*J. Phycol.* **34,** 312–318 (1998)

# ORIGINS AND AFFINITIES OF THE FILAMENTOUS GREEN ALGAL ORDERS CHAETOPHORALES AND OEDOGONIALES BASED ON 18S rRNA GENE SEQUENCES[1]

*Gregory C. Booton*

Department of Molecular Genetics

*Gary L. Floyd*

Department of Plant Biology

*and*

*Paul A. Fuerst*[2]

Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

*The order Chaetophorales includes filamentous green algae whose taxonomic relationships to other chlorophycean orders is uncertain. Chaetophoralean taxa include filamentous species which are both branched and unbranched. Ultrastructural studies of zoospores have revealed similar flagellar apparatuses in a number of genera, including* Uronema, Stigeoclonium, *and* Fritschiella, *suggesting a close phylogenetic relationship among these taxa. The order Oedogoniales represents a second group of branched and unbranched filamentous green algae whose relationships to other chlorophycean orders also has been unclear. A possible close relationship between the Chaetophorales and Oedogoniales has been suggested. Using DNA sequences from the small-subunit ribosomal RNA gene (SSU rRNA) of several members of each order, we have examined the monophyly of the Chaetophorales and Oedogoniales, as well as the nature of their relationship to other chlorophycean orders. Our results show that chaetophoralean and oedogonialean taxa form separate monophyletic groups. Results also suggest that the two orders are not closely related to each other.*

*Key index words: Chaetophorales; Chlorophyceae; phylogeny; Oedogoniales; SSU rDNA*

In this study we examine the relationships of two orders of phylogenetically problematic filamentous green algae: the Chaetophorales and Oedogoniales. We seek to answer questions regarding the origins and affinities of these two groups. The Chaetophorales encompasses green algae with parenchymatous, branched or unbranched filaments; motile cells usually quadriflagellate; phycoplast-assisted cytokinesis; and plasmodesmata present (Mattox and Stewart 1984). The common ultrastructure of the flagellar apparatus of several members of the Chaetophorales has been documented (Manton 1964, Floyd et al. 1980, Bakker and Lokhorst 1984, Melkonian 1984, O'Kelly and Floyd 1984, Watanabe and Floyd 1989). It is widely accepted that *Uronema, Stigeoclonium, Chaetophora,* and *Fritschiella* form a group of closely related organisms sharing the features described above. The Chaetophorales displays a progression of morphologic complexity from the uniseriate filament with holdfast to the most complex heterotrichous form found in *Fritschiella.* Even with this morphologic data, the phylogenetic relationship of the Chaetophorales to the other orders of the Chlorophyceae has been problematic.

The phylogenetic placement of the Oedogoniales is even less well resolved than the Chaetophorales. Pickett-Heaps (1975) hypothesized that the Oedogoniales may have been the earliest offshoot of an evolutionary line giving rise to several groups of filamentous taxa, whereas the Chaetophorales is one of the more derived groups. The ultrastructure of the zoospores and gametes of the Oedogoniales and the cytokinetic apparatus is well known (Hoffman and Manton 1962, 1963, Hoffman 1970, Pickett-Heaps 1975, Markowitz 1978). Proposals for the probable phylogenetic relationships of the Oedogoniales are vague, with most writers merely suggesting only that they are chlorophycean. Mattox and Stewart (1984) proposed a possible relationship between the Oedogoniales and the Sphaeropleales based on members of both groups possessing lateral cell walls consisting of segments rather than being a one-piece, continuous structure. Pickett-Heaps (1975) has also suggested that the Oedogoniales may be derived from autosporic algae in which the mother cell wall rupture site was not at the center of the cell, but was displaced to one end, leading eventually to the wall morphology of the extant Oedogoniales. In addition, three genera of the Oedogoniales demonstrate a clear pattern of differentiation from the unbranched filaments of *Oedogonium* to the branched filaments of *Oedocladium* to the

[1] Received 3 January 1997. Accepted 10 November 1997.

[2] Author for reprint requests; e-mail fuerst.1@osu.edu.

TABLE 1. *Ribosomal RNA gene sequences from the following taxa were utilized in this study.*

| Species | SSU reference (or UTEX culture no.) | GenBank accession no. |
|---|---|---|
| *Ankistrodesmus stipitatus* (= *A. falcatus* var. *stipitatus*) (Chod.) Komárková-Legnerová | Huss and Sogin 1990 | X56100 |
| *Asteromonas gracilis* Artari (= *Stephanoptera gracilis*) | Wilcox et al. 1993 | M95614 |
| *Bulbochaete hiloensis* (Nordst.) Tiffany | (952) this study | U83132 |
| *Chaetophora incrassata* (Huds.) Hazen | (1289) this study | U83130 |
| *Characium hindakii* Lee & Bold | Lewis et al. 1992 | M63000 |
| *Chlamydomonas reinhardtii* Dang | Gunderson et al. 1987 | M32703 |
| *Chlamydopodium vacuolatum* (Korschikoff) Ettl et Komárek | Lewis et al. 1992 | M63001 |
| *Chlorella fusca* Shihara & Krauss | Huss and Sogin 1990 | X56104 |
| *Chlorella kessleri* Fott & Nováková | Huss and Sogin 1990 | X56105 |
| *Chlorella minutissima* Fott & Nováková | Huss and Sogin 1990 | X56102 |
| *Ettlia minuta* (Arce et Bold) Komárek | Lewis et al. 1992 | M62996 |
| *Dunaliella salina* (Dunal) Teod. | Lewis et al. 1992 | M84320 |
| *Fritschiella tuberosa* Iyengar | (1821) this study | U83129 |
| *Fusochloris perforata* (Lee & Bold) Floyd, Watanabe, and Deason | Lewis et al. 1992 | M62999 |
| *Gloeotilopsis planctonica* Iyengar & Philipose | Friedl and Zeltner 1994 | Z28970 |
| *Gongrosira papuasica* (Borzi) Tupa | Rootes and Chapman | U18503 |
| *Hydrodictyon reticulatum* (L.) Lagerh. | Wilcox et al. 1992a | M74497 |
| *Myrmecia israelensis* (Chantanachat et Bold) Friedl | Lewis et al. 1992 | M62995 |
| *Neochloris aquatica* Starr | Lewis et al. 1992 | M62861 |
| *Nephroselmis olivacea* Stein | Steinkötter et al. 1994 | X74754 |
| *Nitella* sp. Kütz | Wilcox et al. 1993 | M95615 |
| *Oedocladium carolinianum* Beaney & Hoffman | (1686) this study | U83135 |
| *Oedogonium angustistomum* Hoffman | (1557) this study | U83134 |
| *Oedogonium cardiacum* Witt. | (40) this study | U83133 |
| *Pediastrum duplex* Meyen | Lewis et al. 1992 | M62997 |
| *Protoderma sarcinoidea* (Grover & Bold) Tupa | Bhattacharya et al. 1996 | Z47998 |
| *Pseudoscourfieldia marina* (Throndsen) Manton | Steinkötter et al. 1994 | X75565 |
| *Scenedesmus obliquus* (Turp.) Kütz | Huss and Sogin 1990 | X56103 |
| *Stigeoclonium helveticum* Vischer | (441) this study | U83131 |
| *Uronema acuminata* (= *Ulothrix acuminata*) (Mattox & Bold) | (1178) this study | U83128 |
| *Volvox carteri* (Iyengar) | Rausch et al. 1989 | X53904 |

branched filaments with bulbous-based bristles of *Bulbochaete*. All the genera have the feature of stephanokont zoospores and male gametes, oogamy, distinctive cytokinesis, and plasmodesmata.

In cases involving the green algae where ultrastructural data has been unable to resolve evolutionary relationships between taxa, molecular data, as well as combined data sets, have proven useful (Gunderson and Sogin 1986, Rausch et al. 1989, Huss and Sogin 1990, Lewis et al. 1992, Wilcox et al. 1992a, b, 1993, Friedl and Zeltner 1994, Mishler et al. 1994). Here we use small-subunit ribosomal RNA (SSU rRNA) gene sequence data to further investigate the Chaetophorales and Oedogoniales. This study addresses whether these orders of filamentous algae are coherent groups of branched and unbranched filamentous taxa and examines their phylogenetic relationships to other chlorophycean taxa.

TABLE 2. *Sequencing and amplification primers for SSU rRNA study.*

| Primer | Sequence[a] | Orientation |
|---|---|---|
| CRN5 | tggttgatcctgccagtag | Forward |
| 170 | gcatgtattagctctaga | Reverse |
| 373 | aggctccctctccggaatc | Reverse |
| 373C | gattccggagagggagcct | Forward |
| 570 | gcattggagctggaattac | Reverse |
| 570C | gtaattccagctccaatagc | Forward |
| 892 | ccaagaatttcacctctgac | Reverse |
| 892C | gtcaaggtgaaattcttgg | Forward |
| 1137 | gtgcccttccgtcaat | Reverse |
| PCR2 | gaaacttaaaggaattga | Forward |
| 1262 | gaacggccatgcaccac | Reverse |
| 1262C | gtggtgcatggccgttctta | Forward |
| 1200 | gggcatcacagacctg | Reverse |
| 1200C | caggtctgtgatgccc | Forward |
| 1/F | cacaccgcccgtcg | Forward |
| SSU2 | ccgcggccgcggatcctgatccct ccgcaggttcac | Reverse |

[a] All primers are presented 5′ → 3′.

## MATERIALS AND METHODS

*Taxa studied.* All taxa utilized in this study were obtained from The Culture Collection of Algae at the University of Texas, Austin (UTEX) (Starr and Zeikus 1987) and are summarized in Table 1. From the Oedogoniales we examined *Oedogonium cardiacum, Oedogonium angustistomum, Oedocladium carolinianum,* and *Bulbochaete hiloensis.* In the Chaetophorales we included *Chaetophora incrassata, Fritschiella tuberosa, Stigeoclonium helveticum,* and *Uronema acuminata.*

*DNA extraction.* An aliquot of ca. 25 μL of algal culture was pelleted and resuspended in 50 μL of lysis buffer (50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, and 1% beta-mercaptoethanol). Samples were then microwaved on high setting (45 s, 3 times) in a Sears model 747.9987821 microwave. Following microwaving, an additional 350 μL of lysis buffer were added and samples were incubated at 80° C for 1 h. DNA was then extracted using standard phenol/chloroform procedures. DNA was precipitated using 250 μL isoproponol and 10 μL 3 M NaOAc. Samples were washed in 70% EtOH and dried, and DNA was resuspended in 30 μL TE buffer (10 mM Tris/Cl, pH 7.5, 1 mM EDTA, pH 8.0).

*Polymerase chain reaction amplification and sequencing.* SSU rRNA gene amplifications were generated using 3 μL of resuspended genomic DNA as a template in 100 μL PCR reactions using 5 μL (10 μM stock solution) of each amplification primer, 2.5 mM $MgCl_2$, 1.5 mM dNTPs, and 1–2.5 units Taq DNA polymerase (Gibco BRL, Inc., Gaithersburg, Maryland). SSU genes were amplified in two overlapping halves. The 5′ portion was amplified using primers CRN5 and 1137, the 3′ half using primer pair 892C and SSU2 (Table 2). Three microliters of pooled duplicate PCR product was sequenced directly by double-stranded cycle sequenc-

ing using a commercial kit (dsDNA Cycle Sequencing System, Gibco BRL, Inc.). Sequencing accuracy was determined by sequencing both strands of the rRNA gene.

*Phylogenetic reconstruction and data analysis.* SSU 18S rRNA gene sequences obtained from the four chaetophoralean and the four oedogonialean taxa contained nearly the entire gene with the exclusion of small regions near the 5′ and 3′ ends of the gene and the terminal amplification primers. A chaetophoralean taxon with biflagellated zoospores (*Gongrosira papuasica*) whose SSU rRNA sequence has been determined previously was included in this study (Rootes and Chapman, unpubl.; GenBank accession #U18503). Other chlorophycean and outgroup taxa also examined in this study are presented in Table 1.

The primary sequences of the taxa used in this study were aligned with other SSU gene sequences using the sequence alignment program Eyeball Sequence Editor for the PC (ESEE, Cabot and Beckenbach 1989). Homology of sites and accuracy of the alignments was determined by examination of SSU rRNA secondary structure. A proposed secondary structure for one of the chaetophoralean taxa (*Chaetophora incrassata*) was used for homologous site comparisons (Fig. 1). The final sequence alignment was used to produce a data matrix of 1784 sites, which corresponds to the range between bases 34 and 1764 of the published *Chlamydomonas reinhardtii* SSU sequence (Gunderson et al. 1987). Two regions were identified in this alignment where robust alignment of the sequences across all taxa examined was not possible. These regions correspond to bases 1349–1370 and 1679–1710 of the published *C. reinhardtii* sequence. For most analyses and for the phylogenetic trees presented, these regions were removed. However, they were included in some instances to examine their effect on gene tree reconstructions (see Results). The charophycean taxon *Nitella* sp. was chosen as an outgroup taxon. The algal sequence alignment is available via WWW at http://www.biosci.ohio-state.edu/~gbooton/filabs.htm.

Distance calculation and gene tree reconstruction analysis was done using the phylogenetic analysis package MEGA (Molecular Evolutionary Genetic Analysis, Kumar et al. 1993). Sites corresponding to the two regions cited above were removed prior to distance calculations. Corrected sequence divergences were quantified using the Kimura two-parameter model option in MEGA (Kimura 1980). Distances derived by this method were then used to produce phylogenetic gene trees using the neighbor-joining algorithm with bootstrapping (1000 bootstraps) of the data, also using MEGA.

Maximum parsimony analysis was performed using PAUP 3.0 (Swofford 1990). Sites not used in the distance analysis were also removed for maximum parsimony analysis. Trees were produced using a heuristic search algorithm, with random addition of taxa (10 replicates). Consistency and homoplasy indices were obtained for all trees. Alternative searches were also performed using all sites for comparison of gene tree reconstruction. Maximum parsimony trees produced in PAUP were examined for alternative branching and character analysis using MacClade 3.04 (Maddison and Maddison 1992).

## RESULTS

Distance reconstruction and maximum parsimony analysis methods resulted in trees with nearly identical branching orders and are therefore presented in one combined tree (Fig 2). Maximum parsimony analysis resulted in two equally parsimonious trees of 1187 steps (CI = 0.58). These two trees differed in the branching order of the *Oedogonium* and *Oedocladium* taxa. Strict consensus of the two trees results in a multifurcation of the these taxa as presented in Figure 2. The *Chaetophorales* and *Oedogoniales* comprised monophyletic clades in both maximum parsimony trees. Bootstrap analysis resulted in the same branching order as found in the maximum parsimony analysis with the Chaetophorales and Oedogoniales comprising monophyletic clades supported by a 100% bootstrap.

Within the Chaetophorales, both reconstructions supported a divergence of a lineage leading to the extant *Uronema* prior to the divergence of the remainder of the Chaetophoralean taxa. This is supported with very high bootstrap support and this branching order was also observed in both maximum parsimony trees.

Results from both reconstructions show that the lineage leading to the extant Oedogoniales diverged prior to the remainder of the chlorophycean orders examined here. This branching order suffers from low bootstrap support but is also observed in both maximum parsimony trees. Within the Oedogoniales there is high bootstrap support, and agreement in both maximum parsimony trees, of a bifurcation leading to the extant *Bulbochaete hiloensis* and another lineage leading to the *Oedogonium* and *Oedocladium* taxa. The two trees differ with regard to the *Oedogonium* and *Oedocladium* taxa. One tree supports the two *Oedogonium* species as sister taxa, and the other places *Oedogonium angustistomum* with *Oedocladium cardiacum*. In the tree presented here, these three taxa have been collapsed to represent a trifurcation at this node.

To investigate the relationships of these filamentous orders to the Trebouxiophyceae, Ulvophyceae, Prasinophyceae, Charophyceae, and other chlorophycean orders, members of these groups were also included in these analyses. Examination of the tree reveals that both reconstructions support the sister group relationship of the Trebouxiophyceae (Friedl 1995), formerly the Pleurastrophyceae sensu Mattox and Stewart (1984), and the Chlorophyceae. In the neighbor-joining tree, this support is evidenced by the 82% bootstrap result for this node, which is greater than the support observed for this relationship in a previous analysis (Bhattacharya et al. 1996).

Furthermore, both reconstruction methods support a branching order in which the lineage that led to the Oedogoniales diverged prior to the remainder of the other chlorophycean taxa examined. Although the bootstrap support for this divergence pattern is rather weak, it was nonetheless also supported by both maximum parsimony trees.

To examine the stability of the consensus maximum parsimony tree, alternative branching orders were examined in MacClade. As stated above, it has been suggested that the two filamentous groups studied here are closely related to each other. Neither the cladistic nor phenetic results support that hypothesis. The maximum parsimony tree consisted of 1187 steps (CI = 0.58). Twenty-seven changes supported the branch leading to the Oedogoniales. When the two filamentous groups are placed together as sister clades, the alternative tree requires seven more steps (1194) than the consensus maximum parsimony tree. In addition, the alternative sis-

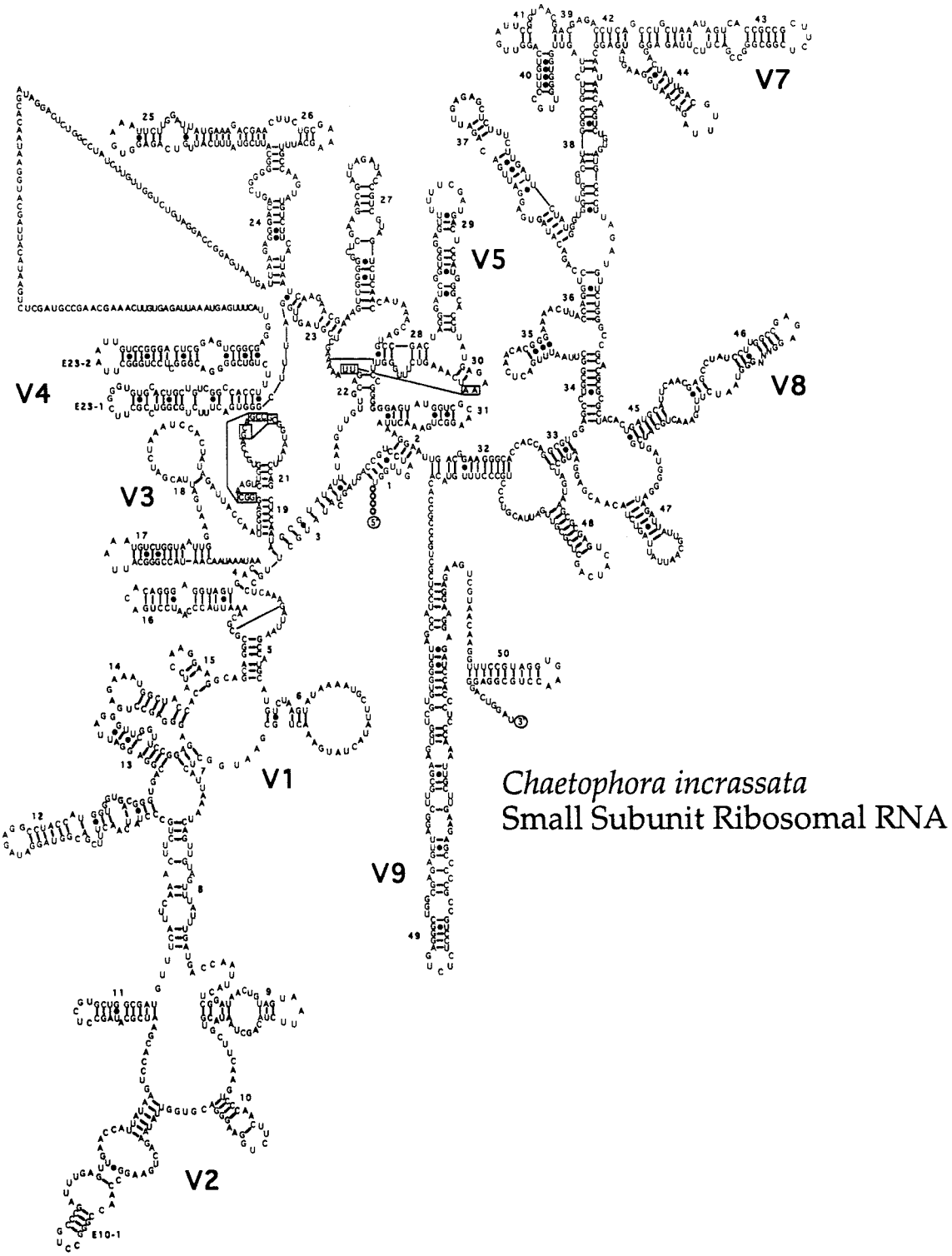


FIG. 1. Proposed secondary structure of the SSU rRNA of *Chaetophora incrassata,* based on the proposed secondary structure model of Gutell (1994).

ter clade relationship is supported by only two characters in the combined filamentous tree. If we are to consider trees seven steps from the most parsimonious tree as viable alternatives, it is of interest to note that another alternative tree, which places the ulvophycean taxa within the trebouxiophycean clade, is also only seven steps from the most parsimonious tree. Clearly this ulvophycean/trebouxiophycean alternative hypothesis is one that few would accept, and similarly, the alternative filamentous

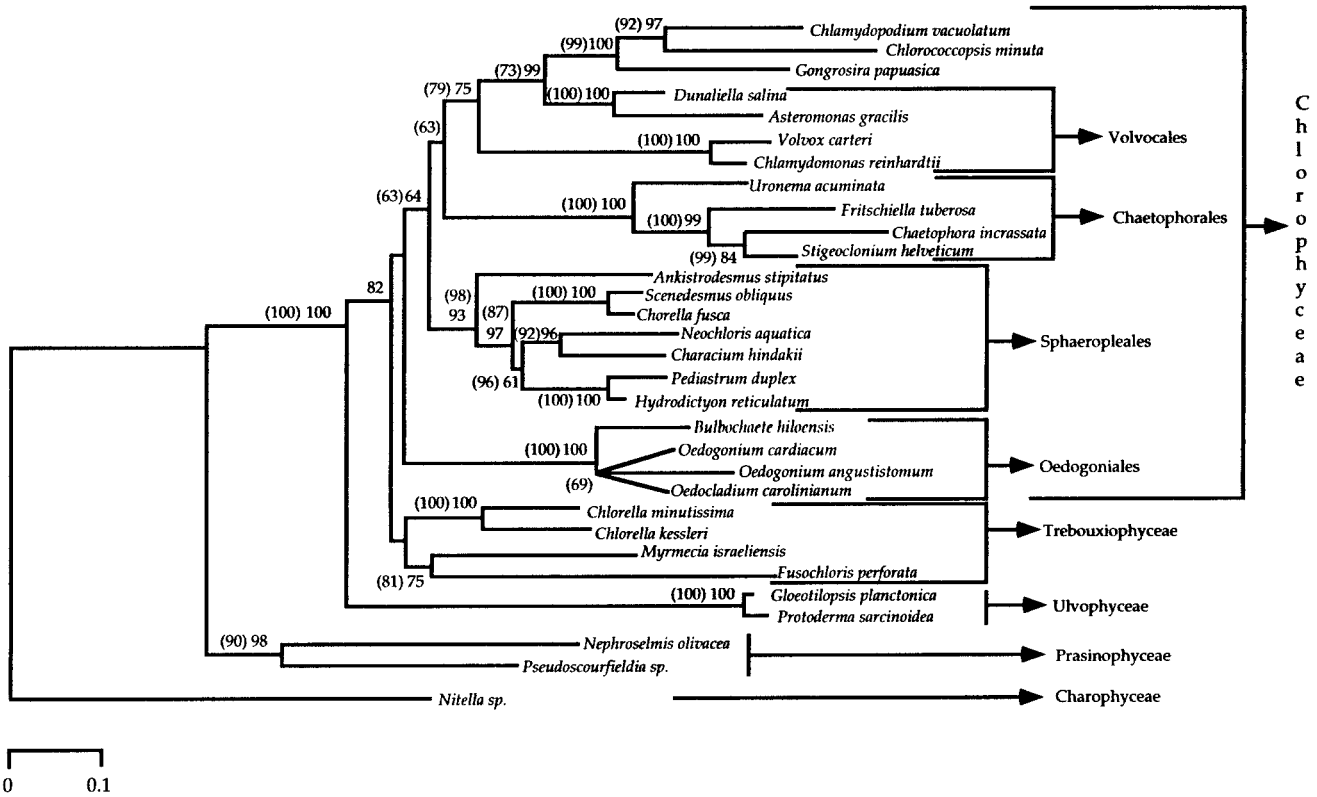


FIG. 2. Combined neighbor-joining and maximum parsimony tree. Branch lengths were produced using distances calculated using the Kimura two-parameter methods. Data was bootstrapped 1000 times. Scale bar is on the lower left of figure and represents the corrected number of nucleotide substitutions using the Kimura method. Numbers at nodes are bootstrap percentages. Only bootstrap values ≥50% are indicated. The ordinal definition of the Sphaeropleales is incomplete at present, but those taxa that produce motile cells would be included in the Sphaeropleales as described in Deason et al. (1991).

tree which would add seven steps appears equally unacceptable.

To further examine the possibility of the relatedness of the two filamentous clades, another maximum parsimony analysis was performed in PAUP using all 1784 sites (tree not shown). This included the 65 sites that were removed previously because they could not be reliably aligned in all taxa. If these sites were synapomorphies for the two filamentous orders of green algae, alternative trees joining these groups might result. However, analysis of the entire data set produced exactly the same two most parsimonious trees discussed above, consisting of 1589 steps (CI = 0.53). The consensus tree was the same as the one shown for the truncated data set. We again examined the alternative hypotheses regarding the filamentous clades in MacClade. Using all the data, 33 characters supported the oedogonialean clade, whereas 37 characters support the chaetophoralean clade. Forcing these two clades together as sister groups results in a tree six steps longer (1595) than the minimum (1589) and is supported by only five characters. To examine the number of trees that were six steps longer than the minimum, another heuristic analysis was performed in PAUP which maintained all trees less than or equal to 1595 steps. There are 206 trees six or fewer steps from the two minimal trees, 101 of which are 1595 steps. Therefore, it does not appear that the alternative grouping which places the two filamentous groups together is probable.

## DISCUSSION

The results obtained in this study support our conclusion that the Oedogoniales and Chaetophorales form distinct monophyletic clades of filamentous algae not closely related to each other. Specifically with regard to the Chaetophorales, both gene tree reconstructions strongly support this group as a monophyletic clade. Both methods also concur that the filamentous lineage leading to the Chaetophorales split from a lineage leading to a clade of Volvocales and other chlorophycean taxa: *Volvox carteri, Chlamydomonas reinhardtii, Chlorococcopsis minuta, Dunaliella parva, Asteromonas gracilis, Chlamydopodium vacuolatum,* and *Gongrosira papuasica.* We note with considerable interest that the biflagellated zoospore producing *Gongrosira papuasica* groups with high bootstrap support within this volvocalean/*Chlamydopodium*/*Chlorococcopsis* clade. This nonchaetophoralean placement is consistent with the suggestion made by Watanabe et al. (1992) that *Gongrosira* is

possibly related to other chlorophycean algae; that is, they hypothesized a chlorococcalean placement for *Gongrosira,* and not chaetophoralean based on the ultrastructure of the flagellar apparatus. In the consensus maximum parsimony analysis, the relationship of *Gongrosira* with other chlorophycean algae is the same.

We were also interested in an examination of the relationships within the filamentous groups regarding the hypothetical progression of branching morphologies. Within the chaetophoralean taxa, both reconstruction methodologies strongly support the hypothesis that the simpler morphologic form (*Uronema*) branches prior to the remainder of genera, which exhibit increasingly more complex branching morphologies.

Looking at relationships within the Oedogoniales, we again hypothesized an evolutionary progression from the morphologically simple to the relatively more complex taxa. However, this is not what we observed in the Oedogoniales. Both reconstruction methods agree regarding the basal placement of the morphologically more complex *Bulbochaete* to both *Oedogonium* and *Oedocladium* taxa. This is at variance with the typically hypothesized relationship between these genera, which postulates that the more complex *Bulbochaete* is the more derived genus. Within the genera *Oedogonium* and *Oedocladium,* the consensus maximum parsimony reconstruction results in a trifurcation between the two *Oedogonium* species and the single *Oedocladium* taxon. Distance reconstruction using the neighbor-joining algorithm is somewhat more resolved regarding these taxa placing *Oedogonium cardiacum* basal to the remaining two *Oedogonium* species, but with weak bootstrap support. Finally, both reconstructions suggest that the more complex *Bulbochaete* may represent a separate lineage within the Oedogoniales and may not necessarily be derived from simpler *Oedocladium*-like ancestors. However, the relationships between these genera must be interpreted cautiously given lack of methodologic consensus.

Also of interest in our examination of the Oedogoniales is the apparent early divergence of the lineage leading to this order and its basal position relative to the other chlorophycean orders examined. This branching order is consistent with the hypothesis of Pickett-Heaps (1975) that the Oedogoniales may have been the earliest offshoot of the evolutionary line giving rise to several groups of filamentous taxa, with the Chaetophorales being one of the more derived groups. Both maximum parsimony trees produced this branching order. However, it is only weakly supported by the bootstrap analysis. In addition, it is troubling that with the addition of the filamentous orders, bootstrap support for Trebouxiophyceae and Chlorophyceae as monophyletic clades falls below 50% in all analyses. One interpretation of this result is that the Oedogoniales may comprise a third, separate lineage, which is a sister group to both the Trebouxiophyceae and the Chlorophyceae. Finally, results of this study do not allow us to fully resolve this question.

In summary, SSU rRNA sequence data support the coherence of the chaetophoralean and oedogonialean groups as monophyletic clades but does not completely resolve the relationship between these two groups. Due to the relatively short distances calculated within the Oedogoniales, we are led to the conclusion that addition of more oedogonialean SSU rRNA sequences would not significantly improve the resolution of the tree at the more basal nodes, which currently have low bootstrap support. However, examination of a different locus may prove useful. Recently, studies that examined the protein coding the *rbc*L (ribulose-1,5-bisphosphate carboxylase/oxygenase) gene of the chloroplast genome concluded that this gene is useful for resolving phylogenetic relationships at the generic or higher level in algae (e.g. Freshwater et al. 1995, Nozaki et al. 1995). Therefore, we have begun examination of this gene in the taxa studied here to determine its utility in answering questions regarding the origins of these orders.

We thank the National Science Foundation for their support of this research under grant DEB-9408193 to G.L.F and P.A.F.

Bakker, M. E. & Lokhorst, G. M. 1984. Ultrastructure of *Draparnaldia glomerata* (Chaetophorales, Chlorophyceae). I. The flagellar ultrastructure of the zoospores. *Nord. J. Bot.* 4:261–73.

Bhattacharya, D., Friedl, T. & Damberger, S. 1996. Nuclear-encoded rDNA group I Introns: origin and phylogenetic relationships of insertion site lineages in the green algae. *Mol. Biol. Evol.* 13:978–89.

Cabot, E. L. & Beckenbach, A. T. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comput. Appl. Biosci.* 5:233–4.

Deason, T. R., Silva, P. C., Watanabe, S. & Floyd, G. L. 1991. Taxonomic status of the species of the green algal genus *Neochloris. Pl. Syst. Evol.* 177:213–19.

Floyd, G. L., Hoops, H.J. & Swanson, J. A. 1980. Fine structure of the zoospores of *Ulothrix belkae* with emphasis on the flagellar apparatus. *Protoplasma* 104:17–31.

Freshwater, D. W., Fredericq, S. & Hommersand, M. 1995. A molecular phylogeny of the Gelidiales (Rhodophyta) based on analysis of plastid rbcL nucleotide sequences. *J. Phycol.* 31: 616–32.

Friedl, T. 1995. Inferring taxonomic positions and testing genus level assignments in coccoid green algae: a phylogenetic analysis of 18S ribosomal DNA sequences from *Dictyochloropsis reticulata* and species of *Myrmecia* (Chlorophyta, Trebouxiophyceae cl. nov.). *J. Phycol.* 31:632–9.

Friedl, T. & Zeltner, C. 1994. Assessing the relationships of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18S ribosomal gene sequence comparisons. *J. Phycol.* 30:500–6.

Gunderson, J. H., Elwood, H., Ingold, A., Kindle, K. & Sogin, M. L. 1987. Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. *Proc. Natl. Acad. Sci. USA* 84: 5823–7.

Gunderson, J. H. & Sogin, M. L. 1986. Length variation in eukaryotic rRNAs: small subunit rRNAs from the protists *Acanthamoeba castellanii* and *Euglena gracilis. Gene* 44:63–70.

Gutell, R. R. 1994. Collection of small subunit (16S and 16S-like) ribosomal RNA structure. *Nuc. Acids Res.* 22:3502–7.

Hoffman, L .R. 1970. Observations on the fine structure of *Oed-*

*ogonium.* IV. The striated component of the compound flagellar ''roots'' of *O. cardiacum. Can. J. Bot.* 48:189–96.

Hoffman, L .R. & Manton, I. 1962. Observations of the fine structure of the zoospore of *Oedogonium cardiacum* with special reference to the flagellar apparatus. *J. Exp. Bot.* 13:443–9.

——— 1963. Observations of the fine structure of *Oedogonium.* II. The spermatozoid of *O. cardiacum. Am. J. Bot.* 50:455–63.

Huss, V. A. & Sogin, M. L. 1990. Phylogenetic position of some *Chlorella* species within the Chlorococcales based upon complete small-subunit ribosomal RNA sequences. *J. Mol. Evol.* 31:432–42.

Kimura, M. 1980. A Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–20.

Kumar, S., Tamura, K. & Nei, M. 1993. *MEGA—Molecular Evolutionary Genetic Analysis,* Ver. 1.02. The Pennsylvania State University, State College. (computer software program)

Lewis, L. A., Wilcox, L. W., Fuerst, P. A. & Floyd, G. L. 1992. Concordance of molecular and ultrastructural data in the study of zoosporic chlorococcalean green algae. *J. Phycol.* 28: 375–80.

Maddison, W. P. & Maddison, D. R. 1992. *MacClade: Analysis of Phylogeny and Character Evolution,* Ver. 3.0. Sinauer Associates, Sunderland, Massachusetts.

Manton, I. 1964. Observations on the fine structure of the zoospore and young germling of *Stigeoclonium. J. Exp. Bot.* 15: 399–411.

Markowitz, M. M. 1978. Fine structure of the zoospore of *Oedocladium carolinianum* (Chlorophyta) with special reference to the flagellar apparatus. *J. Phycol.* 14:289–302.

Mattox, K. R. & Stewart, K. D. 1984. The classification of the green algae: a concept based on comparative cytology. *In* Irvine, D. E. G. & John, D. M. [Eds.] *The Systematics of the Green Algae.* Academic Press, New York, pp. 29–72.

Melkonian, M. 1984. Flagellar apparatus ultrastructure in relation to green algal classification. *In* Irvine, D .E. G. & John, D. M. [Eds.] *Systematics of the Green Algae.* Academic Press, New York, pp. 73–120.

Mishler, B., Lewis, L., Buchheim, M., Renzaglia, K, Delwiche, C., Zechman, F., Kantz, T. & Chapman, R. 1994. Phylogenetic relationships of the ''Green Algae'' and ''Bryophytes.'' *Ann. Missouri Bot. Gard.* 81:451–83.

Nozaki, H., Itoh, M., Sano, R., Uchida, H., Watanabe, M. & Kuroiwa, T. 1995. Phylogenetic relationships within the colonial Volvocales (Chlorophyta) inferred from rbcL gene sequence data. *J. Phycol.* 31:970–9.

O'Kelly, C. J. & Floyd, G. L. 1984. Flagellar apparatus orientations and the phylogeny of the green algae. *BioSystems* 16: 227–51.

Pickett-Heaps, J. D. 1975. *Green Algae: Structure, Reproduction, and Evolutions in Selected Genera.* Sinauer Associates. Sunderland, Massachusetts, 606 pp.

Rausch, H., Larson, N. & Schmitt, R. 1989. Phylogenetic relationships of the green alga *Volvox carteri* deduced from small-subunit ribosomal RNA comparisons. *J. Mol. Evol.* 29:255–65.

Starr, R. C. & Zeikus, J. A. 1987. UTEX—the culture collection of algae at the University of Texas at Austin. *J. Phycol.* 23: (Suppl.) 1–47.

Steinkötter, J, Bhattacharya, D., Semmelroth, I., Bibeau, C. & Melkonian, M. 1994. Prasinophytes form independent lineages within the chlorophyta: evidence from ribosomal RNA sequence comparisons. *J. Phycol.* 30:340–5.

Swofford, D. L. 1990. *PAUP—Phylogenetic Analysis Using Parsimony,* Ver. 3.0L. Illinois Natural History Survey, University of Illinois, Champaign. (computer software program)

Watanabe, S., Chappell, D. F. & Floyd, G. L. 1992. Ultrastructure of the flagellar apparatus of the gametes of *Gongrosira papuasica* (Chlorophyta). *Br. Phycol. J.* 27:21–8.

Watanabe, S. & Floyd, G. L. 1989. Ultrastructure of the quadriflagellate zoospores of the filamentous green algae *Chaetophora incrassata* and *Pseudoschizomeris caudata* (Chaetophorales, Chlorophyceae), with emphasis on the flagellar apparatus. *Bot. Mag. Tokyo* 102:533–46.

Wilcox, L. W., Fuerst, P. A. & Floyd, G. L. 1993. Phylogenetic relationships of four charophycean green algae inferred from complete nuclear-encoded small subunit rRNA gene sequences. *Am. J. Bot.* 80:1028–33.

Wilcox, L. W., Lewis, L. A., Fuerst, P. A. & Floyd, G. L. 1992a. Group I introns within the nuclear-encoded small-subunit rRNA gene of three green algae. *Mol. Biol. Evol.* 9:1103–18.

——— 1992b. Assessing the relationships of autosporic and zoosporic Chlorococcalean green algae with 18S rDNA sequence data. *J. Phycol.* 28:381–6.