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The Origin of Land Plants: Phylogenetic Relationships Among Charophytes, Bryophytes, and Vascular Plants Inferred from Complete Small-Subunit Ribosomal RNA Gene Sequences

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Abstract. Complete nuclear-encoded small-subunit 18S rRNA (=SSU rRNA) gene sequences were determined for the prasinophyte green alga *Mantoniella squamata;* the charophycean green algae *Chara foetida, Coleochaete scutata, Klebsormidium flaccidum,* and *Mougeotia scalaris;* the bryophytes *Marchantia polymorpha, Fossombronia pusilla,* and *Funaria hygrometrica;* and the lycopod *Selaginella galleottii* to get a better insight into the sequential evolution from green algae to land plants. The sequences were aligned with several previously published SSU rRNA sequences from chlorophytic and charophytic algae as well as from land plants to infer the evolutionary relationships for major evolutionary lineages within the Chlorobionta by distance matrix, maximum parsimony, and maximum likelihood analyses. Phylogenetic trees created by the different methods consistently placed the Charophyceae on the branch leading to the land plants. The Charophyceae were shown to be polyphyletic with the Charales (''charalean'' algae) diverging earlier than the Coleochaetales, Klebsormidiales, Chlorokybales, and Zygnematales (''charophycean'' algae) which branch from a point closer to the land plants in most analyses. Maximum parsimony and maximum likelihood analyses imply a successive evolution from ''charophycean'' algae, particularly Coleochaetales, to bryophytes, lycopods, and seed plants. In contrast, distance matrix methods group the bryophytes together with the ''charophycean'' algae, suggesting a separate evolution of these organisms compared with the club moss and the seed plants.

Key words: 18S rRNA — Sequence analysis — Phylogeny — Molecular clock — Chlorophyceae — Charophyceae — Prasinophyceae — Bryophyta —*Selaginella*

Introduction

The evolutionary origin of the land plants has always been a subject of general interest and controversial debate. Morphological, biochemical, and molecular data gathered during the last 10 years strongly imply a common ancestry of land plants and the Charophyceae *sensu* Mattox and Stewart (1984), separating the remaining chlorophytes as a different lineage (cf. De Jesus et al. 1989; Manhart and Palmer 1990; Graham et al. 1991; Graham and Kaneko 1991; Graham 1993). Although it is now widely accepted that the Charophyceae are a sister group to the land plants, there is considerable disagreement about the systematics of different charophycean orders and about whether an alga similar either to *Chara* or *Coleochaete* is the sister group to the land plants.

Cladistic analyses based on ultrastructural and biochemical characters indicate that *Coleochaete* is the member of the Charophyceae most closely related to land plants (Mishler and Churchill 1985; Graham et al.

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Table 1. List of organisms

Taxonomic designation	Species	Strain[a]	EMBL acc. No.
Chlorophyta			
Prasinophyceae			
Mamiellales	*Mantoniella squamata*	CCAP 1965/1	X73999
Charophyceae			
Charales	*Chara foetida*	Bot. Gard. Cologne	X70704
Zygnematales	*Mougeotia scalaris*	Cult. Coll. Univ. Erlangen	X70705
Klebsormidiales	*Klebsormidium flaccidum*	SAG 335-2b	X75520
Coleochaetales	*Coleochaete scutata*	SAG 110.80	X68825
Bryophyta			
Marchantiopsida			
Marchantiidae			
Marchantiales	*Marchantia polymorpha*	Bot. Gard. Erlangen	X75521
Jungermanniidae			
Metzgeriales	*Fossombronia pusilla*	Cult. Coll. Univ. Heidelberg	X78341
Bryopsida			
Bryidae			
Funariales	*Funaria hygrometrica*	Cult. Coll. Univ. Heidelberg	X74114
Pteridophyta			
Lycopodiopsida			
Selaginellales	*Selaginella galleottii*	Bot. Gard. Erlangen	X75517

[a] CCAP, Culture Center of Algae and Protozoa at Cambridge, England; SAG, Sammlung von Algenkulturen der Universität Göttingen, Germany; Bot. Gard., Botanical Garden; Cult. Coll. Univ. Erlangen, Culture Collection at the University of Erlangen, Germany; Cult. Coll. Univ. Heidelberg, Culture Collection at the University of Heidelberg, Germany

1991; Graham 1993). As an example, the occurrence of placental transfer cells similar to those of land plants is so far known only from *Coleochaete orbicularis* (Graham and Wilcox 1983). The presence of ligninlike material in the placenta of *Coleochaete* also supports the placement of *Coleochaete* as a sister taxon to embryophytes in cladistic analyses (Delwiche et al. 1989). It is far beyond the scope of this contribution to give a comprehensive survey of ultrastructural or biochemical attributes supporting *Coleochaete, Chara,* or any other alga suggested to be ancestral to land plants; therefore the excellent book by Graham (1993) is recommended as a review.

In analyses based on 5S ribosomal RNA (=rRNA) sequences (Devereux et al. 1990), as well as partial small-subunit (=SSU) and large-subunit (=LSU) rRNA data, the "charalean" algae *Chara* and *Nitella* were placed next to the land plants (Chapman and Buchheim 1991, 1992). However, the resolution of these results taken alone is limited due to the small number of phylogenetically informative sites used (cf. Steele et al. [1991] and Halanych [1991] for a critical review of 5S rRNA–derived phylogenies).

Wilcox et al. (1993) have compared complete SSU rRNA gene sequences of four "charalean" and "charophycean" algae with sequences of several chlorophycean algae and seed plants. These analyses again placed *Nitella* closest to land plants, whereas *Coleochaete, Klebsormidium,* and *Chlorokybus* appeared as ancestors of both the chlorophytes and land plants, a scenario which is most unlikely. As the authors state, this result must be viewed with great caution, because it might be caused by the lack of sequences from appropriate green algae and other relevant taxa. Recently published analyses of zygnematalean (Surek et al. 1994) and two charalean algae (Ragan et al. 1994) also show an inconsistent branching pattern with respect to the Charophyceae.

Our study was particularly designed to examine the phylogenetic relationships between charophyceae and archegoniate land plants, based on a more comprehensive dataset, including complete SSU rRNA gene sequences of the prasinophyte green alga *Mantoniella squamata;* representatives of all four orders of the Charophyceae; two liverworts, one moss, and the lycopod *Selaginella galleottii.*

Materials and Methods

The taxonomic assignment and the origin of organisms from which complete nuclear-encoded SSU rRNA gene sequences were determined for this study are listed in Table 1.

DNA Isolation, Amplification of SSU rRNA Genes and Sequencing. Mantoniella squamata, Coleochaete scutata, Klebsormidium flaccidum, and *Mougeotia scalaris* were grown in appropriate culture media (Schönbohm 1963; McFadden and Melkonian 1986). The bryophytes were cultivated according to Bopp and Knoop (1984). For *Chara* and the land plants, total genomic DNA was isolated from fresh plant material.

Total DNA from *Mougeotia scalaris, Marchantia polymorpha,* and *Selaginella galleottii* was isolated according to Huss et al. (1986). From sterile cultures of the bryophytes *Funaria hygrometrica* and *Fossombronia pusilla,* DNA was extracted following the method of Murray and Thompson (1980). The cells of *Mantoniella, Coleochaete,* and

Klebsormidium were disrupted in a French Press, and the *Chara* plants were homogenized in a volume of sodium-EDTA solution (0.15 M NaCl, 0.1 M Na_2EDTA; pH 7.8) using a Waring blender (Behn and Herrmann 1977). The homogenates were diluted with two volumes of Tris-EDTA solution (50 mM Trizma-base, 20 mM Na_2EDTA; pH 8.0); 20% sodium-dodecylsulfate (w/v) in 20% ethanol was added to a final concentration of 2% SDS (v/v); 100 µl of proteinase K [Boehringer, Mannheim] (1 mg/ml) was added and the solutions were incubated for 12 h at 56°C in 50 ml Falcon tubes. Subsequently, the solutions were extracted with phenol:chloroform:isoamylalcohol (25:24:1), pH 7.0, and centrifuged 20 min at 4,000*g*. The upper, aqueous phases were transferred to new Falcon tubes and extracted for 20 min with chloroform. The aqueous phases were separated from the organic phase by 20-min centrifugation at 4,000*g*. The aqueous phases were dialyzed two times overnight against 0.2× SSC (30 mM NaCl, 3 mM sodium citrate; pH 7.0); then the dialysates were concentrated to a final volume of 8 ml; 1 g CsCl per ml solution and 200 µl Hoechst dye 33258 (1 mg/ml) were added and the solutions were centrifuged for 48 h at 150,000*g* in an ultracentrifuge using a swing-out rotor. Plastid and nuclear DNAs formed separate bands in the gradient and were harvested. Hoechst dye 33258 was extracted with isobutanol, and the CsCl was removed by dialysis against 0.2× SSC. The total volume was finally reduced to 500 µl using isobutanol, and the DNAs were precipitated by adding absolute ethanol to a final concentration of 70% (v/v) at –80°C for 30 min. The DNAs were pelleted at 18,300*g* for 15 min. The supernatants were decanted and the pellets dried in a speed-vac and resuspended in TE buffer (10 mM Trizma-base, 1 mM Na_2EDTA; pH 7.8) to a final concentration of 0.5–1 µg/µl.

The 18S rRNA genes were amplified from total genomic or from isolated nuclear DNA by the polymerase chain reaction (PCR) as described previously (Huss and Sogin 1990). The amplified DNA fragments were either cloned into bacteriophages M13mp18 and M13mp19 (Medlin et al. 1988), vector pUC18, or directly sequenced taking advantage of the Dynabeads-280 streptavidin system (Hultman et al. 1991). In each case the sequences of the coding and noncoding strand were determined with the dideoxynucleotide chain-terminating sequencing method (Sanger et al. 1977) using oligonucleotide primers that are complementary to evolutionary conserved regions of the SSU rRNA gene (Elwood et al. 1985).

Genomic DNA from *Funaria hygrometrica* was cut with the restriction enzyme *Eco*RI, and the fractions were separated on an 0.8% agarose gel. After blotting, the gel was hybridized to 18S rDNA from *Sinapis alba*. Bands hybridizing with the 18S rDNA were cut from the gel, electroeluted, and ligated into pUC18 vector with which *E. coli* HD-5 cells were transformed.

All sequences have been deposited in the EMBL database. The accession numbers are listed in Table 1.

Sequence Alignment and Phylogenetic Analyses. Secondary-structure models were constructed for all sequences according to Huss and Sogin (1990) and Neefs and De Wachter (1990) in order to optimize alignment of homologous nucleotide positions resulting in a total of 1,705 positions that could be used in the phylogenetic analyses.

The following 18S rRNA sequence data were integrated into the analysis from the GenBank/EMBL databases: *Dictyostelium discoideum* (McCarroll et al. 1983; X00134), *Saccharomyces cerevisiae* (Rubtsov et al. 1980; Mankin et al. 1986; J01353), *Ustilago maydis* (De Wachter et al. 1992; X62396), *Anemonia sulcata* (Hendriks et al. 1990; X53498), *Rattus norvegicus* (Chan et al. 1984; X01117), *Homo sapiens* (Gonzalez and Schmickel 1986; K03432), *Oryza sativa* (Takaiwa et al. 1984; X00755), *Sinapis alba* (Rathgeber and Capesius 1990; X17062), *Glycine max* (Eckenrode et al. 1985; X02623), *Zamia pumila* (Nairn and Ferl 1988; M20017), *Pinus wallichiana* (Sensen et al. 1994; X75080), *Coleochaete orbicularis* (Wilcox et al. 1993; M95611), *Chlorokybus atmophyticus* (Wilcox et al. 1993; M95612), *Nitella* spec. (Wilcox et al. 1993; M95615), *Staurastrum* spec. (Surek et al. 1994; X74752), *Genicularia spirotaenia* (Surek et al. 1994; X74753), *Mesotaenium caldariorum* (Surek et al. 1994; X75763), *Spermatozopsis similis* (Sensen et al. 1992; X65557), *Chlamydomonas reinhardtii* (Gunderson et al. 1987; M32703), *Scenedesmus obliquus* (Huss and Sogin 1990; X56103), and *Chlorella vulgaris* (Huss and Sogin 1989; X13688).

Maximum likelihood and distance analyses were calculated using the PHYLIP program package (Felsenstein 1993). A distance matrix of the aligned sequences was generated using the program DNADIST and corrected with the two-parameter method of Kimura (1980). The distances were then converted to phylogenetic trees using FITCH (Fitch and Margoliash 1967) and the neighbor-joining method of Saitou and Nei (1987) provided by the NEIGHBOR program. DNAML (with global rearrangement) was used for maximum likelihood analyses (Felsenstein 1981). Bootstrap resampling (Felsenstein 1985) was accomplished by the use of the programs SEQBOOT and CONSENSE. Maximum parsimony analyses (Camin and Sokal 1965) were calculated with the PAUP computer program (Swofford 1993) using a heuristic search procedure, a branch-swapping algorithm, and the Mulpars option. The same options were used in the parsimony bootstrap analysis. The distance and maximum likelihood tree showing the relationships of major lineages within the Chlorobionta were rooted using the SSU rRNA sequences of the slime mold *Dictyostelium discoideum,* three animals, and two fungi as a multiple outgroup (Figs. 1 and 3). To avoid additional homoplasy, the parsimony analysis was done without the fungi and animals, using the chlorophyte and prasinophyte algae as an outgroup (Fig. 2).

The sequence alignment and the weighting mask which determines the nucleotide positions taken for the analyses are available from the authors upon request.

Results

The size of the PCR products for all amplified SSU rRNA genes was about 1,800 bp except for *Mougeotia scalaris* and *Klebsormidium flaccidum,* with about 2,300 bp. This size could be attributed to single insertions possessing typical structural characteristics of group I introns (Cech 1988). The insertion site of the 535-bp intervening sequence of *M. scalaris* (28 bp from the 3′-terminus of the SSU rRNA gene) is identical to the one found in other zygnematalean algae (Surek et al. 1994). The SSU rRNA genes of *K. flaccidum* SAG 335-2b show an insertion of 525 extra bases between positions 564 and 565 of the mature rRNA molecule (data not shown), in contrast to strain UTEX 2017 of *K. flaccidum* used by Wilcox et al. (1993), which lacks this intron. In addition, the two strains can be distinguished by the substitution of three nucleotides and the insertion/deletion of a single nucleotide (not shown). The insertion site of this intron is identical to the site where similar introns in the SSU rRNA genes of *Chlorella luteoviridis* and *C. saccharophila* strain SAG 211-9b can be found (Huss et al., unpublished). All three intervening sequences possibly belong to a new subgroup of group I introns. A more detailed discussion of these and other group I introns found in SSU rRNA genes of green algae will be published elsewhere.

For *Funaria hygrometrica,* sequence data were accomplished from genomic DNA and PCR-amplified fragments. Identical sequences for the 18S rDNA were obtained by each method.

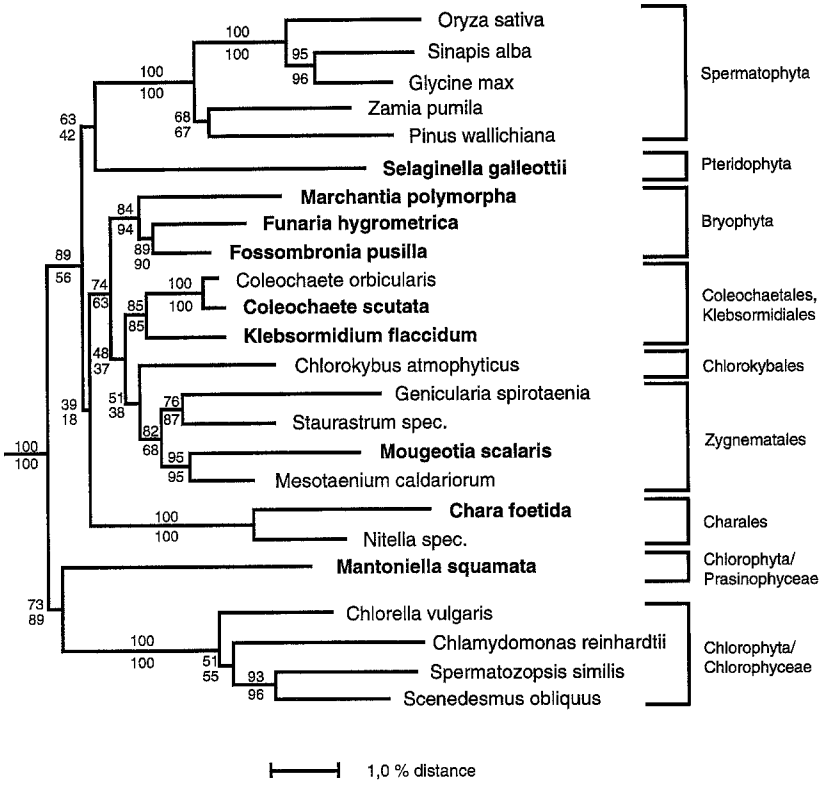

Fig. 1. Phylogenetic relationships within land plants and green algae. The tree is constructed with the Fitch and Margoliash (1967) algorithm based on structural distances (Kimura 1980) and rooted with the sequences of one slime mold, two fungi, and three animals. The distance that corresponds to 1% sequence divergence is indicated by the scale. The percentage that corroborates topological elements in the bootstrap analysis (500 resamplings) is shown above branches. Values below branches indicate the percentage of a bootstrap analysis (1,000 resamplings) using the neighbor-joining method (Saitou and Nei 1987).

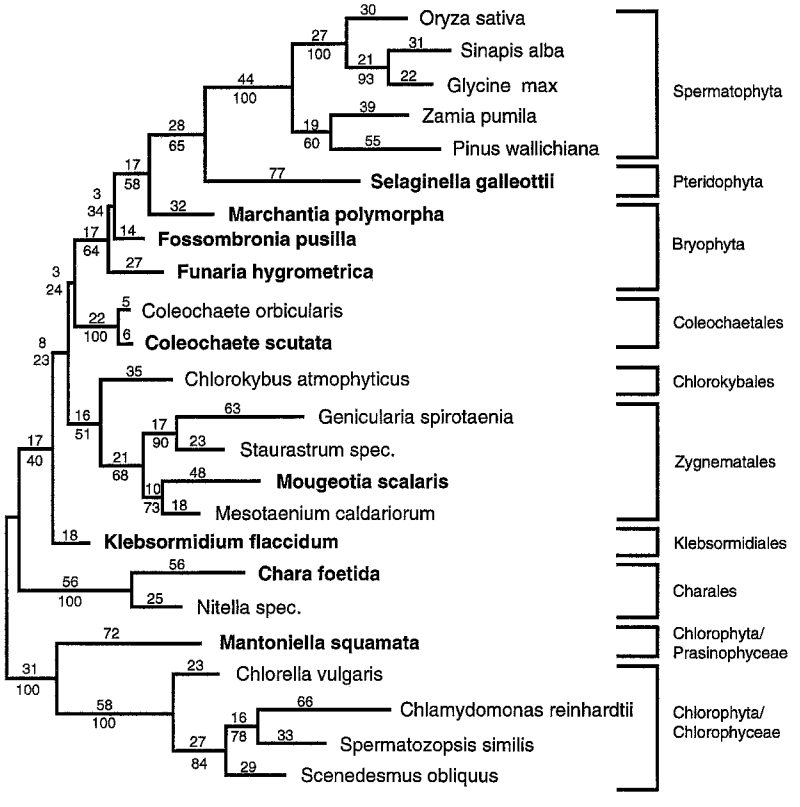

Fig. 2. Single most-parsimonious tree inferred from 1,705 aligned nucleotides of the 18S rRNA genes using a heuristic search procedure and a branch-swapping algorithm. The total length of the tree is 1,325 steps. The number of steps separating two nodes is shown above branches. Bootstrap values based on 100 resamplings with ten heuristic searches each are shown below the internal nodes. This phylogram has a consistency index (CI) of 0.547. The tree was rooted with *Mantoniella* and the chlorophycean algae.

The distance analyses, the FITCH and the NEIGHBOR method, resulted in very similar tree topologies. The Charophyceae *sensu* Mattox and Stewart (1984) and land plants form a monophyletic lineage excluding the chlorophycean algae and the prasinophyte *Mantoniella* (Fig. 1; only the FITCH tree is shown). Within this lineage, however, the small branch length separating the vascular plants from the Charales (''charalean'' algae)

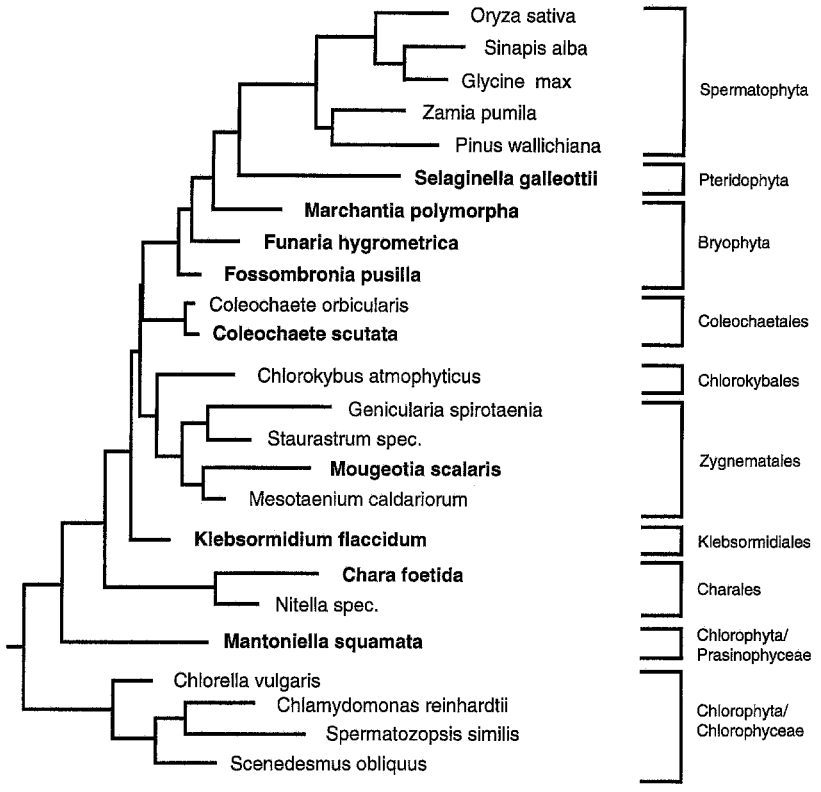

Fig. 3. Maximum-likelihood tree deduced from small-subunit rRNA sequences. The horizontal length of each branch is proportional to the estimated number of base substitutions. The log likelihood of the tree is −14,667.52. The same outgroups as listed in Fig. 1 were used to root the tree.

and the remaining Charophyceae (''charophycean'' algae) and bryophytes indicates an almost simultaneous radiation of these lineages in the distance analyses. Using neighbor-joining, the vascular plants form a sister group with the ''charophycean'' algae, while the ''charalean'' algae form the deepest branch (data not shown). Considering the low bootstrap values of 39% (FITCH) and 18% (NEIGHBOR) in favor of a monophyletic origin of the Charophyceae (Fig. 1), it is impossible to say whether the Charales are more closely related to the ''charophycean''/bryophyte or the lycopod/spermatophyte lineage. Nevertheless, the distance analyses suggest a distinct evolution of the Charales with regard to the other orders of the Charophyceae.

The ''charophycean'' algae form a single group together with the liverworts and the moss, supported by bootstrap values of 74% and 63% in the distance analyses (Fig. 1). This suggests a common origin of the ''charophycean'' algae and the bryophytes, and is strongly supported by distance values as low as 0.0309 between *Coleochaete orbicularis* and *Fossombronia pusilla* compared to 0.0745 and 0.0914 between *F. pusilla* and *Nitella* spec. and *Chara foetida,* respectively (Table 2). The bryophytes are separated from *Selaginella* and the spermatophytes by a much larger distance (0.0790 on an average) than they are from *Coleochaete* or *Klebsormidium* (0.0418).

Within the bryophytes it is remarkable that the foliaceous liverwort *F. pusilla* is not grouping with the thalloid liverwort *Marchantia polymorpha* but with the moss *Funaria hygrometrica* (Fig. 1). The high bootstrap values (89%/90%) strongly support a polyphyletic origin of the foliaceous Jungermanniidae and the thalloid Marchantiidae (cf. Schuster 1984).

The Zygnematales appear as a sister group to *Klebsormidium* and *Coleochaete* in the distance analyses, whereas *Chlorokybus* takes an intermediate position between both groups (Fig. 1).

The phylogenetic trees inferred from the maximum parsimony and the maximum likelihood analysis (Figs. 2 and 3) have a similar topology to the most parsimonious tree concerning the land plants. Both analyses show a successive branching pattern referring to the different orders of the ''charophycean'' algae and the bryophytes rather than forming a common group.

The result of a maximum parsimony analysis shown in Fig. 2 is a single most parsimonious tree with a length of 1,325 steps (two steps less than required for the topology of the maximum likelihood tree and 21 steps less than the distance tree). While an independent evolution of the Charales is again supported by a deep branch, *Klebsormidium* occupies a basal position relative to the other ''charophycean'' algae, followed by a branch leading to *Chlorokybus* and the Zygnematales. *Coleochaete* appears most closely related to, but not in a common cluster with the bryophytes, as is the case in the distance analyses. Instead, a successive branching order from ''charophycean'' algae to *Funaria, Fossombronia, Marchantia, Selaginella,* and seed plants is suggested by the parsimony analysis. This scenario is more consistent with the classical view of land plant evolution but is not strongly supported by bootstrapping (Fig. 2). It is, how-

ever, supported by the maximum likelihood analysis, which results in the same tree topology except that *Funaria* and *Fossombronia* change positions.

Discussion

The method of comparative sequence analysis of small-subunit rRNA genes has been used successfully to elucidate phylogenetic relationships of prokaryotes and eukaryotes. A growing database of small-subunit rRNA molecule sequences allows one to trace back the evolutionary history of most of the major lineages of organisms (Sogin 1989). However, the origin of the land plants, although of notable common interest, has not been systematically investigated using this method. Drawbacks of previous reports concerning the evolution of land plants are based on either the use of insufficient information as shown in analyses of the very small 5S rRNA sequences (Hori and Osawa 1987; Krishnan et al. 1990; Van de Peer et al. 1990) and analyses of partial SSU or LSU rRNA sequences (Chapman and Buchheim 1991, 1992), or on the lack of appropriate reference organisms (Wilcox et al. 1993; Ragan et al. 1994; Surek et al. 1994).

We have now determined complete SSU rRNA sequences from one or more representatives of the major lineages that should be important in determining the phylogenetic relationships between chlorophytes, charophytes (=Charophyceae *sensu* Mattox and Stewart [1984]), and land plants. Prasinophytes such as *Mantoniella squamata* are commonly regarded as ancestral to the chlorophytes (Melkonian 1990). The SSU rRNA gene sequence of *M. squamata* should therefore be helpful in determining the borderline between chlorophytes and charophytes. The position of *Mantoniella* in the distance analyses with bootstrap values of 73% and 89% supports the above view (Fig. 1), although maximum likelihood places *Mantoniella* closer to the charophytes (Fig. 3). A signature nucleotide analysis of the SSU rRNA data (cf. Rouvière et al. 1992) suggests, in support of the distance trees, that *Mantoniella* and the chlorophytes shared a short time of common evolution, while most of the mutations that delimit chlorophytes from charophytes and land plants have accumulated after the divergence of the *Mantoniella* ancestor (data not shown). Regardless of the exact position of *Mantoniella* and other prasinophytes, it is compatible with the features of a hypothetical ancestor of the charophytes as described by Graham (1993) to assume that a common ancestor of chlorophytes and charophytes is closely related to the prasinophytes.

Our analyses consistently show the charophytes and land plants as a sister group to the chlorophytes. This is concordant with the results of two recently published papers dealing with the phylogeny of the Zygnematales (Surek et al. 1994) and the Charales (Ragan et al. 1994). In his review article about the delimitation of the kingdom Protozoa, Cavalier-Smith (1993) presents an 18S rRNA–derived phylogeny of 150 eukaryotes, which also demonstrates the monophyly of the Streptophyta (=land plants + charophytes). The contradictory results of Wilcox et al. (1993) might be an effect of the homoplasy achieved by inclusion of two distant related outgroups in their parsimony analyses. We further show that the charophytes are not monophyletic. The "charalean" algae form an early diverging and evolutionary distinct lineage compared to the "charophycean" algae. The possession of a well-conserved chloroplast *tuf*A gene which is strongly divergent or absent from all "charophycean" algae and land plant cpDNAs but present in all chlorophytes examined so far (Baldauf and Palmer 1990; Baldauf et al. 1990) does support a position of the Charales next to the chlorophycean algae. In a comparison of the cell-wall compositions of *Mougeotia, Klebsormidium,* and *Chara* it was also shown that Zygnematales and Klebsormidiales are more closely related to each other than to the Charales (Domozych et al. 1980; Hotchkiss et al. 1989).

Unlike the Charales, the Zygnematales in all our analyses show close affinities to the remaining "charophycean" algae. The separation of the Zygnematales as an individual phylum Conjugaphyta by Hoshaw et al. (1990) therefore does not appear to be justified. In the parsimony and maximum likelihood analyses the deepest branch within the "charophycean" algae is occupied by *Klebsormidium* instead of *Chlorokybus* supposed to be the most basal member of the Charophyceae (Mattox and Stewart 1984). However, the high bootstrap values in both distance analyses (85%/85%) and very low evolutionary distances (0.0284 and 0.0300; Table 2) between *Klebsormidium* and both species of *Coleochaete* indicate a close relationship between these algae. Moreover, the distance matrix tree topology in Fig. 1 in favor of a common origin for the "charophycean" algae requires only two steps more in the parsimony analysis and is also not significantly worse using the maximum likelihood algorithm (Kishino-Hasegawa log-likelihood difference test: $\ln L = -14671.86$; $\Delta \ln L = -4.34$).

The bryophytes are represented in our study by the liverworts *Marchantia polymorpha* (Marchantiidae) and *Fossombronia pusilla* (Jungermanniidae) and by the moss *Funaria hygrometrica.* According to the rRNA data, however, *F. pusilla* is more closely related to the moss than to *M. polymorpha.* Analyses of more Jungermanniidae (*Calypogeia arguta; Scapania nemorea*), Marchantiidae (*Riccia fluitans, Reboulia hemispherica; Conocephalum conicum*), and Bryidae (*Physcomitrella patens; Leptobryum pyriforme; Mnium hornum*) show that the Marchantiidae are a sister group to the Jungermanniidae and Bryidae, which themselves are sister groups (Capesius, submitted).

Table 2. Structural similarity and distance data between small-subunit rRNA gene sequences[a]

Organism	M.s.	C.f.	N.s.	C.a.	M.s.	M.c.	S.s.	G.s.
Mantoniella squamata	—	0.889	0.896	0.913	0.903	0.919	0.913	0.898
Chara foetida	0.1153	—	0.952	0.912	0.901	0.914	0.913	0.901
Nitella sp.	0.1068	0.0492	—	0.924	0.918	0.931	0.931	0.911
Chlorokybus atmophyticus	0.0865	0.0931	0.0801	—	0.943	0.955	0.948	0.929
Mougeotia scalaris	0.0973	0.1036	0.0851	0.0572	—	0.960	0.944	0.924
Mesotaenium caldariorum	0.0792	0.0891	0.0702	0.0453	0.0403	—	0.965	0.942
Staurastrum sp.	0.0855	0.0898	0.0696	0.0511	0.0570	0.0340	—	0.948
Genicularia spirotaenia	0.1030	0.1032	0.0927	0.0724	0.0791	0.0592	0.0527	—
Klebsormidium flaccidum	0.0748	0.0893	0.0693	0.0437	0.0679	0.0370	0.0464	0.0681
Coleochaete orbicularis	0.0722	0.0915	0.0731	0.0404	0.0600	0.0348	0.0443	0.0694
Coleochaete scutata	0.0736	0.0932	0.0744	0.0432	0.0614	0.0370	0.0433	0.0700
Funaria hygrometrica	0.0897	0.0930	0.0793	0.0534	0.0745	0.0527	0.0503	0.0767
Fossombronia pusilla	0.0842	0.0914	0.0745	0.0463	0.0653	0.0438	0.0489	0.0688
Marchantia polymorpha	0.0917	0.0996	0.0845	0.0629	0.0772	0.0617	0.0652	0.0794
Selaginella galleottii	0.1168	0.1095	0.0955	0.0901	0.1015	0.0869	0.0795	0.0941
Zamia pumila	0.1075	0.1124	0.0990	0.0833	0.0976	0.0822	0.0830	0.1015
Pinus wallichiana	0.1240	0.1136	0.0941	0.0936	0.1092	0.0889	0.0836	0.0995
Sinapis alba	0.1133	0.1249	0.1127	0.0887	0.1085	0.0924	0.0923	0.1048
Glycine max	0.1158	0.1206	0.1058	0.0893	0.1091	0.0962	0.0889	0.0994
Oryza sativa	0.1168	0.1186	0.1072	0.1008	0.1147	0.0997	0.0976	0.1096

[a] The upper right half of the table gives the weighted structural similarity (fraction of homologous sites that are identical), and the lower-left half of the table shows the distance data (average number of base substitutions per sequence position) corrected by the Kimura two-parameter model. The boxes indicate distance values referred to in the text

The crucial question of our study was, which group of green algae is most closely related to the land plants? Cladistic analyses based on ultrastructural and biochemical data do favor *Coleochaete* to be the green alga which should be the closest relative to hornworts (Mishler and Churchill 1985), but molecular studies based on complete SSU rRNA sequence comparisons (Wilcox et al. 1993; Surek et al. 1994) placed the Charales next to land plants. In these studies, however, no bryophytes or pteridophytes were included and thus a large gap existed between the presumed ancestors of land plants and the more distant spermatophytes. The inclusion of a moss, *Funaria hygrometrica,* and two liverworts in our analyses clearly show a close relationship of the early land plants to the "charophycean" algae, especially to *Coleochaete,* rather than to *Chara* or *Nitella.* This result is independent of the tree building algorithm but is most obvious viewing the evolutionary distances (Table 2). The distances between *Chara/Nitella* and the bryophytes are about twice as large (0.0871 on an average) as those between bryophytes and *Coleochaete/Klebsormidium* (0.0418). The latter value is comparable to the distance found between the monocotyledone plant *Oryza sativa* and the dicotyledone plant *Glycine max* (0.0426). Even *Chara* and *Nitella,* which clearly belong to a single group supported by a bootstrap value of 100% (Figs. 1 and 2), are separated by a distance of 0.0492.

According to the low evolutionary distances, the distance analyses result in a separate group containing the "charophycean" algae and bryophytes (Fig. 1). In contrast, the maximum parsimony and maximum likelihood analyses suggest a sequential branching order of "charophycean" algae, bryophytes, lycopods, and seed plants, which is more congruent with the classical view of land plant evolution. If the picture of a distinct evolutionary lineage of "charophycean" algae and bryophytes, as suggested by the use of the distance methods, is caused by an artefact of method, and the parsimony and likelihood trees would give the "true" answer, then the evolutionary distances of "charophycean" algae to bryophytes and seed plants should be the same, supposing that all rRNA genes evolve in a tachytelic manner. Only if the "charophycean" algae have evolved at a very slow rate, the bryophytes at an intermediate rate, and seed plants at a comparatively fast rate, could the distance values be concordant with the phylogenetic relationships shown in Figs. 2 and 3.

Unequal mutation rates in rRNA genes have indeed been observed (cf. Liesack et al. 1992) and are known to cause potential errors in reconstructing phylogenetic trees (Li et al. 1987; Felsenstein 1988). Fortunately, such tachytelically evolving sequences can be easily recognized (cf. Kimura 1980; Tajima 1993). To estimate whether the distance trees might be influenced by variable rates of evolution we have evaluated mutation rates in the different groups of organisms by calculation of the

Table 2. Continued

K.f.	C.o.	C.s.	F.h.	F.p.	M.p.	S.g.	Z.p.	P.w.	S.a.	G.m.	O.s.
0.924	0.923	0.925	0.911	0.916	0.909	0.887	0.896	0.882	0.885	0.888	0.888
0.915	0.910	0.912	0.912	0.914	0.907	0.897	0.896	0.895	0.883	0.888	0.890
0.934	0.927	0.929	0.925	0.929	0.920	0.910	0.908	0.912	0.896	0.901	0.900
0.957	0.957	0.958	0.948	0.955	0.940	0.914	0.921	0.912	0.913	0.915	0.906
0.933	0.937	0.939	0.928	0.936	0.925	0.904	0.907	0.897	0.898	0.897	0.892
0.962	0.961	0.962	0.947	0.956	0.939	0.916	0.921	0.915	0.909	0.908	0.905
0.953	0.951	0.956	0.949	0.951	0.936	0.923	0.920	0.919	0.910	0.914	0.907
0.933	0.929	0.931	0.926	0.933	0.923	0.910	0.904	0.906	0.900	0.905	0.896
—	0.969	0.971	0.961	0.966	0.954	0.916	0.927	0.921	0.916	0.920	0.914
0.0284	—	0.990	0.955	0.966	0.947	0.921	0.926	0.921	0.913	0.913	0.909
0.0300	0.0065	—	0.958	0.968	0.951	0.925	0.927	0.921	0.914	0.917	0.911
0.0399	0.0435	0.0437	—	0.975	0.959	0.928	0.933	0.927	0.919	0.922	0.920
0.0343	0.0309	0.0324	0.0258	—	0.963	0.928	0.940	0.929	0.923	0.924	0.919
0.0481	0.0518	0.0513	0.0426	0.0381	—	0.922	0.929	0.921	0.918	0.924	0.917
0.0877	0.0786	0.0778	0.0755	0.0753	0.0816	—	0.908	0.907	0.900	0.904	0.905
0.0777	0.0745	0.0778	0.0708	0.0629	0.0752	0.0983	—	0.945	0.937	0.937	0.939
0.0837	0.0806	0.0845	0.0780	0.0753	0.0840	0.0990	0.0578	—	0.929	0.929	0.928
0.0864	0.0839	0.0871	0.0814	0.0772	0.0839	0.1009	0.0642	0.0741	—	0.968	0.952
0.0836	0.0877	0.0876	0.0819	0.0792	0.0793	0.1011	0.0647	0.0747	0.0301	—	0.958
0.0918	0.0940	0.0945	0.0853	0.0859	0.0887	0.1006	0.0635	0.0761	0.0438	0.0426	—

arithmetic means and standard deviations of the distance values between three outgroups (*Dictyostelium discoideum, Ustilago maydis,* and *Saccharomyces cerevisiae*) and the different green algal and land plant lineages (Table 3). For the data based on *D. discoideum* as an outgroup, the Student's t-tests indicated a significantly slower evolutionary rate for the "charophycean" algae and the bryophytes compared with the other green algae and spermatophytes ($P < 0.01$; data not shown). A similar observation is reported for "charophycean" algae by Wilcox et al. (1993). However, if *U. maydis* or *S. cerevisiae* were used as outgroups, nearly identical rates of nucleotide substitutions in the SSU rRNA genes were indicated. Although a moderate tendency for slow substitution rates in "charophycean" algae and bryophytes can be recognized, this tendency seems unlikely to be strong enough to account for the high similarity of their rRNA sequences. Therefore we cannot rule out that *Coleochaete* and related algae share a common evolutionary history with the bryophytes distinct from the lineage that gave rise to *Selaginella* and seed plants. With respect to the relatively low bootstrap support and the different solution given by the parsimony and likelihood methods, we have to acknowledge, however, that it is not possible to give a definite answer to this question with the available data. An extended study, which includes sequences of more bryophytes and particularly of more pteridophytes, should help to clarify the relationship between bryophytes and "charophycean" algae.

According to the fossil record, the bryophytes and lycopsids appeared at about the same time, 350 million and 340 million years ago (Stewart 1983). A similar conclusion is drawn by Raubeson and Jansen (1992). They show that bryophytes and lycopsids share a common primitive gene order in the chloroplast DNA that is inverted in all other vascular plants. Both findings are in good agreement with the position of *Selaginella galleottii* in our study, which branches shortly after the divergence of the bryophytes. This might be responsible for the low bootstrap values of 42% and 63% supporting a common origin of *Selaginella* and seed plants (Fig. 1). In contrast, a monophyletic origin of gymnosperms and angiosperms is supported in 100% of the bootstrap resamplings.

Our study indicates that *Coleochaete* and *Klebsormidium,* and not *Chara* or *Nitella,* share the most recent common ancestor with the bryophytes. Moreover, the Charales represent an early branching and distinct lineage in agreement with the separation of the Charales as a phylum Charophyta by Bold and Wynne (1985). However, the exact branching order of algal and land-plant lineages that eventually gave rise to seed plants remains uncertain. One reason is the lack of information available about the reliability of distance or maximum likelihood methods compared with the well-understood "powers and pitfalls" of parsimony (Felsenstein 1978, 1988; Stewart 1993). Without additional data, particularly from ferns and allies, it will be difficult to decide which tree reflects the real phylogeny best.

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Table 3. Distance data, arithmetic mean (x_m), and standard deviations (σ_{n-1}), between green algal/land plant lineages and different outgroups

	D. discoideum	*U. maydis*	*S. cerevisiae*
Chlorophyceae/Prasinophyceae:			
C. vulgaris	0.3585	0.2006	0.1801
S. obliquus	0.3571	0.2155	0.1917
C. reinhardtii	0.3631	0.2210	0.1910
S. similis	0.3630	0.2182	0.1879
M. squamata	0.3516	0.2108	0.1867
$x_m \pm \sigma_{n-1}$	0.3587 ± 0.0043	0.2132 ± 0.0080	0.1875 ± 0.0046
Charophyceae (Charales):			
C. foetida	0.3466	0.2373	0.1923
N. sp.	0.3529	0.2189	0.1851
$x_m \pm \sigma_{n-1}$	0.3498 ± 0.0032	0.2281 ± 0.0130	0.1887 ± 0.0051
Charophyceae (remaining orders):			
C. atmophyticus	0.3217	0.1960	0.1629
K. flaccidum	0.3266	0.2035	0.1709
C. orbicularis	0.3220	0.2000	0.1667
C. scutata	0.3231	0.2055	0.1720
M. scalaris	0.3234	0.2104	0.1775
M. caldariorum	0.3252	0.2053	0.1683
S. sp.	0.3278	0.2083	0.1767
G. spirotaenia	0.3359	0.2106	0.1820
$x_m \pm \sigma_{n-1}$	0.3257 ± 0.0043	0.2050 ± 0.0051	0.1721 ± 0.0063
Bryophyta:			
M. polymorpha	0.3240	0.1988	0.1805
F. hygrometrica	0.3347	0.2068	0.1731
F. pusilla	0.3316	0.2074	0.1784
$x_m \pm \sigma_{n-1}$	0.3301 ± 0.0055	0.2043 ± 0.0048	0.1773 ± 0.0038
Pteridophyta:			
S. galleottii	0.3339	0.2067	0.1866
Spermatophyta:			
Z. pumila	0.3454	0.2180	0.1862
P. wallichiana	0.3491	0.2221	0.1987
S. alba	0.3508	0.2120	0.1884
G. max	0.3471	0.2148	0.1864
O. sativa	0.3538	0.2205	0.1971
$x_m \pm \sigma_{n-1}$	0.3492 ± 0.0042	0.2175 ± 0.0041	0.1914 ± 0.0061

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References

Baldauf SL, Palmer JD (1990) Evolutionary transfer of the chloroplast *tuf*A gene to the nucleus. Nature 344:262–265

Baldauf SL, Manhart JR, Palmer JD (1990) Different fates of the chloroplast *tuf*A gene following its transfer to the nucleus in green algae. Proc Natl Acad Sci USA 87:5317–5321

Behn W, Herrmann RG (1977) Circular molecules in the β-satellite DNA of *Chlamydomonas reinhardtii*. Mol Gen Genet 157:25–30

Bold HC, Wynne MJ (1985) Introduction to the algae. Prentice-Hall, Englewood Cliffs, NJ

Bopp M, Knoop B (1984) Culture methods for bryophytes. In: Vasil I (ed) Cell culture and somatic cell genetics of plants. Academic Press, New York, pp 96–106

Camin JH, Sokal RR (1965) A method for deducing branching sequences in phylogeny. Evolution 19:311–326

Capesius I (1994) New information about the phylogeny of bryophytes from the nuclear encoded 18S rRNA genes. J Plant Physiol (submitted)

Cavalier-Smith T (1993) Kingdom Protozoa and its 18 phyla. Microbiol Rev 57:953–994

Cech TR (1988) Conserved sequences and structures of group I introns: building an active site for RNA catalysis—a review. Gene 73:259–271

Chan YL, Gutell R, Noller HF, Wool IG (1984) The nucleotide sequence of a rat 18S ribosomal ribonucleic acid gene and a proposal for the secondary structure of 18S ribosomal ribonucleic acid. J Biol Chem 259:224–230

Chapman RL, Buchheim MA (1991) Ribosomal RNA sequences: analysis and significance in the phylogeny and taxonomy of green algae. Crit Rev Plant Sci 10:343–368

Chapman RL, Buchheim MA (1992) Green algae and the evolution of land plants: inferences from nuclear-encoded rRNA gene sequences. Biosystems 28:127–137

De Jesus M, Tabatabai F, Chapman DJ (1989) Taxonomic distribution of copper-zinc superoxide dismutase in green algae and its phylogenetic importance. J Phycol 25:767–772

Delwiche CF, Graham LE, Thomson N (1989) Lignin-like compounds and sporopollenin in *Coleochaete,* an algal model for land plant ancestry. Science 245:399–401

Devereux R, Loeblich III AR, Fox GE (1990) Higher plant origins and the phylogeny of green algae. J Mol Evol 31:18–24

De Wachter R, Neefs J-M, Goris A, Van de Peer Y (1992) The gene coding for small ribosomal RNA in the basidiomycete *Ustilago maydis* contains a group I intron. Nucleic Acids Res 20:1251–1257

Domozych DS, Stewart KD, Mattox KR (1980) The comparative aspects of cell wall chemistry in the green algae (*Chlorophyta*). J Mol Evol 15:1–12

Eckenrode VK, Arnold J, Meagher RB (1985) Comparison of the nucleotide sequence of soybean 18S rRNA with the sequences of other small-subunit rRNAs. J Mol Evol 21:259–269

Elwood HJ, Olsen GJ, Sogin ML (1985) The small subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata.* Mol Biol Evol 2:399–410

Felsenstein J (1978) Cases in which parsimony and compatibility methods will be positively misleading. Syst Zool 27:401–410

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Felsenstein J (1988) Phylogenies from molecular sequences: inference and reliability. Annu Rev Genetics 22:521–565

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package); Version 3.5c. University of Washington, Seattle

Fitch WM, Margoliash E (1967) Construction of phylogenetic trees. Science 155:279–284

Gonzalez IL, Schmickel RD (1986) The human 18S ribosomal RNA gene: Evolution and stability. Am J Hum Genet 38:419–427

Graham LE (1993) Origin of land plants. John Wiley & Sons, New York

Graham LE, Kaneko Y (1991) Subcellular structures of relevance to the origin of the land plants (embryophytes) from green algae. Crit Rev Plant Sci 10:323–342

Graham LE, Wilcox LW (1983) The occurrence and phylogenetic significance of putative placental transfer cells in the green alga *Coleochaete.* Am J Bot 70:113–120

Graham LE, Delwiche CF, Mishler BD (1991) Phylogenetic connections between the "green algae" and the "bryophytes." Adv Bryol 4:213–244

Gunderson JH, Elwood H, Ingold A, Kindle K, Sogin ML (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. Proc Natl Acad Sci USA 84:5823–5827

Halanych KM (1991) 5S ribosomal RNA sequences inappropriate for phylogenetic reconstruction. Mol Biol Evol 8:249–253

Hendriks L, Van de Peer Y, Van Herck M, Neefs J-M, De Wachter R (1990) The 18S ribosomal RNA sequence of the sea anemone *Anemonia sulcata* and its evolutionary position among other eukaryotes. FEBS Lett 269:445–449

Hori H, Osawa S (1987) Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. Mol Biol Evol 4:445–472

Hoshaw RW, McCourt RM, Wang JC (1990) Phylum Conjugaphyta. In: Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds) Handbook of Protoctista, Jones and Barlett, Boston, pp 119–131

Hotchkiss Jr AT, Gretz MR, Hicks KB, Brown Jr RM (1989) The composition and phylogenetic significance of the *Mougeotia* (Charophyceae) cell wall. J Phycol 25:646–654

Hultman T, Bergh S, Moks T, Uhlen M (1991) Bidirectional solid-phase sequencing of *in vitro*-amplified plasmid DNA. Biotechniques 10:84–93

Huss VAR, Sogin ML (1989) Primary structure of the *Chlorella vulgaris* small subunit ribosomal RNA coding region. Nucleic Acids Res 17:1255

Huss VAR, Sogin ML (1990) Phylogenetic position of some *Chlorella* species within the Chlorococcales based upon complete small-subunit ribosomal RNA sequences. J Mol Evol 31:432–442

Huss VAR, Dörr R, Grossmann U, Kessler E (1986) Deoxyribonucleic acid reassociation in the taxonomy of the genus *Chlorella.* I. *Chlorella sorokiniana.* Arch Microbiol 145:329–333

Kimura M (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Krishnan S, Barnabas S, Barnabas J (1990) Interrelationships among major protistan groups based on a parsimony network of 5S rRNA sequences. Biosystems 24:135–144

Li W-H, Wolfe KH, Sourdis J, Sharp PM (1987) Reconstruction of phylogenetic trees and estimation of divergence times under nonconstant rates of evolution. Cold Spring Harb Symp Quant Biol 52:847–856

Liesack W, Söller R, Stewart T, Haas H, Giovannoni S, Stackebrandt E (1992) The influence of tachytelically (rapidly) evolving sequences on the topology of phylogenetic trees—intrafamily relationships and the phylogenetic position of Planctomycetaceae as revealed by comparative analysis of 16S ribosomal RNA sequences. Syst Appl Microbiol 15:357–362

Manhart JR, Palmer JD (1990) The gain of two chloroplast tRNA introns mark the green algal ancestors of land plants. Nature 345: 268–270

Mankin AS, Skryabin KG, Rubtsov PM (1986) Identification of ten additional nucleotides in the primary structure of yeast 18S rRNA. Gene 44:143

Mattox KR, Stewart KD (1984) Classification of the green algae: a concept based on comparative cytology. In: Irvin DEG, John DM (eds) Systematics of the green algae. Academic Press, London, pp 29–72

McCarroll R, Olsen GJ, Stahl YD, Woese CR, Sogin ML (1983) Nucleotide sequence of the *Dictyostelium discoideum* small-subunit ribosomal ribonucleic acid inferred from the gene sequence: Evolutionary implications. Biochemistry 22:5858–5868

McFadden GI, Melkonian M (1986) Use of Hepes buffer for microalgal culture media and fixation for electron microscopy. Phycologia 25:551–557

Medlin L, Elwood HJ, Stickel S, Sogin M (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71:491–499

Melkonian M (1990) Phylum Chlorophyta: class Prasinophyceae. In: Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds) Handbook of Protoctista. Jones and Barlett, Boston, pp 600–607

Mishler BD, Churchill SP (1985) Transition to a land flora: phylogenetic relationships of the green algae and bryophytes. Cladistics 1:305–328

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 84:321–4325

Nairn CJ, Ferl RJ (1988) The complete nucleotide sequence of the small-subunit ribosomal RNA coding region for the cycad *Zamia pumila:* phylogenetic implications. J Mol Evol 27:133–141

Neefs J-M, De Wachter R (1990) A proposal for the secondary structure of a variable area of eukaryotic small ribosomal subunit RNA involving the existence of a pseudoknot. Nucleic Acids Res 18: 5695–5704

Ragan MA, Parsons TJ, Sawa T, Straus NA (1994) 18S ribosomal DNA sequences indicate a monophyletic origin of Charophyceae. J Phycol 30:490–500

Rathgeber J, Capesius I (1990) Nucleotide sequence of the intergenic spacer and the 18S ribosomal RNA gene from mustard (*Sinapis alba*). Nucleic Acids Res 18:1288

Raubeson LA, Jansen RK (1992) Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. Science 255: 1697–1699

Rouvière P, Mandelco L, Winkler S, Woese CR (1992) A detailed phylogeny for the Methanomicrobiales. System Appl Microbiol 15:363–371

Rubtsov PM, Musakhanov MM, Zakharyev VM, Krayev AS, Skryabin KG, Bayev AA (1980) The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae.* Nucleic Acids Res 8: 5779–5794

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schönbohm E (1963) Untersuchungen über die Starklichtbewegung des *Mougeotia*-Chloroplasten. Zeitschr Bot 51:233–276

Schuster RM (1984) Evolution, phylogeny and classification of the Hepaticae. In: Schuster RM (ed) New manual of bryology. The Hatori Botanical Laboratory, Nichinan, Miyazaki, Japan, pp 1071–1092

Sensen CW, Banrevi A, Capesius I (1994) The sequence of the 18S rDNA gene from *Pinus wallichiana.* Plant Mol Biol 24:259

Sensen CW, Hinkle GJ, Sogin ML, Melkonian M (1992) The sequence of the 18S rDNA gene from *Spermatozopsis similis* Preisig et Melkonian, 1894 (Chlorophyceae). Plant Mol Biol 20:1209

Sogin ML (1989) Evolution of eukaryotic microorganisms and their small subunit ribosomal RNAs. Am Zool 29:487–499

Steele KP, Holsinger KE, Jansen RK, Taylor DW (1991) Assessing the reliability of 5S rRNA sequence data for phylogenetic analysis in green plants. Mol Biol Evol 8:240–248

Stewart C-B (1993) The powers and pitfalls of parsimony. Nature 361:603–607

Stewart WN (1983) Paleobotany and the evolution of plants. Cambridge University Press, Cambridge, pp 394–395

Surek B, Beemelmanns U, Melkonian M, Bhattacharya D (1994) Ribosomal RNA sequence comparisons demonstrate an evolutionary relationship between Zygnematales and charophytes. Plant Syst Evol 191:171–181

Swofford DL (1993) PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natl Hist Surv, Champaign, IL

Tajima F (1993) Simple methods for testing the molecular evolutionary clock hypothesis. Genetics 135:599–607

Takaiwa F, Oono K, Sugiura M (1984) The complete nucleotide sequence of a rice 17S rRNA gene. Nucleic Acids Res 12:5441–5448

Van de Peer Y, De Baere R, Cauwenberghs J, De Wachter R (1990) Evolution of green plants and their relationship with other photosynthetic eukaryotes as deduced from 5S ribosomal RNA sequences. Plant Syst Evol 170:85–96

Wilcox LW, Fuerst PA, Floyd GL (1993) Phylogenetic relationships of four 'charophycean' green algae inferred from complete nuclear-encoded small subunit rRNA gene sequences. Am J Bot 80:1028–1033