Phylogenetic affinities of the Trentepohliales inferred from small-subunit rDNA

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Phylogenetic analyses of the nuclear-encoded small-subunit rDNA sequences from taxa representing all of the major lineages of green algae, including new sequences for the Trentepohliales, consistently indicated that the subaerial Trentepohliales are closely related to ulvophycean marine green algae, particularly to the siphonous and hemisiphonous orders. The presence of phragmoplast-type cytokinesis in the order Trentepohliales remains enigmatic, and it is interesting that this type of cell division is associated with terrestrial (subaerial) habits.

INTRODUCTION

Recent reviews (e.g. Chapman et al., 1999; Waters & Chapman, 1996) of analyses of the nuclear-encoded small-subunit (SSU) rDNA as well as the chloroplast-encoded large-subunit Rubisco gene (rbcL) provide support for the original suggestion, based on ultrastructural data, that there are two main lineages among the green plants (Pickett-Heaps & Marchand, 1972). One of the lineages comprises the charophycean algae and their descendents, the land plants (charophycean lineage sensu Pickett-Heaps & Marchant, 1972), forming a monophyletic group named Streptophyta (sensu Cavalier-Smith, 1993). The charophycean algae include taxa from at least five orders: the Chlorokybales, Klebsormidiales, Zygmematales, Coleochaetales and Charales. The second lineage (chlorophycean algae sensu Pickett-Heaps & Marchant, 1972) consists exclusively of the remaining green algae (containing at least Chlamydomonas and its allies, the coccoid green algae, Dasyyclades, Cladophorales and Trebouxiophyceae), forming the monophyletic group Chlorophyta (sensu Cavalier-Smith, 1993; Friedl, 1997; Melkonian et al., 1995). The term ‘chlorophyte’ has often been used to denote all green algae; however, it should now be used only as an informal designation for green algae in the Chlorophyta [see Chapman et al. (1999) for a revisionary approach to the systematics of the green algae].

Conflicting hypotheses have been offered about the systematic position of the Trentepohliales among the several classes of green algae. The presence of multilayered structures in the flagellar apparatus and the demonstration of a phragmoplast-type cytokinesis in Cephaleuros parasiticus (Chapman & Henk, 1986) and Trentepohlia odorata (Chapman et al., 2001; Waters et al., 1998) suggest an affinity with the class Charophyceae. However, taxonomic features of the Trentepohliales such as the anticlockwise flagellar apparatus components can be cited as evidence for an affinity with the Ulvophyceae (Roberts, 1984). Based on biochemical, biophysical and physiological features, Raven (1987) classified the Trentepohliales among a third class, the Pleurostrophyceae. Moreover, it was noted that the Trentepohliales even share a rare ultrastructural feature (presumptive mating structures in the gametes) with members of a fourth class, the Chlorophyceae (Chapman & Henk, 1983, 1985). Therefore, the Trentepohliales exhibit some features associated with four of the five major classes of green algae in the system proposed by Mattox & Stewart (1984); however, the major discussions have been focused on ulvophycean versus charophycean affinities of this enigmatic order. Members of the Trentepohliales are most abundant in tropical and subtropical regions worldwide. A distinct assemblage of green algae characterized by special adaptations to subaerial habitats, the group includes vascular plant epiphytes, some of which are considered to be economically important, such as Cephaleuros virescens Kunze in Fries, which is a parasite on tea leaves (Chapman, 1984). The order remains incompletely characterized for phylogenetic purposes (O’Kelly & Floyd, 1990).

Where does the Trentepohliales belong in the various proposed classification schemes of green algal classes? What are the closest relatives of the Trentepohliales? In this study, we have addressed these questions by using phylogenetic analysis of newly obtained SSU rDNA sequences from several genera of Trentepohliales and representatives of the major classes and orders of green algae.

The GenBank/EMBL/DDBJ accession numbers for the SSU rDNA sequences of Cephaleuros parasiticus, Cephaleuros virescens, Physolium monile, Trentepohlia arborum, Trentepohlia aurea, Trentepohlia dialepta, Trentepohlia sp. and Phycopeltis sp. are respectively AY052563–AY052570.
RESULTS AND DISCUSSION

The phylogenetic position of the Trentepohliales

In order to elucidate the overall position of the Trentepohliales in a nuclear-encoded SSU rDNA phylogeny of the Viridiplantae, a global analysis was performed with a subset of 57 taxa (Fig. 1) and 1729 (equally weighted) aligned characters. Two glaucocystophytes (Cyanoptyle gloecystis and Glaucocystis nostochinearum) were included as the outgroup. Fifty-five green plants were included as representatives of the Viridiplantae, from the streptophyte lineage (Charophyceae and Embryophyta), as well as from the chlorophyte lineage. Both the distance and maximum-parsimony analyses positioned the Trentepohliales unequivocally within the chlorophytan lineage, the Chlorophyta. The Chlorophyta lineage forms a monophyletic group (bootstrap support 97–82 %). The present results based on nuclear-encoded SSU rDNA confirm previous reports from both SSU rDNA data and chloroplast-encoded Rubisco large subunit data on the existence of two lineages of Viridiplantae. The land plants within the Streptophyte clade diverged from the Chlorophyceae, as expected from previous reports.

As predicted by Mattox & Stewart (1984), the mononadophycean taxa (Mamiella sp., Mantoniella spp., Micromonas pusilla, Pycnococcus provasoli, Pseudococcorfieldia marina, Nephroselmins olivacea, Tetraselmis striata and Scherffelia dubia) are not a natural or monophyletic group but rather a series of basal divergences forming a
grade. This paraphyletic group is also known as Prasinophyceae, and the use of this term has been recommended by Sym & Pienaar (1993). Similar results supporting the paraphyletic nature of this group have been reported (Nakayama et al., 1998; Fawley et al., 1999). Our results for the clade formed by *Pseudoscourfieldia marina* and *Pycnococcus provasolii* (bootstrap support 100/100 %) confirm the recent study by Fawley et al. (1999) using SSU rDNA sequence data in grouping these two taxa in one family, Pycnococcaceae. Furthermore, recent rDNA and actin analysis of *Mesostigma* (not shown in Fig. 2) has placed this taxon in the charophycean lineage (Melkonian et al., 1995; Bhattacharya et al., 1998), thus indicating polyphyly for the algae we call the prasinophytes. Therefore, it may be time to end the use of 'Prasinophyceae' or 'Micromonadophyceae', and maybe even the very convenient term 'prasinophytes'. However, in the absence of new names for the seven distinct lineages, the term 'prasinophytes' will be used in this report. In general, the prasinophytes are considered as the modern representatives.
of the earliest green algae (Graham & Wilcox, 2000) and, despite the fact that they are not a monophyletic group, their basal position is well supported.

Three classes of chlorophytes

The representatives of the ulvophycean taxa in this study consistently form a monophyletic group (bootstrap support 80/53 %) that includes the taxa of the order Trentepohliales. This clade is the sister group of the remaining green algae, the Chlorophyceae and the Pleurastrophyceae. There is no strong support for the recognition of the monophyletic nature of the Chlorophyceae and the Pleurastrophyceae sensu Mattox & Stewart. The Pleurastrophyceae of Mattox & Stewart has been shown to be a polyphyletic group (Friedl & Zeltner, 1994). Friedl (1995) erected a new class name, the Trebouxiophyceae, to include many coccoid green algae that completely lack a motile stage (autosporic coccoids) and members of the Microthamniales sensu Mattox (1982, 1990) or Pleurastrales sensu Mattox & Stewart (1984) based on rDNA sequence comparisons (Friedl, 1995, 1997). In our analyses, the Trebouxiphyceae form a clade with moderate support (64–79 %).

The Chlorophyceae analysed in the present study formed a well-supported clade (97–91 %) and comprise two distinct monophyletic lineages defined by ultrastructural details of the flagellar apparatus; one group (Chlamydomonas reinhardtii, Volvox carterii, Gongrosira papuasia, Botryococcus braunii, Protosiphon botryoides and Dunaliella salina) with a clockwise basal body configuration (CW group, Fig. 1) and the other group (Ankistrodesmus stipitatus, Scenedesmus obliquus, Neochloris aquatica, Pediastrum duplex and Hydrodictyon reticulatum) with directly opposed basal bodies (DO group, Fig. 1) (Lewis et al., 1992; Nakayama et al., 1996).

In the present study, the ulvophycean taxa formed a monophyletic group in all analyses. Distance methods and maximum-parsimony analysis consistently positioned the taxa of the Trentepohliales within this ulvophycean clade. The monophyly of the Trentepohliales (bootstrap support 100/100 %) is not surprising, since some features such as the sporangium-associated apparatus and the flagellar apparatus are unique to this order. An affinity with the Pleurastrophyceae (Trebouxiophyceae), as suggested by Raven (1987), is not supported. An ulvophycean affinity for the Trentepohliales has been expressed before (Roberts, 1984) based on ultrastructural features of the flagellar apparatus. The ulvophycean and trentepohlialean taxa share an anticlockwise basal body configuration as well as an alternation of generations. Based on preliminary partial nuclear-encoded SSU rRNA sequence data, Zechman et al. (1990) also related the Trentepohliales to the ulvophycean clade.

One question arising from these results is how to explain the presence of the phragmoplast in Cephaleuros (Chapman & Henk, 1986) and Trentepohlia (Chapman et al., 2001). The presence of a phragmoplast-type cytokinesis is well documented in some charophycean algae and is the typical mode of cytokinesis in land plants. The presence of a phragmoplast-type cytokinesis in the chlorophycean lineage raises the question of the ‘homology’ of this process: it is possible that the phragmoplast evolved more than once? It is difficult to understand how a highly sophisticated complex cytological process involving many genes evolved in two different lineages. However, the genus Koliella provides another example of very similar forms of cell division occurring in both major lineages of green algae. The general similarities in cytokinesis and karyokinesis in Koliella and Klebsormidium suggested a close phylogenetic affinity (Lokhorst & Star, 1998), but molecular analyses (Katana et al., 2001) indicate that the Koliella species belong to the Trebouxiophyceae in the chlorophycean lineage. Thus, the similarities in cytokinesis and karyokinesis are homoplasious. Explanations of the enigmatic presence of a phragmoplast in the Trentepohliales in the ulvophycean clade will come from thorough ultrastructural and immunological analyses of the evolution of the phragmoplast in the basal lineages of the streptophytes as well as in the Trentepohliales.

Relationships within the Ulvophyceae

In order to elucidate the precise position of the Trentepohliales within the nuclear-encoded SSU rDNA phylogeny of the ulvophycean green algae, a subset of 38 taxa from the complete data matrix was analysed with 1714 (equally weighted) characters (Fig. 2). Two trebouxiophycean algae (Myrmecia israeliensis and Trebouxia impressa) and two chlorophycean algae (Pediastrum duplex and Scenedesmus obliquus) were included as the outgroup taxa. Thirty-four algal taxa were included in the ingroup, representing most of the major orders of the ulvophytes.

In all phylogenetic analyses, including neighbour-joining, parsimony and likelihood methods, the Trentepohliales formed a monophyletic group that was well supported by bootstrap analysis in the neighbour-joining method (100 %) and maximum-parsimony method (100 %) as well as the maximum-likelihood approach (Figs 2 and 3). In the distance and parsimony analyses, the Trentepohliales emerged as a sister group to the clade containing the Siphonocladales/Cladophorales complex and Dasycladales, both of which contain representatives mainly from the marine environment. However, in the maximum-likelihood analysis, the Trentepohliales are resolved as a sister clade only to the Dasycladales.

Previous concepts of the Ulvophyceae

In the scheme of Mattox & Stewart (1984), the Ulvophyceae were defined in terms of ultrastructural features that make them a group distinct from the Chlorophyceae and Charophyceae. In the same paper, ulvophycean algae were considered more ‘advanced’, since their vegetative state is non-motile and non-flagellated and is presumably derived from scaly green flagellates. O’Kelly & Floyd (1984)
presented a more detailed study of the Ulvophyceae, defining them in terms of ultrastructural, reproductive and biochemical features. The scheme recognized five orders: Ulotrichales, Ulvales, Siphonocladales (including Clado-
phorales), Dasycladales and Caulerpales. In this study, the Ulvales and Ulotrichales were considered primitive orders of the Ulvophyceae and the Siphonocladales, Dasycladales and Caulerpales more advanced orders. The authors also implied that the Siphonocladales and Dasycladales are sister groups. This arrangement of orders, as well the relationships of the orders in the Ulvophyceae, was based on (i) the absence of quadriflagellated motile cells or modification of the flagellar apparatus, (ii) development of basal body orientation perpendicular to the long axis of the cell during forward swimming, (iii) loss of the isomorphic life history, (iv) an increasing complexity in the zoosporangial and gametangial structure and development and (v) structural and chemical composition of the cell walls. O’Kelly & Floyd (1984), although recognizing that trentepohlialean motile cells are consistent with their definition of the class Ulvophyceae, decided to exclude the order Trentepohliales from the Ulvophyceae because of the presence of multilayered structures associated with two of the four rootlets and plasmodesmata in the cross walls between vegetative cells.

Sluiman (1989) considered the Ulvophyceae to be represented by eight orders: Ulotrichales, Ctenocladales, Trentepohliales, Pleurastrales, Acrosiphonales, Cladophorales, Bryopsidales and Dasycladales. His system was based mainly on ultrastructural features of the flagellar apparatus, de-emphasizing the importance of cell division at the
ordinal level. It is important to mention that this was the first paper to assign the order Trentepohliales formally to the Ulvophyceae. Mattox & Stewart (1984) mentioned the order Trentepohliales in the Ulvophyceae by referring to the ultrastructural studies of Roberts (1984), and O’Kelly & Floyd (1984) did not mention the order Trentepohliales in their Ulvophycean scheme. However, Sluiman’s concept of the orders in the class Ulvophyceae, acknowledging the ulvophycean nature of the order Trentepohliales, implied that some features such as cell division may be homoplasious (independently derived), particularly the phragmoplast-type cytokinesis in the overall scheme of the chlorophytes.

**Current concepts**

Results from the present study support two groups within the ulvophycean algae. The first group is represented by the orders Ulotrichales (*Gloecolitopsis planctonica*, *Parotoderma sarcinoidea*, *Pseudodendronium basiliense*, *Ulothrix zonata*, *Monostroma grevillei*, *Urosperma penicilliformis* and *Acrosiphonia*) and Ulvales (*Ulva rigida* and *Enteromorpha intestinalis*). The second monophyletic group of ulvophytes contains the order Trentepohliales and the siphonous and hemisiphonous ulvophycean algae (bootstrap support of 100 and 90%). Within this group, the order Trentepohliales either is a sister clade to the Siphonocladeles/Cladophorales complex (represented by *Chamaedoris peniculatum*, *Cladophora coelothrix*, *Siphonocladus tropicus*, *Ernodesmis verticillata*, *Chaetomorpha* sp., *Cladophora rupestris*, *Microdictyon boergesioii*, *Cladophora coelothrix*, *Cladophora vagabunda* and *Valonia utricularis*) and the Dasycladales (distance and parsimony methods) or belongs to a more derived clade (maximum-likelihood analyses), with the sister clade formed only by Dasycladales (*Acetabularia major*, *Acicularia schenki*, *Polyphysa parvula*, *Batophora occidentalis*, *Chlorocladus australasicus*, *Cymopolia*).
vanbossea and Neomeris dumentosa). Representatives of the order Caulerpales were not available for comparison at the time of this study and were therefore not included in the analyses. Our results confirm previous analyses with partial nuclear-encoded SSU rRNA sequences (Zechman et al., 1990), suggesting a close relationship between the orders Ulvales and Ulotrichales, as well as the relationships among the Siphonocladales/Cladophorales complex, Dasycladales and Trentepohliales. However, Zechman et al. (1990) reported non-monophyly for Ulvophyceae. The two groups of ulvophytes, that is, the Ulvales + Dasycladales and Trentepohliales. However, Zechman et al. (1990) reported non-monophyly for Ulvophyceae. The two groups of ulvophytes, that is, the Ulvales + Ulotrichales and the Siphonocladales/Cladophorales + Dasycladales (‘primitive’ versus ‘advanced’ orders sensu O’Kelly & Floyd, 1984) were separated by the Pleurastrophyceae and Chlorophyceae. However, Zechman et al. (1990) also noted that these ‘intermediate clades’ (chlorophycean and pleurastrophycean algae) were resolved by just a few evolutionary steps and were susceptible to rearrangement by changes in taxon and/or character sampling. Our results do not support the concept of Sliuiman (1989) that includes representatives of the Pleurastroles (pleurastrophycean algae sensu Mattox & Stewart, 1984) within the Ulvophyceae. Similar results based on cladistic analysis of nuclear rDNA sequence data have been reported by Kantz et al. (1990) and reviewed by Chapman et al. (1999).

In conclusion, our study provides robust support to position the order Trentepohliales within the ulvophycean algae in the chlorophyta lineages. Our results clearly indicate that the ‘advanced’ marine taxa (orders Siphonocladales/Cladophorales complex and Dasycladales) are the most closely related to the trentepohlialean algae. Our analyses provide only moderate support for the monophyly of the class Ulvophyceae. The evolutionary implications of these results for the origin of the Trentepohliales indicate that they presumably diverged from an ulvophycean-like macroscopic filamentous marine ancestor. The presence of the phragmoplast-type cytokinesis in the order Trentepohliales remains enigmatic, but, if such a system evolved in the freshwater Streptophyta lineage, there is no reason why an almost identical system could not have evolved in the marine portion of the Chlorophyta lineage. Finally, it is interesting that, in both lineages, phragmoplast-mediated cytokinesis is associated with terrestrial (subaerial) habits.

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REFERENCES


