

Phylogenetic position of the Sphaeropleaceae (Chlorophyta)

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Abstract. The complete 18S rRNA gene sequences of four *Sphaeroplea* C.A. Agardh strains (Sphaeropleales, Sphaeropleaceae), two *Atractomorpha* Hoffman strains (Sphaeropleales, Sphaeropleaceae) and two *Ankyra* Fott strains (Chlorococcales, Characiaceae) were determined and subjected to phylogenetic analyses. The analyses indicated that all these taxa belong to a monophyletic lineage (Sphaeropleaceae) and are related to a group of chlorophycean algae comprising autosporic taxa and taxa that reproduce by zoospores which are characterized by directly opposed basal bodies. The taxonomic assignment of the Sphaeropleaceae as a family within the Sphaeropleales (Chlorophyta, Chlorophyceae) is discussed.

Key words: 18S rDNA, *Ankyra*, *Atractomorpha*, Chlorophyta, molecular phylogeny, *Sphaeroplea*, Sphaeropleaceae, Sphaeropleales.

According to an emended diagnosis by Deason et al. (1991) the order Sphaeropleales Luerksen 1877 (Chlorophyta, Chlorophyceae), formerly represented by only one family Sphaeropleaceae Kützing 1849, comprises three families, the Sphaeropleaceae (Kützing) emend. Deason et al. 1991, the Hydrodictyaceae (Dumortier) emend. Deason et al. 1991 and the Neochloridaceae (Ettl et Komárek) emend. Deason et al. 1991. The order includes unicellular, filamen-

tous, or coenobitic green algae that produce motile cells with directly-opposed basal bodies. Oogamous, anisogamous or isogamous sexual reproduction is known to occur.

The family Sphaeropleaceae is characterized by unicellular or filamentous algae that exhibit incomplete septa (coenocytic organization) and naked motile cells. The basal bodies of the flagellar apparatus are directly opposed and oogamous or anisogamous sexual reproduction appears.

Within the green algae, directly opposite (DO), counterclockwise (CCW) and clockwise (CW) flagellar apparatus orientations are found (Mattox and Stewart 1984). According to Lewis et al. (1992) these flagellar apparatus configurations are indicative of evolutionary histories. Taxa possessing DO flagellar orientations were transferred to the Sphaeropleales, Chlorophyceae (Deason et al. 1991), those with CCW flagellar orientations were united in the Trebouxiophyceae (Friedl 1995) or the Ulvoephyceae and those coccoid taxa that exhibit a CW orientation were relegated to the Chlorococcales sensu stricto within the Chlorophyceae (Deason et al. 1991).

O’Kelly et al. (1994) proposed the erection of a second order that includes DO taxa, the

Chaetopeltidales, because the DO configuration has been discovered in both basal body pairs of the quadriflagellated *Chaetopeltis* and allies (O'Kelly and Floyd 1984, O'Kelly et al. 1994). Finally, the DO condition was shown for the upper pair of basal bodies in zoospores of at least some chaetophoralean taxa (Manton 1964, Melkonian 1975, Bakker and Lokhorst 1984, Watanabe and Floyd 1989).

Molecular analyses of 18S rDNA revealed, that the Sphaeropleales plus *Bracteacoccus* and autosporic chlorococcalean taxa (e.g. *Scenedesmus* and *Ankistrodesmus*) form a monophyletic lineage (Chapman et al. 1998). Booton et al. (1998a) suggest that the Sphaeropleales is the sister group to the chlorophycean order Chaetopeltidales, whereas the Chaetophorales are resolved as the sister group to the chlorophycean order Volvocales (Booton et al. 1998b). However, the molecular phylogenetic position of sphaeropleacean taxa (*Sphaeroplea* and *Atractomorpha*) remained, until now, an open question.

The genus *Sphaeroplea* comprises seven freshwater species and is characterized by unbranched vegetative filaments of large uniseriately arranged cells, whereas the two coccoid *Atractomorpha* species are characterized by multinucleate, spindle-shaped cells with "sharply pointed extremities" (Hoffman 1983).

Sphaeroplea and *Atractomorpha* both have pyrenoids that exhibit invaginations of the cytoplasm. This distinctive characteristic has been reported from the Sphaeropleaceae, the Oedogoniales and the chlorococcalean genus *Ankyra* (Mattox and Stewart 1984). In the case of the Oedogoniales, a basal position within the Chlorophyceae was supported by molecular phylogenetic analyses (Booton et al. 1998b), whereas *Ankyra* has not yet been investigated. The genus *Ankyra* frequently appears in the phytoplankton of lakes, rivers and ponds and is characterized by single, heteropolar spindle-shaped or cylindrical cells with a basal anchor-like protrusion of the cell wall. Zoospores, aplanospores and cysts are known to occur. The genus *Ankyra* currently comprises six species and was assigned to the

Characiaceae (Nägeli) Wille in Warming 1884 within the Chlorococcales, Chlorophyceae (Komárek and Fott 1983). According to Wilcox et al. (1992) and Floyd et al. (1993) who studied the 18S rDNA phylogeny of *Characium* species, the Characiaceae are a polyphyletic assemblage and, therefore, the position of *Ankyra* in the phylogenetic system of green algae is unknown.

Conflicting opinions concerning the taxonomic affinities of the Sphaeropleaceae have been discussed for more than 150 years, mainly by Agardh (1824), Kützing (1849), Engler and Prantl (1897), West and Fritsch (1927), Pascher (1931, 1939), Rieth (1952, 1953), Stewart and Mattox (1975), Mattox and Stewart (1984), Bold and Wynne (1978), Cáceres and Robinson (1980, 1981), Hoffman (1983, 1984a, b), Buchheim and Hoffman (1986), Deason et al. (1991), Chapman et al. (1998) and by Booton et al. (1998a, b). Depending on the author, the Sphaeropleaceae were classified either within what are now recognized as the class Ulvophyceae or within the class Chlorophyceae. They have been ascribed to the orders Siphonocladales, Ulotrichales, Cladophorales and in recent times to the separate order Sphaeropleales.

In this study we present the most extensive survey of the Sphaeropleaceae, to date, using results from analysis of 18S rRNA gene sequence data. We included 18S rDNA data from *Sphaeroplea robusta* Buchheim et Hoffman, *Sphaeroplea soleirolii* (Duby) Montagne ex. Kützing, *Sphaeroplea annulina* (Roth) C.A. Agardh (two strains), *Atractomorpha echinata* Hoffman (two strains), *Ankyra lanceolata* (Korshikov) Fott and *Ankyra judayi* (G. M. Smith) Fott, in order to elucidate the relationships of these taxa, one to another, and to examine their phylogenetic position within the Chlorophyta.

Material and methods

Atractomorpha echinata (SAG 70-90, AF302772), *Ankyra judayi* (SAG 17.84, U73469), and two strains of *Sphaeroplea annulina* (SAG 377-1a, AF302770;

Table 1. List of organisms used in the phylogenetic analyses, strain and GenBank accession numbers for 18S rRNA gene sequences. Sequences marked with an asterisk were determined in this study

Organisms	Strain no.	GenBank acc. no.
<i>Ankistrodesmus stipitatus</i>	SAG 202-5	X56100
<i>Ankyra judayi</i>	SAG 17.84	U73469*
<i>Ankyra lanceolata</i>	Hegewald 1998-5	AF302769*
<i>Asteromonas gracilis</i>	UTEX LB 635	M95614
<i>Atractomorpha echinata</i>	L. R. Hoffman	U73470*
<i>Atractomorpha echinata</i>	SAG 70-90	AF302772*
<i>Bracteacoccus</i> sp.	Broady #668	U63104
<i>Bulbochaete hiloensis</i>	UTEX 952	U83132
<i>Chaetopeltis orbicularis</i>	UTEX 422	U83125
<i>Chaetophora incrassata</i>	UTEX LB 1289	U83130
<i>Characiopodium hindakii</i>	UTEX 2098	M63000
<i>Chlamydomonas reinhardtii</i>	CC-400	M32703
<i>Chlamydomodium vacuolatum</i>	UTEX 2111	M63001
<i>Chlorella kessleri</i>	SAG 211-11g	X56105
<i>Chlorella minutissima</i>	Bethesda C-1.1.9	X56102
<i>Dunaliella parva</i>	UTEX LB 1983	M62998
<i>Ettlia minuta</i>	UTEX 776	M62996
<i>Fritschiella tuberosa</i>	UTEX 1821	U83129
<i>Fusochloris perforata</i>	UTEX 2104	M62999
<i>Gloeotilopsis planctonica</i>	SAG 29.93	Z28970
<i>Hormotilopsis gelatinosa</i>	UTEX 104	U83126
<i>Hydrodictyon reticulatum</i>	CBS	M74497
<i>Myrmecia israeliensis</i>	UTEX 1181	M62995
<i>Nanochlorum eucaryotum</i>	SAG 55.87	X06425
<i>Neochloris aquatica</i>	UTEX 138	M62861
<i>Nephroselmis olivacea</i>	SAG 40.89	X74754
<i>Oedocladium carolinianum</i>	UTEX LB 1686	U83135
<i>Oedogonium cardiacum</i>	UTEX 40	U83133
<i>Planophila terrestris</i>	UTEX 1709	U83127
<i>Prototheca wickerhamii</i>	SAG 263-11	X74003
<i>Pseudoscourfieldia marina</i>	CCMP 717	X75565
<i>Scenedesmus obliquus</i>	SAG 276-3a	X56103
<i>Sphaeroplea annulina</i>	SAG 377-1a	AF302770*
<i>Sphaeroplea annulina</i>	SAG 377-1e	AF302771*
<i>Sphaeroplea robusta</i>	L.R. Hoffman	U73472*
<i>Sphaeroplea soleirolii</i>	L.R. Hoffman	U73473*
<i>Stigeoclonium helveticum</i>	UTEX 441	U83131
<i>Trebouxia magna</i>	UTEX 902	Z21552
<i>Trebouxia impressa</i>	UTEX 892	Z21551
<i>Ulothrix zonata</i>	SAG 38.86	Z47999
<i>Volvox carteri</i>	UTEX 1885	X53904

Bethesda = Culture collection at Bethesda, Maryland, USA; CBS = Carolina Biol. Supply; CC = Chlamydomonas Genetics Center at Duke University, Durham, North Carolina, USA; CCMP = Culture collection of Marine Phytoplankton, McKown Point, West Boothbay, USA; SAG = Culture collection of Algae at the University of Göttingen, Germany; UTEX = Culture collection of Algae at the University of Texas, Austin, USA

A

(helix 10)

<i>Nephroselmis olivacea</i>	AC-UCCCGACUUC-GGAAGGGACG
<i>Ulothrix zonata</i>	.A-.....CAC-.....
<i>Gloeotilopsis planctonica</i>	.A-.....CAC-.....
<i>Nanochlorum eucaryotum</i>	CA-.....U.....
<i>Chlorella kessleri</i>	.A.....U.....
<i>Trebouxia impressa</i>	CA-.....CUU.....
<i>Chaetophora incrassata</i>	.AG...A...U.....
<i>Chlamydomonas reinhardtii</i>	CA-A.....U.....U..
<i>Volvox carteri</i>	CA-A.....U.....U..
<i>Dunaliella parva</i>	.A-.....U.....
<i>Ankistrodesmus stipitatus</i>	.A-.....U.....
<i>Bracteacoccus spec.</i>	.A-.....U.....
<i>Neochloris aquatica</i>	.A-A.....U.....U..
<i>Scenedesmus obliquus</i>	.A-.....U.....
<i>Oedogonium cardiacum</i>	.A-.....U.....
<i>Hydrodictyon reticulatum</i>	.A-A.....U.....U..
<i>Characiopodium hindakii</i>	.A-A.....U.....U..
<i>Chaetopeltis orbicularis</i>	.A-.....U.....
<i>Ankyra judayi</i>	.A-GGGU.....-.....CCUU.
<i>Ankyra lanceolata</i>	.A-GGGU.....-.....CCUU.
<i>Sphaeroplea annulina</i>	.A-GGGU.....-.....CCUU.
<i>Sphaeroplea robusta</i>	.A-GGGU.....N-.....CCUU.
<i>Sphaeroplea soleirolii</i>	.A-GGGU.....-.....CCUU.
<i>Atractomorpha echinata</i>	.A-GGGU.....-.....CCUU.

B

(helix 45)

<i>Nephroselmis olivacea</i>	UAGAUUAUUGCAACUAUUAUCUU
<i>Ulothrix zonata</i>	...AC.....U...GU...
<i>Gloeotilopsis planctonica</i>	...AC.....U...GU...
<i>Nanochlorum eucaryotum</i>U.....
<i>Chlorella kessleri</i>U.....
<i>Trebouxia impressa</i>U.....
<i>Chaetophora incrassata</i>U.....G...A
<i>Chlamydomonas reinhardtii</i>U.....G...
<i>Volvox carteri</i>U.....G...
<i>Dunaliella parva</i>U.....G...
<i>Ankistrodesmus stipitatus</i>U.....G...
<i>Bracteacoccus spec.</i>U.....G...
<i>Neochloris aquatica</i>U.....G...
<i>Scenedesmus obliquus</i>U.....G...
<i>Oedogonium cardiacum</i>	G.....U.....G...
<i>Hydrodictyon reticulatum</i>U.....G...
<i>Characiopodium hindakii</i>	...C.U.....U.....G...
<i>Chaetopeltis orbicularis</i>U.....G...
<i>Ankyra judayi</i>U.....G...
<i>Ankyra lanceolata</i>	...CCC.....U...GGG...
<i>Sphaeroplea annulina</i>	...CCC.....U...GGG...
<i>Sphaeroplea robusta</i>	...NCC.....U...GGG...
<i>Sphaeroplea soleirolii</i>	...CCC.....U...GGG...
<i>Atractomorpha echinata</i>	...CCC.....U...GGG...

Fig. 1. Proposed alignment of autapomorphic sites within the investigated group and selected green algae. **A** Helix 10 included seven autapomorphic sites for all sphaeropleacean taxa (compare to Fig. 2). **B** Helix 45 included an autapomorphic pattern for all sphaeropleacean taxa except *Ankyra judayi* (compare to Fig. 2). All nucleotides that were identical with the first sequence are indicated by a period. The outer square indicates chlorophycean taxa, the inner square indicates taxa investigated in this study

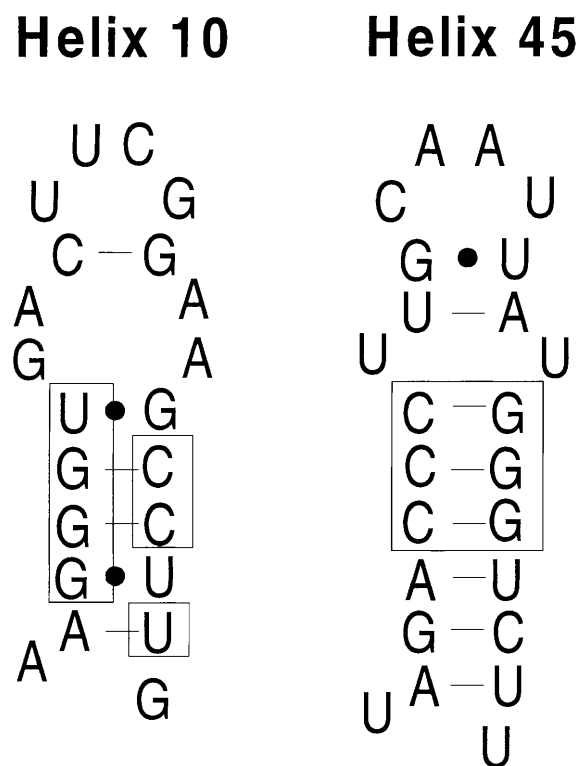


Fig. 2. Autapomorphic sites in proposed secondary structures of helix 10 and helix 45. Helix 10 included seven autapomorphic sites for all sphaeropleacean taxa (marked by rectangles, compare to Fig. 1A). Helix 45 included an autapomorphic pattern (marked with a rectangle) for all sphaeropleacean taxa except *Ankyra judayi* (compare to Fig. 1B)

SAG 377-1e, AF302771) were obtained from the Sammlung von Algenkulturen (SAG, Göttingen). An additional culture of *Atractomorpha echinata* (U73470), and cultures of *Sphaeroplea robusta* (U73472), and *Sphaeroplea soleirolii* (U73473) were provided by L.R. Hoffman. *Ankyra lanceolata* (strain Hegewald 1998-5, AF302769) was isolated from a sample received from A. Schmidt & G. Fehér from a dead arm of the Danube near Hercegszántó (Hungary) by E. Hegewald in 1998. Organisms were cultured at 20 °C under a 16:8 h L:D cycle (100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in medium 7 (Schlösser 1994).

After harvesting cells by centrifugation, the genomic DNA was extracted and purified using Dynabeads DNA DIRECT system I (Deutsche Dynal GmbH, Hamburg). PCR amplification of the 18S rRNA gene was carried out using PCR Ready-To-Go Beads (Amersham Pharmacia Biotech Inc., Freiburg), universal eukaryote specific

oligonucleotide primers (Elwood et al. 1985, Gunderson et al. 1986, Medlin et al. 1988) and a Progene thermocycler (Techne, Cambridge, U.K.). The PCR products were purified through Nucleon QC columns (Amersham Pharmacia Biotech Inc., Freiburg). For cycle-sequencing of the complete 18S rRNA gene, a Thermo Sequenase DYEnamic Direct cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech Inc., Freiburg), an automated ALF DNA Sequencer (Amersham Pharmacia Biotech Inc., Freiburg) as well as oligonucleotide primers that are complementary to conserved regions of the 18S rRNA gene were used.

All sequences were aligned with other green algal sequences according to conserved patterns of the primary structure and the secondary structure model of the 18S rRNA (Gutell et al. 1985). Alignment and direct comparison of sequences were carried out with the Windows-based multi-sequence alignment editor of Hepperle (1999). Those parts of the sequences where a proper alignment was not possible were excluded from the analysis. The alignment is available from the authors upon request. RNA secondary structures were calculated using the RNAstructure program 3.5c of Mathews et al. (1999).

For the construction of cladograms, the dataset was restricted to 41 green algae (Table 1). Phylogenetic analyses of aligned sequences were conducted using PAUP* version 4.0b8 win32 (Swofford 2001). Heuristic searches with 10 random taxon addition replicates and tree bisection-reconnection (TBR) swapping were applied. MulTrees and Collapse options of PAUP were used and character changes were interpreted under ACCTRAN optimization. For parsimony analyses (MP), characters were equally weighted and coded as unordered. For all analyses, gaps were treated as missing data. Bootstrap support for parsimony trees was estimated based on 1000 replicates. Minimum evolution (ME) trees were generated using maximum likelihood genetic distances, heuristic searches, TBR swapping, and a starting tree obtained via neighbor-joining. Bootstrap support also was estimated based on 1000 replicates. PUZZLE analyses were performed with 1000 quartet puzzling steps (set criterion = likelihood). Likelihood settings from the best-fit model (TIM + I + G, number of substitution types = 6) were determined using the AIC-criterion in Modeltest version 3.06 (Posada and Crandall 1998). Estimated parameters were used in ME and

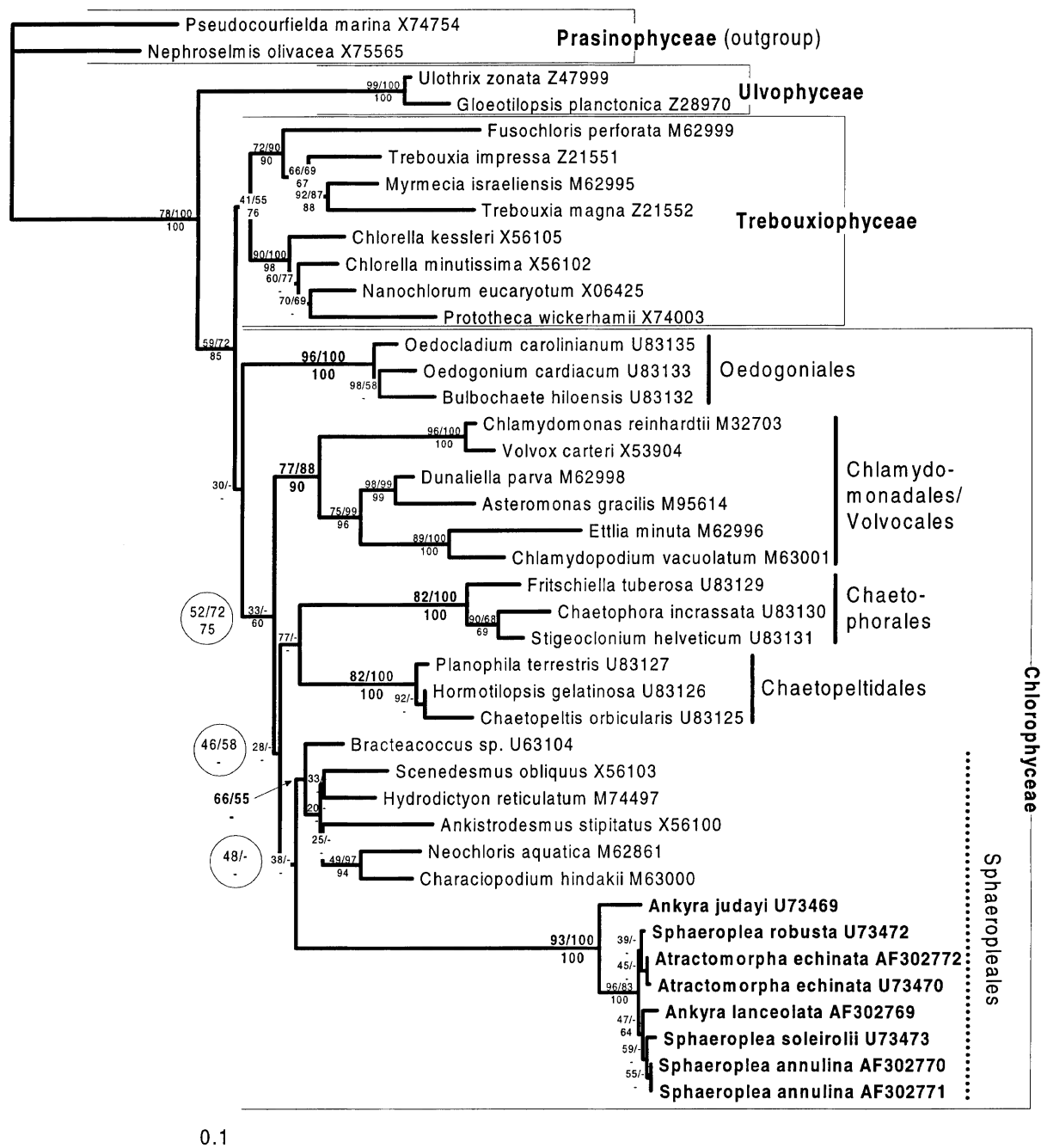


Fig. 3. Maximum likelihood tree as derived from PUZZLE analyses of 18S rRNA gene sequences using TIM + I + G (-Ln likelihood 8993.88779, estimated proportion of invariable sites 0.5295, gamma shape parameter 0.7186). Length of the alignment was 1623 sites of which 352 were parsimony informative. Numbers at the nodes indicate PUZZLE and bootstrap support values for the clusters to the right of them as calculated by PUZZLE, ME (above branches) and MP (below branches). ME (minimum evolution score = 0.86144) and MP (L = 1279, CI = 0.4450, RI = 0.7008) were combined with a 1000-fold bootstrap analysis. PUZZLE was calculated with 1000 puzzling steps. Support values in circles derived from taxon deletion experiments

PUZZLE analyses. Minimum evolution score, tree lengths (L), consistency index (CI), and retention index (RI) were recorded from results of PAUP analyses. All trees were rooted using an user specified outgroup (two prasinophycean taxa). Several taxon deletion experiments were performed to avoid long branch attraction. Trees were displayed using the TREEVIEW program (Page 1996).

Results

Given the taxon sampling used for most analyses (Table 1) and the reduction of the alignment to those sites that could be unambiguously aligned, the resulting data set comprised 1623 nucleotide positions. The proposed alignment and a comparison of the secondary structures of the 18S rRNAs revealed two remarkable helices, including autapomorphic sites for the taxa investigated: helix 10 included seven sites that were unique for the sphaeropleacean taxa (including *Ankyra*) and different from all other known green algal 18S rDNA sequences (Figs. 1a, 2). Helix 45 exhibited a sequence of three consecutive nucleotides (GGG) that was characteristic for all sphaeropleacean strains except for *Ankyra judayi* (Figs. 1b, 2). Direct comparison of the complete 18S rDNA sequences showed that *Ankyra lanceolata*, *Atractomorpha echinata*, *S. annulina*, *S. robusta*, and *S. soleirolii* differed in a maximum of 22 nucleotide positions from each other, whereas *Ankyra judayi* differed in up to 39 positions from these taxa. In this dataset, the chlorophycean taxon *Bracteacoccus* sp. had the most similar sequence, which

differed in at least 102 nucleotide positions from the sphaeropleacean sequences. All phylogenetic analysis methods yielded identical results with respect to the monophyly of the investigated group. The phylogenetic lineage that comprises *Ankyra*, *Atractomorpha* and *Sphaeroplea* was supported by a bootstrap value of at least 93% by any algorithm that was applied (Fig. 3). *Ankyra judayi* was resolved as the sister taxon to the group that comprised *Atractomorpha* species, *Sphaeroplea* species and *Ankyra lanceolata*. Within the latter group the branching order could not be resolved without ambiguity (Fig. 3).

Preliminary phylogenetic analyses with a series of datasets (not shown) and a BLAST search (Altschul et al. 1997) revealed that the sphaeropleacean strains showed affinities to the Chlorophyceae. This study revealed that the branch supporting the sphaeropleacean cluster was remarkably long in comparison to branches leading to other chlorophycean lineages (Fig. 3).

A branching order of the main chlorophycean lineages could not be resolved without ambiguity. Cladograms, inclusive of oedogonialean, chaetopeltidalean, chaetophoralean and sphaeroplealean taxa, yielded multifurcations (low bootstrap support) for the Chlorophyceae, and different tree topologies according to the algorithms applied (Fig. 3). In particular, the relationship between the Sphaeropleales, the Sphaeropleaceae, the Chaetopeltidales and the Chaetophorales remains unclear. After deletion of oedogonialean species from the align-

← calculating the trees without oedogonialean taxa. Taxa investigated in this study (Sphaeropleaceae) are in boldface. Vertical lines and brackets indicate taxonomical groups. There are six major lineages within the Chlorophyceae (support values in boldface). Note that there are different tree topologies according to the algorithm applied: In ME and MP analyses the Chaetopeltidales appear as sister group to the Sphaeropleaceae (no statistical support). Further in MP analyses the Chaetophorales appear as sister group to the Chlamydomonadales/Volvocales (no statistical support). The relation between the group investigated and the chlorophycean assemblage including autosporic taxa as well as biflagellated DO-taxa (Sphaeropleales) is only supported in PUZZLE analyses (dotted line). Note the long branch that leads to the sphaeropleacean cluster in comparison to branches leading to other chlorophycean lineages

ment, phylogenetic trees constructed by either PUZZLE, ME and MP analyses yielded bootstrap support of up to 75% for the monophyly of the Chlorophyceae (Fig. 3). Within the Chlorophyceae, only PUZZLE analyses placed the Sphaeropleaceae (including *Ankyra*) in close relationship with a clade comprising *Bracteacoccus*, *Ankistrodesmus*, *Scenedesmus*, *Neochloris* and *Hydrodictyon* (Sphaeropleales sensu Deason et al. 1991). The monophyly of the Sphaeropleales (with the Sphaeropleaceae) was weakly supported by a bootstrap value of 48% (Fig. 3). Results from ME and MP analyses supported six major lineages within the Chlorophyceae but the relationships between lineages were weakly resolved (Fig. 3).

Discussion

All phylogenetic analyses applied in this study suggested that the group investigated belongs to the Chlorophyceae. Resolution of the major lineages within the chlorophycean assemblage was ambiguous, when all oedogonialean, sphaeroplealean, sphaeropleacean, chaetopeltidean and chaetophoralean taxa were included (each of these lineages is well supported). The heterogeneous branch lengths derived from the PUZZLE analysis may be due to different mutation rates in different chlorophycean lineages. Consequently, resolution of this issue awaits other sources of data or additional taxon sampling. Regardless, the molecular phylogenetic analyses of the 18S rRNA gene sequences clearly demonstrate, that *Ankyra*, *Atractomorpha* and *Sphaeroplea* are a monophyletic lineage within the Chlorophyta, which can be addressed as Sphaeropleaceae. *Ankyra lanceolata* and *Ankyra judayi* need to be transferred into this family. It is likely that *Ankyra* will be shown to possess directly-opposed basal bodies in zoospores and that the flagellar apparatus will lack a striated microtubuli associated component (SMAC) as well as a partial cap as it is diacritic for all currently recognized sphaeropleacean taxa (sensu Deason et al. 1991).

While the data presented here suggest that the genus *Ankyra* is paraphyletic, the monophyletic Sphaeropleaceae (inclusive of *Ankyra*) are well supported (>93%) by different phylogenetic algorithms and by high identities among the 18S rDNA sequences. The Sphaeropleales sensu Deason et al. (1991) must be emended by the inclusion of several autosporic taxa that presumably lost their ability to reproduce by zoospores (Scenedesmaceae, Ankistrodesmaceae) and, by the inclusion of *Bracteacoccus*. According to Booton et al. (1998a, b) and as mentioned in the introduction, the quadriflagellated and polymorphic (CW + DO) Chaetophorales are weakly resolved as sister taxa to the Chlamydomonadales/Volvocales (which is in agreement with only our MP analyses, but with no statistical support). Depending on the algorithm applied, the quadriflagellated Chaetopeltidales are the sister group to the Chaetophorales (PUZZLE support value 77%) or to the biflagellated Sphaeropleales and/or Sphaeropleaceae (no statistical support). In a review that did not include the Chaetophorales and Chaetopeltidales, Chapman et al. (1998) divided the chlorophycean algae into two clades. The first clade includes taxa traditionally placed in the Chlorococcales, the Volvocales and the Chlorosarcinales. The second clade includes Sphaeropleales plus *Bracteacoccus* and autosporic chlorococcalean taxa such as *Scenedesmus* and *Ankistrodesmus*. The close association of the autosporic taxa with the zoosporic DO-taxa is not particularly surprising if similarities in the cell structure and coenobial growth forms are considered (Chapman et al. 1998). Lewis et al. (1992), Wilcox et al. (1992), Lewis (1997), Friedl (1997), and Booton et al. (1998a, b) already included several autosporic taxa in the DO-group. The ML/PUZZLE results of this investigation, which support a basal position of the family Sphaeropleaceae (Fig. 3) within the second clade sensu Chapman et al. (1998), are consistent with ultrastructural evidence. Consequently, one interpretation is that the autapomorphic feature of the sphaeroplealean clade is the biflagellated condition with a DO

flagellar apparatus configuration where several lineages lost their ability to reproduce by zoospores. However, there is still the possibility, that the DO condition is a symplesiomorphy for the Chlorophyceae (see Buchheim et al. 2001 [in press]). The sister group relations between the biflagellated Sphaeropleales/Sphaeropleaceae and the quadriflagellated Chaetopeltidales/Chaetophorales could not be unequivocally resolved by the 18S rDNA data. In future studies more chaetopeltidalean and chaetophoralean taxa need to be examined.

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