree of their fabaceous inflection (Figs 18, 19). In *E. bilobata* var. *corticola* the autospores tended to be more of elliptical shape, whereas in *E. bilobata* var. *bilobata* they were pronouncedly inflexed.

Other green algae that were assigned to the genus *Elliptochloris* by Ettl & Gärtner (1995) on the basis of traditional morphological assessment differ conspicuously from *E. bilobata* var. *corticola*. *Elliptochloris subsphaerica* possesses a pyrenoid and a more complex, multi-lobed chloroplast (Reisigl 1964). In *E. reniformis*, adult cells are mostly non-globular and chloroplast is typical by deeply lobed chloroplast, usually with three to more lobes (Watanabe 1977; Ettl & Gärtner 1995; Hanagata et al. 1998). Two new species of Hoffmann et al. (2007) differ by their specific plastid structure (presence of "hollows" of perforations in chloroplast in *E. incisiformis* and *E. perforata*) and by the absence of unilateral cell wall thickenings that are characteristic for our species. Therefore, from a purely morphological point of view, the investigated alga would possibly be considered as a new taxon, closely related to *E. bilobata* var. *bilobata*.

The sequenced part of 18S rRNA gene of the CAUP H 7103 strain comprises 1753 bp (excluding the positions matching the primers used for the PCR amplification) and does not contain any introns. In BLAST searches against the nr database at NCBI (last check as of June 2007) the sequence was most similar to the 18S rRNA gene sequence from *Elliptochloris bilobata* SAG 245.80 (accession number AM422984.1). Other top BLAST hits represented sequences from diverse trebouxiophyceans, and phylogenetic analysis including a representative set of sequences from all major lineages of the Chlorophyta revealed that our alga and SAG 245.80 strain form a single clade nested deeply within the Trebouxiophyceae (data not shown). To investigate the position of *E. bilobata* var. *corticola* within Trebouxiophyceae in more detail, we performed a second phylogenetic analysis with a rich sample of the trebouxiophycean diversity. The resulting tree (Fig. 20) shows that sequences from CAUP H 7103 and SAG 245.80 are sisters with 100/100% ML/NJ bootstrap support and the level of their mutual divergence is in order characteristic for independent species in other trebouxiophycean genera. We mapped the differences in the 18S rDNA sequences of CAUP H 7103 and SAG 245.80 on the predicted secondary structure of the 18S rRNA molecule (Fig. 21). Beside substitutions or an indel in loop regions of conserved helices E10_1, 11, and E23_2, there are five differences in the stem region of the conserved helix 49. Interestingly, the substitutions between the two species abrogate base pairing at two positions while restore base pairing at two other positions. Such compensatory base changes indicate a clear genetic separation of CAUP H 7103 and SAG 245.80 and support their status of independent taxa.

The CAUP H 7103/SAG 245.80 clade is sister with strong bootstrap support (95/99%) to a clade comprising sequences attributed to species of the genus *Coccomyxa*. The close relationship of *E. bilobata* and representatives of *Coccomyxa* is consistent with a phylogeny reported previously by Friedl & Bhattacharya (2002)

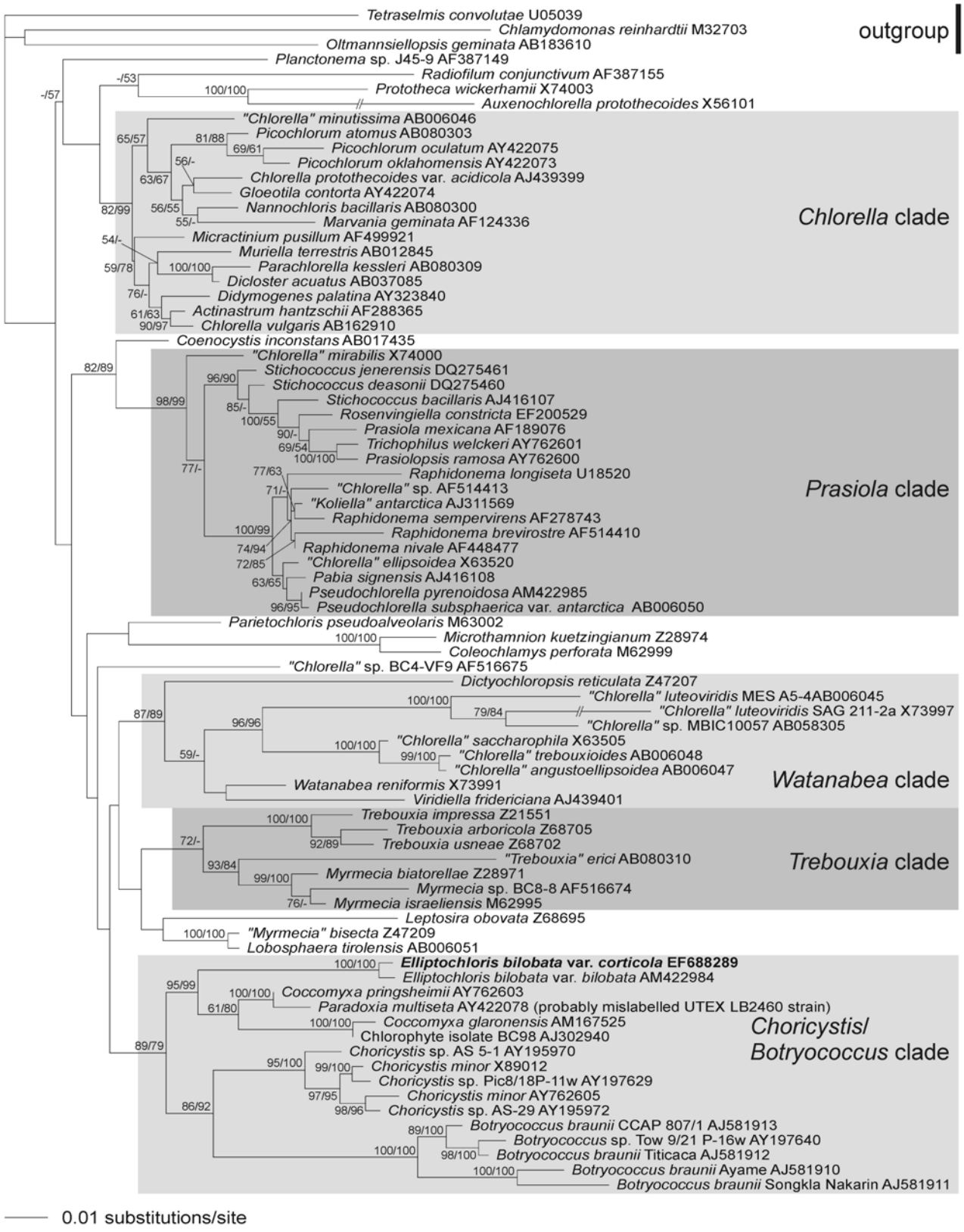


Fig. 20. Maximum likelihood tree ($\ln L = -13916.76238$) of a representative set of 18S rRNA gene sequences from the class Trebouxiophyceae and three outgroup sequences constructed with PhyML-aLRT (GTR+Γ+I, see Material and Methods). Bootstrap support for nodes from ML/NJ analyses is shown when higher than 50%. DDBJ/EMBL/GenBank accesion numbers are provided for each sequence, the sequence determined in this study is indicated in bold. Terminal branches leading to *Auxenochlorella protothecoides* and *"Chlorella" luteoviridis* SAG 211-2a were shortened by a half.

and with shared characteristics of the cell wall reported by Brunner & Honegger (1985). Additional sequences branching in the vicinity of *E. corticola* represent the genera *Choricystis* and *Botryococcus*, and all these se-

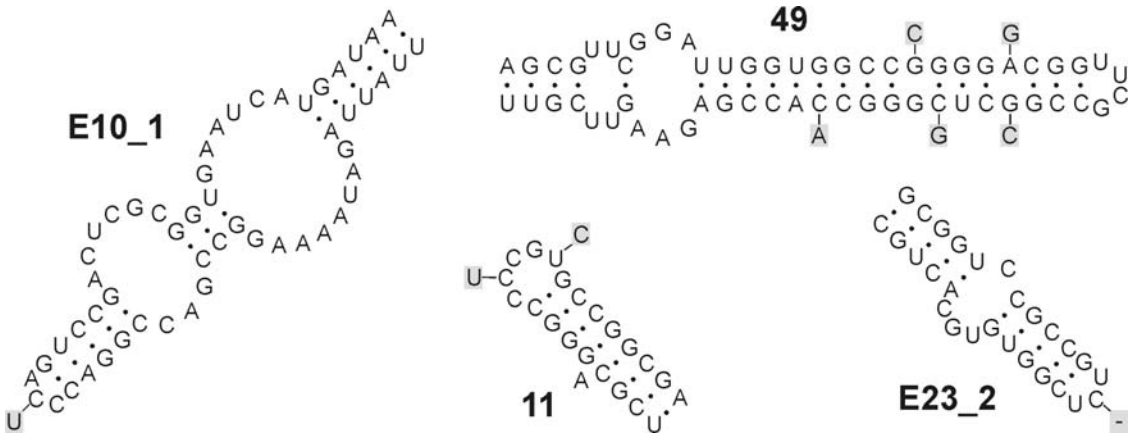


Fig. 21. Differences in the 18S rRNA sequences of *E. bilobata* var. *corticola* and *E. bilobata* var. *bilobata*. The picture shows only those parts of a secondary-structure model of the *E. bilobata* var. *corticola* 18S rRNA molecule that exhibit sequence differences in *E. bilobata* var. *bilobata* (i.e., the helices E10_1, 11, E23_2, and 49 of the general eukaryotic 18S rRNA structure; Wuyts et al. 2000). We omitted the very beginning (the first 14 nucleotides) of the 18S rRNA sequence from *E. bilobata* var. *bilobata* available in DDBJ/EMBL/GenBank (AM422984.1) because it is highly suspicious and possibly affected by sequencing errors. Substitutions in the *E. bilobata* var. *bilobata* 18S rRNA with respect to the *E. bilobata* var. *corticola* are indicated by letters on a grey background connected to the respective position in the *E. bilobata* var. *bilobata* by a short line. The deletion in the terminal loop of the E23_2 helix is indicated by a dash. Note that the substitutions in the helix 49 of the *E. bilobata* var. *bilobata* abrogate base pairing (indicated by dots throughout the picture) at two positions while restore base pairing at the two remaining positions.

quences together form a well-supported clade (89/79% BS) corresponding to the *Choricystis/Botryococcus* clade as delimited in Karsten et al. (2005). The exact placement of the *Choricystis/Botryococcus* clade within Trebouxiophyceae could not be determined due to a lack of resolution along the base of the trebouxiophycean tree, a result echoing previously published phylogenetic analyses with a similar taxon sampling (e.g., Krienitz et al. 2004; Henley et al. 2004; Karsten et al. 2005; Fawley et al. 2005a). Regardless this uncertainty, the genus *Elliptochloris* is clearly not related to *Myrmecia* as suggested by Hoffmann et al. (2007) on the basis of traditional morphological data. On the other hand, the genus *Myrmecia* is related to *Trebouxia* and belongs to Trebouxiales.

Sequences from other taxa that have been considered as members of the genus *Elliptochloris* on the basis of traditional criteria clustered into different clades. A sequence from the original strain of *E. subsphaerica* var. *antarctica* (=*Pseudochlorella subsphaerica* var. *antarctica* CCAP 264/3) branched in a position remote from the sequence from *E. bilobata* var. *bilobata* but close to a sequence from *Pseudochlorella pyrenoidosa* within the *Prasiola* clade (sensu Karsten et al. 2005). The sequence from *Watanabea reniformis* SAG 211-9b, considered by Hanagata et al. (1998) as conspecific with *Elliptochloris reniformis* (S. Watanabe) Ettl & Gärtner, clustered in a clade with *"Chlorella" luteoviridis* and "*Ch." saccharophila*, "*Ch." trebouxioides* and "*Ch." angustoellipsoidea*, in accordance with Krienitz et al. (1996, 2004) and Karsten et al. (2005). This position of *W. reniformis* well corresponds with its deeply multi-lobed plastid structure.

Thus, based on available molecular data it appears that the traditional genus *Elliptochloris* sensu Ettl & Gärtner (1995) actually consists of members of several different trebouxiophycean lineages. The genus *Elliptochloris sensu stricto* now comprises *E. bilobata* as the type species of the genus, with two varieties – the type variety and the newly described *E. bilobata* var. *corticola*. Both of them are characterized by bi-lobed pyrenoid-less plastid and the presence of irregularly elliptical non-motile asexual spores. Although the *E. bilobata* var. *bilobata* and *E. bilobata* var. *corticola* may seem similar in traditional morphological features, the wide use of molecular characters has recently pointed to a general previously underestimated genetic and taxonomic diversity within traditional morphospecies. Such semi-cryptic or pseudo-cryptic taxa with subtle morphological differences have now been reported in diverse algal groups, including haptophytes (Saez et al. 2003), diatoms (Amato et al. 2007), prasinophytes (Slapeta et al. 2006), as well as coccoid green algae (e.g., Fawley et al. 2005b; Vanormelingen et al. 2007). In addition, both varieties differ ecologically, since the former was originally identified as a phycobiont in temperate-zone high mountains (altitude of 2200 m a.s.l.) whereas the latter was found as a free-living alga in a tropical rainforest. Therefore, we believe that with the distinguishing of *E. bilobata* var. *corticola* from the type variety we just started to appreciate the true taxonomic diversity within the genus *Elliptochloris*. In this respect, we cannot preclude future elevation of *E. bilobata* var. *corticola* to a species level made as a part of molecular phylogenetic revision of the genus involving the ITS sequence analyses with complementary base changes (CBC) comparisons on more isolates.

Populations attributed to *Elliptochloris bilobata* on the basis of traditional morphological data were reported several times from different habitats, mostly as lichen phycobionts (Watanabe et al. 1997; Aoki et al. 1998; Trembley et al. 2002) or as free-living aero-

terrestrial organisms (Andreeva 2001; Kostikov et al. 2001; Darienko & Hoffmann 2003; Mikhailyuk et al. 2003). However, as these reports lack phylogenetic information from molecular characters and the cultures of these algae are not available, we cannot confirm true relation of these algae to the genus *Elliptochloris*. Similarly, there are no molecular data available for two new species described by Hoffmann et al. (2007). In addition, strains of these organisms have not been deposited in any public culture collection so that they are no more available for further research. Hence, it is presently not possible to ascertain their phylogenetic status and relationship to both varieties of *E. bilobata* by molecular characters. However, their morphological characteristics outlined by Hoffmann et al. (2007) indicate that they are distinct from, though potentially related to *E. bilobata*.

The probably high hidden diversity of aero-terrestrial trebouxiophycean algae with little morphological differentiation should lead us to a rather cautious approach in assigning individual morphotypes to taxa without parallel molecular investigation. Certainly, morphological data can be used in diversity-based ecological studies, where comparison of individual morphotypes, rather than their exact taxonomic placement, is of interest. Taxonomic investigation of these organisms should inherently involve molecular assessment in all data sets, where it is possible. Nevertheless, morphology still remains at the core of taxonomic research in aero-terrestrial green algae. Minute morphological differences that can be ascertained by careful morphological investigation (as e.g. those of Hoffmann et al. 2007) turn our attention to possibly new or taxonomically problematic taxa whose phylogenetic position can then be ascertained by molecular methods.

Elliptochloris bilobata var. *bilobata* and *E. bilobata* var. *corticola* form a pair of similar organisms, but their different taxonomic status is evident from different morphology, different 18S rDNA sequences and ecology. The highly significant discrimination between both strains was demonstrated also by the quantitative shape analysis of autospore outlines. We believe that this way of geometric morphometric evaluation of closely similar species could soon become a standard tool of phenotypic analysis of green microalgae with variable cell shapes (Neustupa & Šťastný 2006; Neustupa & Nemjová 2008). High diversity of tropical subaerial green algae was already suggested in previous studies (Neustupa & Šejnohová 2003; Neustupa 2004, 2005b; Rindi et al. 2006; Neustupa et al. 2007). However, given their aero-terrestrial habitats and the subsequent selection for low surface-to-volume ratio, most of these algae have very simple morphology. Thus, for a number of subaerial microalgae, we now have to acknowledge the probably common existence of taxa with few discriminating morphological characters. Geometric morphometrics can probably be useful in many such cases as a method parallel to molecular phylogenetic analyses and traditional qualitative morphological observations.

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