J Mol Evol (2003) 56:89–104
DOI: 10.1007/s00239-002-2383-4

JOURNAL OF MOLECULAR EVOLUTION

Molecular Phylogeny of Conjugating Green Algae (Zygnemophyceae, Streptophyta) Inferred from SSU rDNA Sequence Comparisons*

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Received: 15 May 2002 / Accepted: 19 July 2002

Abstract. Nuclear-encoded SSU rDNA sequences have been obtained from 64 strains of conjugating green algae (Zygnemophyceae, Streptophyta, Viridiplantae). Molecular phylogenetic analyses of 90 SSU rDNA sequences of Viridiplantae (including 78 from the Zygnemophyceae) were performed using complex evolutionary models and maximum likelihood, distance, and maximum parsimony methods. The significance of the results was tested by bootstrap analyses, deletion of long-branch taxa, relative rate tests, and Kishino–Hasegawa tests with user-defined trees. All results support the monophyly of the class Zygnemophyceae and of the order Desmidiales. The second order, Zygnematales, forms a series of early-branching clades in paraphyletic succession, with the two traditional families Mesotaeniaceae and Zygnemataceae not recovered as lineages. Instead, a long-branch *Spirogyra*/*Sirogonium* clade and the later-diverging *Netrium* and *Roya* clades represent independent clades. Within the order Desmidiales, the families Gonatozygaceae and Closteriaceae are monophyletic, whereas the Peniaceae (represented only by *Penium margaritaceum*) and the Desmidiaceae represent a single weakly supported lineage. Within the Desmidiaceae short internal branches and varying rates of sequence evolution among taxa reduce the phylogenetic resolution significantly. The SSU rDNA-based phylogeny is largely congruent with a published analysis of the *rbc*L phylogeny of the Zygnemophyceae (McCourt et al. 2000) and is also in general agreement with classification schemes based on cell wall ultrastructure. The extended taxon sampling at the subgenus level provides solid evidence that many genera in the Zygnemophyceae are not monophyletic and that the genus concept in the group needs to be revised.

Key words: Green algae — Streptophyta — Zygnemophyceae — Desmids — Molecular phylogeny — SSU rDNA

Introduction

The conjugating green algae (Zygnemophyceae, Streptophyta) have fascinated professional and amateur microscopists for over 200 years because of their morphological diversity and complex cell symmetry, which make them organisms of great natural beauty. In consequence, however, this attraction resulted in the description of a large number of "species" [approximately 4000; the exact number has never been known with any certainty (Hoshaw and McCourt 1988; Gerrath 1993)] and an even larger number of almost inaccessible references. The distinctness of the Zygnemophyceae, which are characterized by a special type of sexual reproduction (conjugation) and the complete absence of flagellate reproductive stages, has never been in doubt (Brook 1981). However, the relationships of the taxonomic entities (families, genera, species) to each other as

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*Dedicated to Prof. Marianne Mix on the occasion of her 75th birthday

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Table 1. Origin and taxonomic designation of strains and corresponding EMBL/GenBank accession numbers (Acc. No.) of SSU rDNA sequences used (sequences determined for this study are indicated by accession numbers in boldface)

Taxon	Strain or reference	Acc. No.
Actinotaenium (Penium) phymatosporum (Nordst) Coes. et Kouwets	M 1368	**AJ428088**
Actinotaenium cucurbita (Bréb.) Teil.	M 1199	**AJ428099**
Actinotaenium curtum (Bréb.) Teil.	SVCK 163	**AJ428100**
Bambusina borreri (brebissonii) (Ralfs) Cl.	CCAC 0045	**AJ428118**
Closterium acerosum (Schranke) Ehr. ex Ralfs	NIES 125	**AJ428087**
Closterium ehrenbergii Menegh. ex Ralfs	Besendahl and Bhattacharya 1999	AF115437
Closterium littorale Gay	SAG 611-7	**AJ428086**
"*Closterium littorale* Gay"	Besendahl and Bhattacharya 1999	AF115438
Closterium navicula (Bréb.) Lütkem.	SVCK 44	**AJ428085**
Cosmarium botrytis Menegh. ex Ralfs	Bhattacharya et al. 1994	X79498
Cosmarium contractum Kirchn.	SVCK 396	**AJ428112**
Cosmarium elegantissimum Lund.	M 1887	**AJ428115**
Cosmarium isthmium West var. *hibernica* West	SVCK 229	**AJ428116**
Cosmarium lundellii Delp	SVCK 357	**AJ428113**
Cosmarium ovale Ralfs ex Ralfs	SVCK 342	**AJ428114**
Cosmocladium constrictum (Arch.) Arch.	SVCK 321	**AJ428126**
Cosmocladium saxonicum de Bary	Bhattacharya et al. 1994	X79497
Cylindrocystis brebissonii Menegh. ex de Bary	Besendahl and Bhattacharya 1999	AF115439
Cylindrocystis crassa de Bary	SAG 23.97	**AJ428080**
Desmidium (cylindricum) grevillii (Kütz.) de Bary	SVCK 113	**AJ428117**
Desmidium swartzii (Ag.) Ag. ex Ralfs	SVCK 23	**AJ428133**
Euastrum oblongum (Grev.) Ralfs	ASW 07018	**AJ428095**
Euastrum pinnatum Ralfs	SVCK 175	**AJ428096**
Gonatozygon (Genicularia) spirotaenium de Bary	Surek et al. 1994	X74753
Gonatozygon aculeatum Hast.	Bhattacharya et al. 1996	X91346
Gonatozygon brebissonii de Bary	SVCK 210	**AJ428083**
Gonatozygon monotaenium de Bary	SVCK 415	**AJ428084**
Groenbladia neglecta (Racib.) Teil.	SVCK 478	**AJ428119**
Haplotaenium (Pleurotaenium) minutum (Ralfs) Bando	SVCK 302	**AJ428090**
Heimansia (Cosmocladium) pusilla (Hilse) Coes.	SVCK 428	**AJ428125**
Hyalotheca dissiliens (J.E. Smith) Bréb.	NIES 149	**AJ428120**
Hyalotheca mucosa (Mert.) Ehr. ex Ralfs	SVCK 103	**AJ428121**
Mesotaenium caldariorum (Lagerch.) Hansg.	Surek et al. 1994	X75763
[*Mesotaenium endlicherianum* Näg.]	SAG 12.97	**AJ428078**
Mesotaenium kramstai Lemm.	UTEX 1025	**AJ428079**
Micrasterias crux-melitensis (Ehr.) Hass. ex Ralfs	NIES 149	**AJ428097**
Micrasterias fimbriata Ralfs	ASW 07026	**AJ428098**
Mougeotia scalaris Hass.	Kranz et al. 1995	X70705
Mougeotia sp.	SVCK 417	**AJ428073**
Netrium digitus (Her.) Itzigs.	SVCK 48	**AJ428070**
Netrium interruptum (Bréb.) Lütkem.	ASW 07036	**AJ428071**
Onychonema laeve Nordst.	SVCK 142	**AJ428127**
Penium margaritaceum (Ehr.) Bréb.	Besendahl and Bhattacharya 1999	AF115440
Phymatodocis nordstedtiana Wolle	SVCK 327	**AJ428122**
Pleurotaenium ehrenbergii (Bréb.) de Bary	NIES 309	**AJ428132**
Pleurotaenium trabecula (Ehr.) Nag.	ASW 07051	**AJ428131**
Roya anglica G.S West	UTEX 934	**AJ428081**
Roya obtusa (Bréb.) W. et G.S. West	SVCK 45	**AJ428082**
Spirogyra (*Sirogonium*) *stictica* (J.E. Smith) Wille	UTEX 1984	**AJ428076**
Spirogyra sp.	SVCK 253, 261	**AJ428072**
Spirogyra sp.	M 1810	**AJ428074**
Spirogyra sp.	M 1843	**AJ428075**
Spondylosium panduriforme (Heimerl) Teil.	SAG 52.88	**AJ428124**
Spondylosium planum (Wolle) W. et G.S. West	SAG 41.81	**AJ428123**
Spondylosium pulchellum Arch. ex Arch.	SVCK 365	**AJ428130**
Spondylosium pulchrum (Bail.) Arch.	SVCK 331	**AJ428129**
Spondylosium secedens (De Bary) Arch.	SVCK 31	**AJ428128**
Staurastrum [*cristatum*] cf. *granulosum* (Ehr.) Ralfs	UTEX 402	**AJ428110**
Stauratrum arctiscon (Ehr. ex Ralfs) Lund.	M 1885	**AJ428105**
Staurastrum hirsutum (Ehr.) Bréb. in Ralfs (=M752)	Surek etal. 1994	X74752
Staurastrum lunatum Ralfs	SVCK 15	**AJ428106**
Staurastrum ophiura Lund.	M 1027	**AJ428104**

Staurastrum pingue Teil.	Gontcharov, unpublished	**AJ428109**
Staurastrum subavicula (W. West) W. et G.S. West	M 0754	**AJ428107**
Staurastrum teliferum Ralfs (= M 753)	Bhattacharya et al. 1994	X77452
Staurastrum tumidum Bréb. ex Ralfs	SVCK 85	**AJ428108**
Staurodesmus (Arthrodesmus) bulnheimii (Racib.) Round et Brook	SVCK 84	**AJ428111**
Staurodesmus (Staurastrum) dickei (Ralfs) Lillier	SVCK 38	**AJ428101**
Staurodesmus (Staurastrum) mucronatus (Ralfs ex Bréb.) Croasd.	M 1394	**AJ428103**
Staurodesmus convergens Ehr. ex Ralfs	M 1886	**AJ428102**
Teilingia (Sphaerozosma) granulata (Roy et Biss.) Bourr.	Bhattacharya et al. 1994	X79496
Tetmemorus brebissonii (Menegh.) Ralfs ex Ralfs	SVCK 214	**AJ428091**
Triploceras gracile Bail.	SVCK 173	**AJ428089**
Xanthidium antilopaeum (Bréb.) Kütz.	M 1229	**AJ428093**
Xanthidium armatum (Bréb.) Rabenh. ex Ralfs	ASW 07059	**AJ428094**
Xanthidium brebissonii Ralfs	SVCK 134	**AJ428092**
Zygnema sp.	M 1384	**AJ428077**
Zygnemopsis circumcarinata (Czurda) Krieg.	Bhattacharya et al. 1994	X79495
Other streptophyte green algae		
Chaetosphaeridium globosum (Nordst.) Kleb.	Marin and Melkonian 1999	AJ250110
Chlorokybus atmophyticus Geitler	Wilcox et al. 1993	M95612
Coleochaete orbicularis Prings.	Wilcox et al. 1993	M95611
Coleochaete scutata Bréb.	Kranz et al. 1995	X68825
Klebsormidium flaccidum (Kütz.) Silva, Mattox et Blackwell	Kranz et al. 1995	X75520
Klebsormidium nitens (Menegh. in Kütz.) Lokhorst	Marin and Melkonian 1999	AJ250112
Mesostigma viride Lauterborn	Marin and Melkonian 1999	AJ250108
Mesostigma viride Lauterborn	Marin and Melkonian 1999	AJ250109
Prasinophytes		
Cymbomonas tetramitiformis Schiller	Nakayama et al. 1998	AB017126
Mamiella sp.	Nakayama et al. 1998	AB017129
Mantoniella squamata (Manton et Parke) Desikachary	Nakayama et al. 1998	X73999
Pterosperma cristatum Schiller	Nakayama et al. 1998	AJ010407

Note. CCAC—Culture Collection of Algae at the University of Cologne, Germany; M—Culture Collection Melkonian, Botanical Institute, University of Cologne, Germany; NIES—National Institute for Environmental Studies, Tsukuba, Japan (Watanabe et al. 2000); SAG—Sammlung von Algenkulturen, University of Göttingen, Germany (Schlösser 1994); SVCK—Sammlung von Conjugaten-Kulturen, University of Hamburg, Germany (Engels and Mix 1980); UTEX—Culture Collection of Algae at the University of Texas at Austin, U.S.A. (Starr and Zeikus 1993); ASW—Sammlung von Algen-Kulturen, University of Vienna, Austria (Kusel-Fetzmann and Schagerl 1992). Taxa names in parentheses correspond to those used in the culture collection catalog. [*Mesotaenium endlicherianum*] and *Staurastrum* [*cristatum*] presumably represent wrong determinations of strains.

well as the genus concepts in the group have remained controversial and elusive. Ultrastructural and molecular phylogenetic data suggested clear affinities of the conjugating green algae with a paraphyletic assemblage of green algae previously known as the Charophyceae (*sensu* Mattox and Stewart 1984) and the embryophyte land plants (Mattox and Stewart 1984; McCourt et al. 1995; Pickett-Heaps 1975; Surek et al. 1994; recent review by Qiu and Palmer 1999). In a modern cladistic framework (Bremer 1985; Bremer et al. 1987; Kenrick and Crane 1997) the conjugating green algae are designated as class Zygnemophyceae [= Zygnematophyceae (van den Hoek et al. 1995)] within the division Streptophyta [= Streptobionta (Kenrick and Crane 1997)], which, together with the Chlorophyta, form the green plants [Viridiplantae or Chlorobiota (Cavalier-Smith 1981; Kenrick and Crane 1997)].

Classification schemes of the Zygnemophyceae have generally been based on morphology, emphasizing cell wall ultrastructure, cellular organization (unicells, filaments), and chloroplast structure (Gerrath 1993; Hoshaw and McCourt 1988; Prescott et al. 1972, 1975, 1982; Mix 1975). Two orders based on cell wall structure are traditionally distinguished within the Zygnemophyceae, namely, the Zygnematales and the Desmidiales (Brook 1981; van den Hoek et al. 1995). The Zygnematales are characterized by smooth cell walls consisting of a single piece and lacking pores and other ornamentations. Zygnematalean taxa forming filaments are distinguished as the family Zygnemataceae from the unicellular Zygnematales classified as the Mesotaeniaceae, formerly known as "saccoderm desmids" (Gerrath 1993; Kadlubowska 1984; Prescott et al. 1972; Transeau 1951). The Desmidiales (or "placoderm desmids") have cell walls consisting of two or more pieces further characterized by ornamentations and pores (Mix 1972). Based on cellular organization and cell wall features the Desmidiales are subdivided into three or four families (Brook 1981; Gerrath 1993; Kouwets and Coesel 1984; Mix 1975), namely, the Peniaceae, Gonatozygaceae [merged with the Peniaceae by Kouwets and Coesel (1984)], Closteriaceae, and Desmidiaceae.

Genera and species of the Zygnemophyceae are based mostly on differences in cell organization (filaments/unicells), cell symmetry, size and shape, cell wall projections and ornamentations, and chloroplast structure. In most taxa neither the stability of the taxonomic characters nor their evolutionary status (plesiomorphic or apomorphic) has been studied in any detail.

Previous molecular phylogenetic analyses in the Zygnemophyceae, addressing primarily the relationships between orders and families, have clearly supported the monophyly of the group and their affinities to other streptophyte green algae (Besendahl and Bhattacharya 1999; Bhattacharya et al. 1994; Chapman et al. 1998; Denboh et al. 2001; McCourt et al. 1995, 2000; Park et al. 1996; Surek et al. 1994). The SSU rDNA phylogenetic studies so far have suffered from limited taxon sampling [18 taxa studied by Besendahl and Bhattacharya (1999); 36 taxa by Denboh et al. (2001)] and the most recent *rbc*L analysis (McCourt et al. 2000), although comprehensive with respect to the sampling of genera (30 genera within the six recognized families), included only a single representative for each genus and could not address the validity or the artificial nature of genera in the Zygnemophyceae. Therefore, genera studied here are represented by more than one species, whenever possible, as also done by Denboh et al. (2001) in their SSU rDNA analysis of 23 *Closterium* strains. This approach seems necessary since the artificial nature of many zygnemophycean genera has long been recognized [see discussion by Gerrath (1993)], although very few modern monographic treatments and revisions exist [e.g., Ohtani (1990) for *Netrium*].

In the present paper we use SSU rDNA sequences from 78 taxa of the Zygnemophyceae (64 sequences were newly determined) to analyze the molecular phylogeny of the group at different taxonomic levels and to provide a basis for future phylogenetic/systematic research in this group. The sequence data also provide a test for the monophyly of zygnemophycean genera as well as an opportunity to compare the results with those obtained with a different molecular marker [*rbc*L (McCourt et al. 2000)].

Materials and Methods

Cultures. Sixty-four strains of conjugating green algae used for SSU rDNA sequencing were obtained from different culture collections (see Table 1) and grown in modified WARIS-H culture medium (Kies 1967; McFadden and Melkonian 1986) at 20°C with a photon fluence rate of 40 μmol m^{-2} s^{-1} in a 14/10-h light/dark cycle.

DNA Extraction, Amplification, and Sequencing. Cells were harvested after 2 to 4 weeks of growth. To remove mucilage, the algae were subjected to mild ultrasonication (Surek and Sengbusch 1981) and washed several times with distilled water. Total DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987; modified according to Surek et al. 1994) or the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the recommendations of the manufacturer. SSU rDNA was amplified by polymerase chain reactions (PCR) (Saiki et al. 1988) using thermocycling protocols and 5′-biotinylated PCR primers as described previously (Marin et al. 1998). Amplifications of SSU rDNA regions were performed using oligonucleotide primers A (biotinylated) and ITS055 (Marin et al. 1998). PCR products were sequenced with sequencing methods and primers (82F, 528F, 920F, 1400F, BR, 1400R, 920R, 536R) as described by Marin et al. (1998). The rDNA genes of the Zygnemophyceae contain a group I intron (except *Spirogyra sp.*, strains SVCK 253 and 261), but only the presumptive coding regions were used in this study.

Sequence Alignment and Phylogenetic Analyses. Coding regions of the SSU rDNA sequences of the Zygnemophyceae were manually aligned with homologous sequences from other streptophyte green algae and four prasinophyte taxa using the Olson Multiple Sequence Alignment Editing Program (Olsen 1990) with respect to primary and secondary structure conservation. The alignment is available from the authors upon request. Phylogenetic trees were inferred with distance (NJ), maximum parsimony (MP), and maximum likelihood (ML) criteria using the PAUP 4.0b8 program package (Swofford 1998). Four data sets were used: two large alignments (90 or 86 sequences, respectively) with 1699 unambiguously aligned positions, a small data set (37 sequences of Zygnemophyceae from all six families plus five sequences of other streptophyte green algae as an outgroup) with 1712 unambiguously aligned positions, and a data set of 52 sequences from the Desmidiaceae and Peniaceae with 1738 unambiguously aligned positions. To decide which evolutionary model (for ML and NJ analyses) fit the data best, the program Modeltest 3.04 (Posada and Crandall 1998) was used, which employs two statistics, the likelihood ratio test (LRT) and the Akaike information criterion [AIC (Akaike 1974)]. The model selected by the hierarchical LRT for all data sets was the Tamura–Nei (1993) model (TrN) with the proportion of invariable sites (I) and the Γ; shape parameter for among-site rate variation calculated from the data (TrN + I + Γ; base frequencies and substitution parameters were estimated by Modeltest). Distances used for NJ analyses were calculated via ML (TrN + I + Γ). ML and MP analyses used heuristic searches with a branch-swapping algorithm (tree bisection–reconnection). For each ML analysis, three heuristic searches were performed using different starting trees (obtained by stepwise addition, NJ and MP). The robustness of the trees was tested by bootstrap analyses (Felsenstein 1985) using 1000 (NJ) or 100 (ML and MP) replications. In MP the stepwise addition option (10 replicate heuristic searches with a random shuffling of taxon input order) was used for each bootstrap replicate. ML bootstrap used a single heuristic search (starting tree via stepwise addition) per replicate. Relative rate tests of nucleotide substitutions between defined clades and selected outgroups were performed according to Robinson et al. (1998) using two-parameter distances of Kimura (1980) and not taking the tree topologies into account. Constant sites were excluded for relative rate tests to obtain a more reliable estimation of distances.

User-defined trees were generated by manually modifying the treefile of the “best tree” using TreeView [Version 1.6.2 (Page 1996)]. To compare user-defined topologies with the “best tree,” the sequence data file and the tree files were loaded into PAUP and used for Kishino–Hasegawa (1989) test. Comparisons were based on ML (TrN + I + Γ;) and MP criteria (see Table 3 for details).

Results

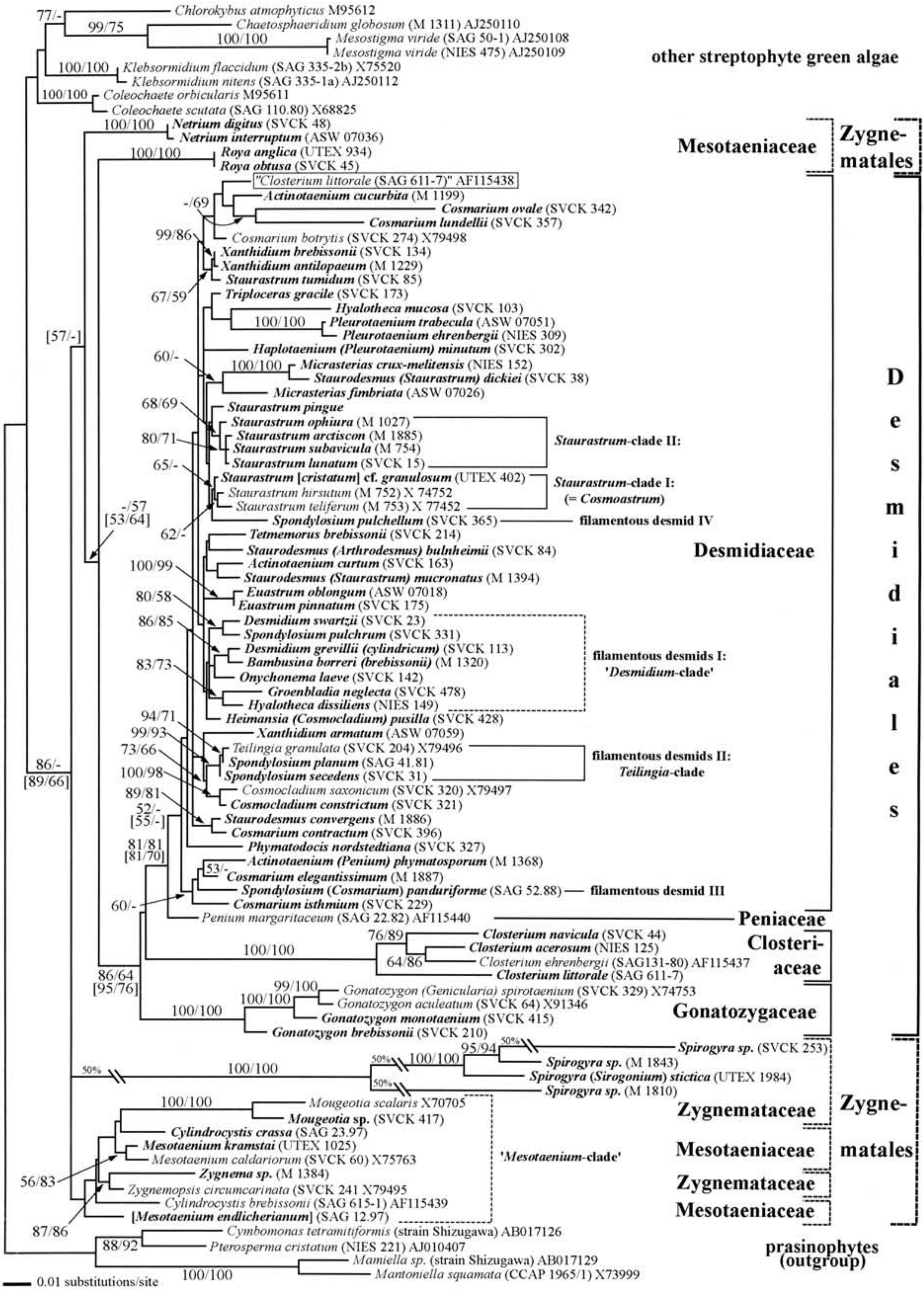
SSU rDNA Coding Regions and 1506 Group I Introns. The PCR products resulting from the amplification of SSU rDNA coding regions were (with three exceptions; see below) approximately 2200 bp in length due to the presence of the well-known zygnemophycean 1506 group I intron near the 3′ terminus of the coding region (Besendahl and Bhattacharya 1999; Bhattacharya et al. 1994). In one strian, *Cosmarium lundellii* (SVCK 357), the PCR product was about 2600 bp in length, caused by an unexpectedly long 1506 group I intron (800 bp), which has insertions near the P1 and P8 domains (results not shown). The PCR products of two strains of *Spirogyra* sp. (SVCK 253 and 261) were only about 1800 bp long and sequencing revealed no group I intron. The SSU rDNA coding regions of the two sequences are identical and the two strains presumably belong to the same unidentified *Spirogyra* species [both strains were isolated from two localities in Hamburg, Germany (Engels and Mix 1980); only strain SVCK 253 was used further for this study].

Phylogenetic Analysis of SSU rDNA Coding Regions. In this study we determined nuclear-encoded SSU rDNA coding regions of 64 strains of Zygnemophyceae and aligned these sequences with other known zygnemophycean sequences and those of other green algae from both the Streptophyta and Chlorophyta. Taxon sampling in the Zygnemophyceae aimed at representing the diversity of the group in the best possible way. Toward this end we sampled SSU rDNA sequences from 78 strains representing 32 genera (about 55% of the total known number of genera) in all six currently recognized families of Zygnemophyceae. If possible, more than one strain from each genus was sampled and morphological diversity within the genus was taken into account.

Sequences were included into four data sets. The largest data set comprised 78 sequences from Zygnemophyceae, 8 sequences from other streptophyte algae, and 4 sequences from prasinophytes (as outgroup). The phylogenetic tree shown in Fig. 1 was inferred from a ML approach using the TrN + I + Γ model of evolution selected for this data set by Modeltest 3.04 (see Materials and Methods). The Zygnemophyceae are topologically recovered as a monophyletic lineage and supported by moderately high (86%) bootstrap values in NJ but not in MP (Fig. 1). Bootstrap support for the monophyly of the Zygnemophyceae increases to 89% in NJ and becomes moderate in MP (66%) when four *Spirogyra*/*Sirogonium* strains are omitted from the analysis (results not shown), strongly suggesting that the extremely long common/individual branches of this lineage interfere with the stability of the basal branches in the tree (see also below). In the *Spirogyra*/*Sirogonium* clade the single *Sirogonium* strain branches within *Spirogyra* resulting in *Spirogyra* not being monophyletic. The *Spirogyra* strain, which lacks the 1506 group I intron (strain SVCK 253), is a late divergence in this clade and also has the longest branch (Fig. 1).

Taxa traditionally placed in the Zygnematales (Mesotaeniaceae and Zygnemataceae) represent the basal divergences in the tree and form several paraphyletic clades (Fig. 1). The order Desmidiales (Gonatozygaceae, Closteriaceae, Peniaceae, Desmidiaceae) diverges later and its monophyly is supported by moderate bootstrap values [NJ, 84%; MP, 64% (Fig. 1)]. Again, support for the Desmidiales increases significantly when the *Spirogyra*/*Sirogonium* clade is removed from the analysis (NJ, 95%; MP, 76%). The branching order between the paraphyletic clades of the Zygnematales is not resolved irrespective of whether or not the *Spirogyra*/*Sirogonium* clade is included in the analysis. The late divergence of *Netrium* and *Roya* lineages (each with two taxa) with respect to the rest of the Zygnematales is weakly supported (53–64% bootstrap values) only when the *Spirogyra*/*Sirogonium* Clade is removed; *Roya* species represent the sister to the Desmidiales (Fig. 1). An early-diverging but nonsupported zygnematalean clade ("*Mesotaenium* lineage") contains nine species of *Mesotaenium*, *Cylindrocystis*, *Zygnema*, *Zygnemopsis*, and *Mougeotia*. In this clade significantly supported sister-group relationships among genera are revealed only for *Zygnema*/*Zygnemopsis* (Fig. 1).

Within the Desmidiales, the families Gonatozygaceae (four taxa) and Closteriaceae (four taxa), both strongly supported in all analyses (100%; Fig. 1), represent basal divergences. The succession of divergence between *Gonatozygon* and *Closterium* is not resolved and both genera have relatively long branches, which could have contributed to their attraction as well as their basal divergence [the branch length of *Closterium* and its phylogenetic position is not changed after the inclusion of additional *Closterium* sequences (Denboh et al. 2001); not shown]. The Desmidiaceae plus the single strain of *Penium* analyzed here (family Peniaceae) represent a moderately supported lineage (81% in NJ and MP; Fig. 1). Though the *Penium* strain topologically appears as a sister taxon to the Desmidiaceae, this position receives only very low support in NJ (52%) and no support in MP (Fig. 1). Within the large clade of the Desmidiaceae most internal branches have no support (Fig. 1; see below). It should be noted that a database sequence designated *Closterium littorale* (AF115438) is significantly resolved within the Desmidiaceae (Fig. 1), suggesting polyphyly of *Closterium* and the Closteriaceae. Therefore, the SSU rRNA gene from the same strain (SAG 611-7) was redeter-


77/-
Chlorokybus atmophyticus M95612
99/75
Chaetosphaeridium globosum (M 1311) AJ250110
100/100
Mesostigma viride (SAG 50-1) AJ250108
Mesostigma viride (NIES 475) AJ250109
other streptophyte green algae
100/100
Klebsormidium flaccidum (SAG 335-2b) X75520
Klebsormidium nitens (SAG 335-1a) AJ250112
100/100
Coleochaete orbicularis M95611
Coleochaete scutata (SAG 110.80) X68825
100/100
Netrium digitus (SVCK 48)
Netrium interruptum (ASW 07036)
Mesotaeniaceae
Zygne-matales
100/100
Roya anglica (UTEX 934)
Roya obtusa (SVCK 45)
"Closterium littorale (SAG 611-7)" AF115438
-/69
Actinotaenium cucurbita (M 1199)
Cosmarium ovale (SVCK 342)
Cosmarium lundellii (SVCK 357)
99/86
Cosmarium botrytis (SVCK 274) X79498
Xanthidium brebissonii (SVCK 134)
Xanthidium antilopaeum (M 1229)
Staurastrum tumidum (SVCK 85)
67/59
Triploceras gracile (SVCK 173)
Hyalotheca mucosa (SVCK 103)
100/100
Pleurotaenium trabecula (ASW 07051)
Pleurotaenium ehrenbergii (NIES 309)
[57/-]
Haplotaenium (Pleurotaenium) minutum (SVCK 302)
60/-
100/100
Micrasterias crux-melitensis (NIES 152)
Staurodesmus (Staurastrum) dickiei (SVCK 38)
Micrasterias fimbriata (ASW 07026)
68/69
Staurastrum pingue
Staurastrum ophiura (M 1027)
80/71
Staurastrum arctiscon (M 1885)
Staurastrum subavicula (M 754)
Staurastrum-clade II:
Staurastrum lunatum (SVCK 15)
65/-
Staurastrum [cristatum] cf. granulosum (UTEX 402)
Staurastrum-clade I:
(= Cosmoastrum)
Staurastrum hirsutum (M 752) X 74752
Staurastrum teliferum (M 753) X 77452
-/57
[53/64]
62/-
Spondylosium pulchellum (SVCK 365)
filamentous desmid IV
Tetmemorus brebissonii (SVCK 214)
Staurodesmus (Arthrodesmus) bulnheimii (SVCK 84)
Desmidiaceae
Actinotaenium curtum (SVCK 163)
100/99
Staurodesmus (Staurastrum) mucronatus (M 1394)
Euastrum oblongum (ASW 07018)
80/58
Euastrum pinnatum (SVCK 175)
Desmidium swartzii (SVCK 23)
86/85
Spondylosium pulchrum (SVCK 331)
Desmidium grevillii (cylindricum) (SVCK 113)
filamentous desmids I:
'Desmidium-clade'
Bambusina borreri (brebissonii) (M 1320)
83/73
Onychonema laeve (SVCK 142)
Groenbladia neglecta (SVCK 478)
Hyalotheca dissiliens (NIES 149)
94/71
Heimansia (Cosmocladium) pusilla (SVCK 428)
Xanthidium armatum (ASW 07059)
99/93
Teilingia granulata (SVCK 204) X79496
73/66
Spondylosium planum (SAG 41.81)
filamentous desmids II:
Teilingia-clade
100/98
Spondylosium secedens (SVCK 31)
Cosmocladium saxonicum (SVCK 320) X79497
89/81
Cosmocladium constrictum (SVCK 321)
86/-
[89/66]
52/-
[55/-]
Staurodesmus convergens (M 1886)
Cosmarium contractum (SVCK 396)
81/81
[81/70]
Phymatodocis nordstedtiana (SVCK 327)
Actinotaenium (Penium) phymatosporum (M 1368)
53/-
Cosmarium elegantissimum (M 1887)
Spondylosium (Cosmarium) panduriforme (SAG 52.88)
filamentous desmid III
60/-
Cosmarium isthmium (SVCK 229)
Penium margaritaceum (SAG 22.82) AF115440
Peniaceae
D
e
s
m
i
d
i
a
l
e
s
76/89
Closterium navicula (SVCK 44)
100/100
Closterium acerosum (NIES 125)
Closteri-aceae
64/86
Closterium ehrenbergii (SAG131-80) AF115437
86/64
[95/76]
Closterium littorale (SAG 611-7)
99/100
Gonatozygon (Genicularia) spirotaenium (SVCK 329) X74753
100/100
Gonatozygon aculeatum (SVCK 64) X91346
100/100
Gonatozygon monotaenium (SVCK 415)
Gonatozygaceae
Gonatozygon brebissonii (SVCK 210)
50%
95/94
Spirogyra sp. (SVCK 253)
50%
100/100
Spirogyra sp. (M 1843)
50%
100/100
Spirogyra (Sirogonium) stictica (UTEX 1984)
50%
Spirogyra sp. (M 1810)
100/100
Mougeotia scalaris X70705
Zygnemataceae
Zygne-
Mougeotia sp. (SVCK 417)
Cylindrocystis crassa (SAG 23.97)
Mesotaenium kramstai (UTEX 1025)
'Mesotaenium-clade'
Mesotaeniaceae
matales
Mesotaenium caldariorum (SVCK 60) X75763
56/83
Zygnema sp. (M 1384)
Zygnemopsis circumcarinata (SVCK 241 X79495
Zygnemataceae
Cylindrocystis brebissonii (SAG 615-1) AF115439
[Mesotaenium endlicherianum] (SAG 12.97)
Mesotaeniaceae
87/86
Cymbomonas tetramitiformis (strain Shizugawa) AB017126
88/92
Pterosperma cristatum (NIES 221) AJ010407
prasinophytes
(outgroup)
Mamiella sp. (strain Shizugawa) AB017129
100/100
Mantoniella squamata (CCAP 1965/1) X73999
0.01 substitutions/site

mined during this study and both sequences differed considerably: the *C. littorale* sequence obtained here is monophyletic with the other strains of *Closterium* in the Closteriaceae (Fig. 1). We conclude that the sequence (AF115438) presented by Besendahl and Bhattacharya (1999) cannot be assigned to *Closterium* and is presumably the result of a misplacement of cultures (as suspected by Denboh et al. 2001).

To investigate phylogenetic relationships between the families of the Zygnemophyceae in more detail, a smaller data set (42 taxa) with 5 other streptophytes as outgroup was subjected to phylogenetic analyses (Fig. 2). This allowed us to increase the number of positions used in the analysis from 1699 to 1712 and to perform ML bootstrap. Taxon sampling within the Zygnemophyceae was guided by the deletion of the long-branched *Spirogyra/Sirogonium* clade and of most sequences of the Desmidiaceae (15 desmidiacean taxa retained; phylogeny within this family was not readdressed here). Basically, the tree topology obtained is very similar to results using the larger data set (Fig. 1), however, with better resolution at some nodes. In particular, we note that the lineage encompassing the Desmidiales plus the *Netrium* and *Roya* clades now receives higher bootstrap support (ML, 85%; NJ, 67%), although there is still no support in MP for this association (Fig. 2; compare with Fig. 1). The Desmidiales again receive high bootstrap support (98% in ML, 82% in NJ, and 75% in MP), however, their relation with the *Netrium* and *Roya* clades (sisters in Fig. 2) remains unresolved. The family Desmidiaceae (excluding P. margaritaceum) gains somewhat better (compared to the large data set; Fig. 1), but still relatively low bootstrap support (59–66% in different analyses; Fig. 2). As in previous analyses the relationships within the "*Mesotaenium* lineage" are not well resolved and the monophyly of this clade is not supported (Fig. 2). The lack of resolution among the zygnematalean sequences might be caused by differences in evolutionary rates between sequences, in particular, by the high evolutionary rates of two *Mougeotia* sequences (see also results from relative rate tests; Table 2). Indeed, when *Mougeotia* is removed from the analyses, the rest of the "*Mesotaenium* lineage" obtains bootstrap support in NJ (70%; but not in the MP analysis; not shown) and some internal branches are also better supported [63% in NJ for a clade including all strains except *M. endlicherianum*; and 74% (NJ) or 76% (MP) for a lineage including *M. kramstai*, *M. caldariorum*, and *C. crassa*; not shown.

To evaluate the significance of the results obtained, user-defined tree topologies were generated and compared with the ML topology (Figs. 1 and 2) using Kishino–Hasegawa (KH) tests (Table 3). When major clades with significant bootstrap support are collapsed, the resulting topologies are rejected (at $p < 0.05$) in all analyses [Zygnemophyceae, tree 2; Desmidiales, tree 14 (Table 3)]. In contrast, when hypotheses are tested which require changes of non-significant branches, topologies are not rejected by KH tests in most cases, e.g., rearrangements concerning the monophyly of the Zygnematales (tree 11), the relationship between *Netrium* and *Roya* (trees 12 and 13) or between the four families of the Desmidiales (trees 17–19), the monophyly of the Desmidiaceae (tree 20), and a possible association of *Phymatodocis nordstedtiana* (the earliest branch in the Desmidiaceae in Fig. 2) and *Penium* (tree 21). Another series of user-defined trees addressed morphological and/or taxonomic hypotheses, which are in obvious conflict with results of phylogenetic analyses. Most rearrangements of this type were significantly rejected, e.g., the monophyly of *Mesotaenium* and *Cylindrocystis* (trees 4 and 7) or zygnematalean taxa with identical chloroplast types (*Mougeotia*, *Mesotaenium*, *Zygnema*, and *Cylindrocystis*; trees 3, 5, 6, 8–10) as well as the association between *Roya* and *Gonatozygon* [as suggested by *rbc*L analyse (McCourt et al. 2000); tree 15)] and the validity of the genus *Genicularia* (tree 16).

One result immediately evident from the previous analyses is the lack of resolution within the Desmidiaceae. All basal branches receive no bootstrap support in any method of analysis and with both data sets (Figs. 1 and 2), and although the level of sequence divergence in the Desmidiaceae/*Penium* lineage is still relatively high: 337 of 1699 positions analyzed in Fig. 1 are variable within this clade. However, sequence variation accounts predominantly for the longer terminal/individual branches since most basal branches are very short. Nonetheless some terminal clades containing few sequences receive significant bootstrap support. A refinement of the

Fig. 1. Phylogeny of conjugates (Zygnemophyceae) based on comparisons of SSU rDNA sequences (1699 positions; group I intron sequences excluded from analyses). Four prasinophytes (Mamiellales and Pyramimonadales) were used as outgroup for 78 taxa of conjugates and 8 taxa of other streptophyte green algae. The tree topology shown was inferred by maximum likelihood (ML using TrN + I + Γ); bootstrap percentage values ≥50% are given for NJ (same model as in ML) and unweighted MP analyses. For selected nodes bootstrap values for an analysis without the long-branched *Spirogyra* clade are given in brackets. New SSU rDNA sequences obtained for this study are indicated in ***boldface*** (for accession numbers, see Table 1). Names in parentheses indicate taxonomic rearrangements or those used in the culture collection catalog; [*Mesotaenium endlicherianum*] and *Staurastrum* [*cristatum*] presumably represent wrong determinations. A database sequence (AF115438) wrongly designated "*Closterium littorale* (SAG 611-7)" is placed in a *box*.

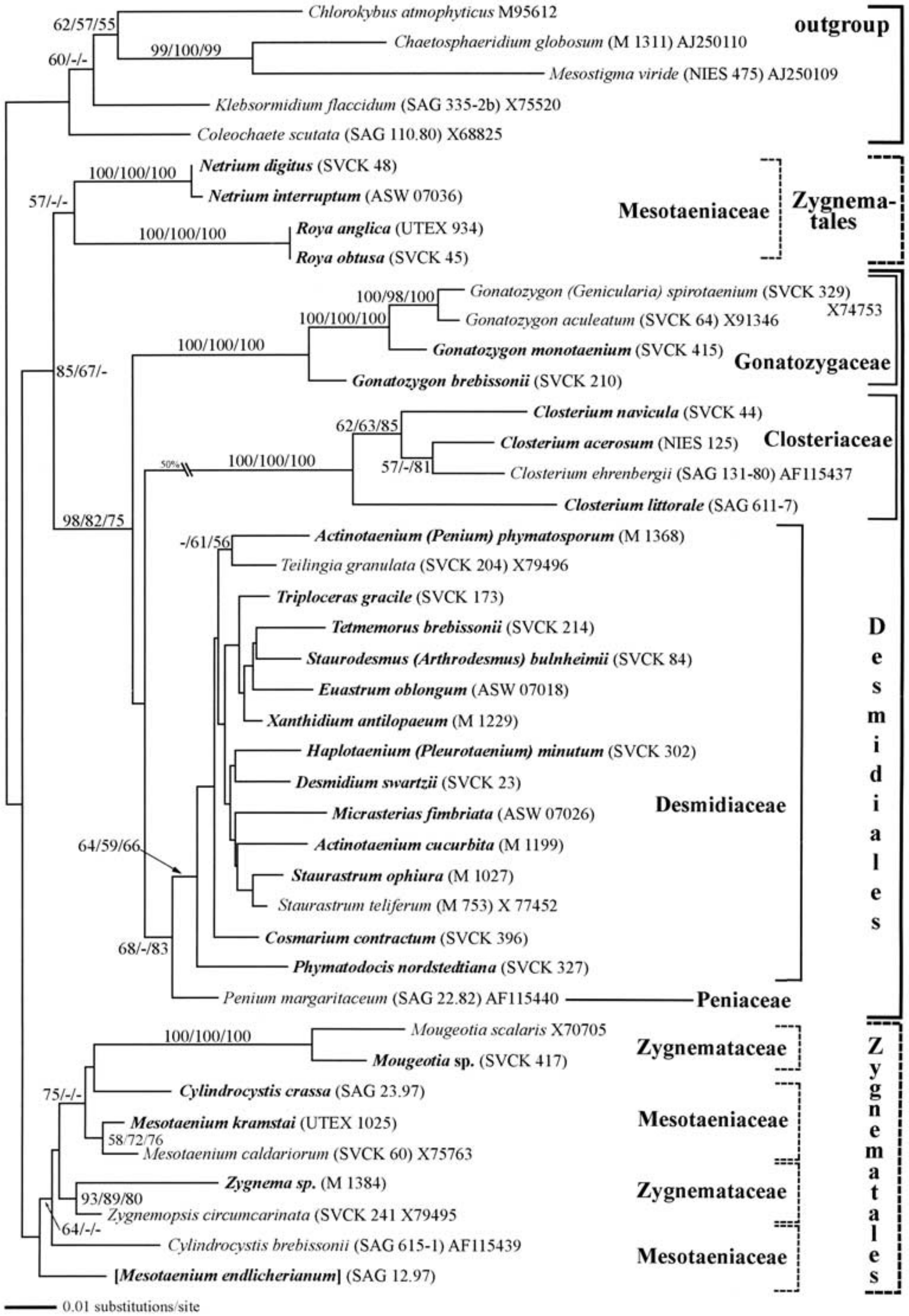


Fig. 2. Refined SSU rDNA phylogeny of 37 conjugates (Zygnemophyceae) representing all recognized families and 5 other streptophyte green algae as outgroup. One thousand seven hundred twelve aligned positions were used for all analyses. The phylogenetic tree shown was constructed with ML (TrN + I + Γ); bootstrap percentage values ≥50% are given for ML, NJ (TrN + I + Γ), and MP analyses.

analysis by limiting the data set to only the Desmidiaceae and *Penium margaritaceum* (52 sequences) and further increasing the number of positions [from 1712 (Fig. 2) to 1739] did not lead to a better topological resolution or to any improvement of bootstrap percentages for basal branches (ML analysis with the evolutionary model TrN + I + Γ; results not shown).

Table 2. Relative rate test (Robinson et al. 1998) using Kimura (1980) distances of 12 conjugate clades/taxa (Fig. 2) and 3 streptophytes (*Chlorokybus atmophyticus*, *Klebsormidium flaccidum*, and *Coleochaete scutata*) as outgroup

	Clade/taxon	1	2	3	4	5	6	7	8	9	10	11	12
1.	*Netrium* spp.												
2.	*Roya* spp.	**											
3.	*Gonatozygon* spp.	***	NS										
4.	*Closterium* spp.	***	***	***									
5.	*Desmidiaceae*	NS	NS	***	***								
6.	*Penium margaritaceum*	NS	*	***	***	*							
7.	*Mougeotia* spp.	*	NS	*	***	NS	**						
8.	*Cylindrocystis crassa*	NS	*	***	***	*	NS	***					
9.	*Mesotaenium kramstai* and *M. caldariorum*	NS	***	***	***	NS	NS	***	NS				
10.	*Zygnema* sp.	*	NS	***	***	NS	NS	NS	*	***			
11.	*Zygnemopsis circumcarinata*	NS	***	***	***	NS	NS	***	NS	NS	***		
12.	*Cylindrocystis brebissonii*	NS	*	***	***	NS	NS	***	NS	*	*	NS	
13.	[*Mesotaenium endlicherianum*]	*	***	***	***	NS	*	***	NS	NS	***	NS	*

Note. NS ($p > 0.05$; relative rates not significantly different at 5% level). Asterisks: $p = 0.05 > * > 0.01 > ** > 0.005 > ***$ (relative rates significantly different). [*Mesotaenium endlicherianum*] presumably represents a wrong determination of strain SAG 12.97

Table 3. Comparison of the maximum likelihood trees in Fig. 1 and 2 with the user-defined trees by Kishino–Hasegawa tests using ML and MP methods

Tree No.	Fig. 1 (90 taxa)				Fig. 2 (42 taxa)			
	ML(TrN + I + Γ)		MP		ML(TrN + I + Γ)		MP	
	Diff-ln$L$[a]	p[b]	L/D[c]	p	Diff-ln$L$	p	L/D	p
1	**15,918.4**	**(best)**	+4	0.103	**9,021.1**	**(best)**	+1	0.706
2	+20.5	0.017*	+12	0.013*				
3	+0.5	0.295	+11	0.023*	+2.5	0.164	+5	0.132
4	+30.7	0.011*	+14	0.001*	+26.7	0.013*	+10	0.018*
5	+32.3	0.005*	+17	<0.001*	+30.0	0.008*	+13	0.007*
6	+0.7	0.279	+7	0.035*	+11.3	0.241	+4	0.248
7	+18.3	0.025*	+12	0.014*	+18.0	0.030*	+8	0.103
8	+18.5	0.022*	+12	0.011*	+17.9	0.024*	+8	0.088
9	+34.2	0.011*	+18	0.004*	+32.0	0.010*	+13	0.028*
10	+33.5	0.010*	+18	0.003*	+32.1	0.011*	+13	0.028*
11	+13.9	0.080	+11	0.041*	+112.1	0.071	+7	0.052
12					+2.3	0.183	+6	0.083
13					+0.5	0.450	+1	0.808
14	+24.5	0.006*	+13	0.001*	+28.5	0.003*	+11	0.008*
15	+24.4	0.005*	+8	0.074	+22.2	0.022*	+6	0.289
16	+72.2	0.001*	+31	<0.001*	+68.8	0.000*	+25	<0.001*
17	+1.3	0.285	**2,681**	**(best)**	+0.9	0.263	**1,271**	**(best)**
18	+13.9	0.027*	+12	0.007*	+9.6	0.052	+9	0.039*
19	+5.6	0.065	+13	0.001*	+3.2	0.134	+8	0.033*
20	+12.6	0.047*	+11	0.002*	+7.9	0.091	+4	0.206
21	+8.4	0.146	+7	0.071	+6.9	0.134	+3	0.366

Note. Tree topologies suggested are as follows. Tree 1, identical to Figs. 1 and 2. Tree 2, Zygnemophyceae clade is collapsed. Tree 3, *Mesotaenium*/*Mougeotia* clade is collapsed. Tree 4, *Mesotaenium endlicherianum* is a sister to *Mesotaenium kramstai*/*caldariorum*. Tree 5, monophyletic *Mesotaenium* is a sister to *Mougeotia*. Tree 6, *Mesotaenium kramstai*/*caldariorum* clade is a sister to *Mougeotio*. Tree 7, *Cylindrocystis* is monophyletic. Tree 8, monophyletic *Cylindrocystis* is a sister to the *Zygnema* clade. Tree 9, *Mougeotia* + *Zygnema* is a sister to *Mesotaenium* + *Cylindrocystis*. Tree 10, *Mougeotia* + *Mesotaenium* is a sister to *Zygnema* + *Cylindrocystis*. Tree 11, Zygnematales monophyletic: (*Spirogyra*, ((*Netrium*, *Roya*), (Zygnemataceae, Mesotaeniaceae))) (Fig. 1); ((*Netrium*, *Roya*), (Zygnemataceae, Mesotaeniaceae)) (Fig. 2). Tree 12, *Netrium*/*Roya* clade is collapsed. Tree 13, *Netrium* and *Roya* are paraphyletic. Tree 14, Desmidiales is collapsed. Tree 15, *Roya* is a sister to the *Gonatozygon* clade. Tree 16, *Genicularia* is a sister to *Gonatozygon*. Tree 17, *Gonatozygon* is a sister to *Closterium*. Tree 18, *Penium* is a sister to *Gonatozygon*. Tree 19, *Penium*/Desmidiaceae clade is collapsed. Tree 20, *Desmidiaceae* is collapsed. Tree 21, *Phymatodocis* is a sister to *Penium*. The topologies not rejected by results of the test are indicated by gray shading. *User-defined tree significantly worse than best tree, at $p < 0.05$.

[a] Difference in −log-likelihood between the best tree and the user-defined tree.

[b] Probability of obtaining a more extreme t value under the null hypothesis of no difference between the two trees (one-tailed test).

[c] Difference in tree length between the best tree and the user-defined tree.

Clades with significant bootstrap support include the two colony-forming *Cosmocladium* species (*C. saxonicum* and *C. constrictum*; 98–100% bootstrap in all analyses; Fig. 1) and three filamentous species (*Spodylosium planum*, *S. secedens*, and *Teilingia granulata*, referred to as the *Teilingia* clade in Fig. 1; 93 and 99% bootstrap). Another cluster of filamentous genera, here recovered only topologically in ML (Fig. 1) and distance analyses (not shown) and thus informally named the *Desmidium* clade, includes seven species of six genera (Fig. 1): *Desmidium*, *Bambusina*, *Onychonema*, *Groenbladia*, *Hyalotheca* (*H. dissiliens*), and *Spondylosium* (*S. pulchrum*). Three internal nodes in the *Desmidium* clade have moderate bootstrap support (Fig. 1) but their branching pattern is not further resolved. Seven of nine *Staurastrum* species sampled here form two weakly supported clades (65–69%; Fig. 1), whereas *S. pingue* and *S. tumidum* are not part of these clades. Instead, *S. tumidum* is resolved with low to moderate bootstrap support [67% (NJ) and 59% (MP); Fig. 1] as a sister to a robust clade of two *Xanthidium* species with very short branches (*X. brebissonii* and *X. antilopaeum*; the third *Xanthidium* species studied, *X. armatum*, is a long-branch taxon and could not be positioned in the clade). A small clade containing *Staurodesmus convergens* and *Cosmarium contractum* received high bootstrap values [89% (NJ) and 81% (MP) in Fig. 1; 94% (ML) in a reduced data set containing only the Desmidiaceae and *P. margaritaceum*; not shown]. Finally, two genera with two species each were found to be monophyletic: the long-branch genus *Pleurotaenium* and the genus *Euastrum* (bootstrap support in all analyses, 99–100%; Fig. 1).

When the various taxa in the Desmidiaceae were investigated by relative rate tests (using the two-parameter model of Kimura; results not shown), it became obvious that evolutionary rates of sequences differed vastly between taxa (genera but also species within genera). For example, the long-branch sequence of *Cosmarium ovale* has a significantly higher evolutionary rate (at $p < 0.005$) than those of all other taxa (except *Cosmarium lundellii*). Other taxa with high evolutionary rates are *Pleurotaenium* spp., *Hyalotheca mucosa*, *Micrasterias crux-melitense*, and *Staurodesmus dickiei*.

Again, several alternative topologies for taxa within the Desmidiaceae were probed by KH tests [using an unrooted ML (TrN $+I+\Gamma$) tree of the Desmidiaceae/*Penium* lineage; results not shown]. Especially, the monophyly of several genera was probed: placing *Desmidium swartzii* and *D. grevillii* as sisters or enforcing the monophyly of *Heimansia pusilla* (which was formerly known as *Cosmocladium pusillum*) with the two *Cosmocladium* species results in significantly rejected topologies. Although a monophyletic *Staurastrum* (*S. tumidum* as sister to the other *Staurastrum* species) is significantly rejected, *Staurastrum* excluding *S. tumidum* is topologically resolved as a clade in the unrooted analyses used here (NJ, MP, and ML; trees not shown). When *Xanthidium armatum* (a long-branch taxon; see above) was placed as sister to the other two *Xanthidium* species, this topology is not significantly "worse" (at $p < 0.05$). Placing *Hyalotheca mucosa* as sister to *Hyalotheca dissiliens*/*Groenbladia neglecta* is not rejected (although *H. mucosa* has a significantly higher evolutionary rate than the *H. dissiliens*/*G. neglecta* clade).

Discussion

The results of the SSU rDNA analyses of the Zygnemophyceae based on sequences of 78 taxa presented here are largely congruent with previous studies using a more limited set of taxa (Besendahl and Bhattacharya 1999; Bhattacharya et al. 1994; Denboh et al. 2001; Surek et al. 1994) or using *rbc*L as phylogenetic marker (McCourt et al. 1995, 2000). The data support the monophyly of the class of conjugating green algae (Zygnemophyceae) in general and of the order Desmidiales in particular. The systematic value of cell wall characters, which were previously used by Mix (1972, 1975) to delineate orders and families within the Zygnemophyceae (see below), is also corroborated by SSU rDNA phylogenies.

Phylogeny of the Zygnematales. In contrast to the well-supported status of the order Desmidiales, the Zygnematales appear as a paraphyletic assemblage of several independent clades occupying a basal position within the class. The paraphyly of the Zygnematales is less surprising when the phylogenetic status of the defining zygnematalean character (a smooth cell wall consisting of a single piece) is regarded as plesiomorphic (see also McCourt et al. 2000). However, in KH tests the monophyly of the Zygnematales was not significantly rejected (see Results). Using a ML approach and a complex model of evolution (TrN $+ I + \Gamma$), the *Spirogyra* (including *Sirogonium*) lineage is positioned within the Zygnemophyceae, although the four sequences have extremely long branches compared to all other zygnemophycean sequences (Fig. 1). In a previous study using SSU rDNA sequence comparisons, a single species of *Spirogyra* (*S. grevilleana*) could not be placed with confidence within the Zygnemophyceae (Besendahl and Bhattacharya 1999), presumably because of an LBA between the *S. grevilleana* sequence and the two (long-branch) sequences of the outgroup taxa chosen (Charales), in combination with the simple evolutionary methods applied (MP and distance using a simple HKY85 model, i.e., without I and Γ). By analyzing four genetically divergent strains of *Spirogra/Sirogonium* in this study, the (still) long basal branch could be subdivided and LBA problems significantly reduced. It is anticipated that additional taxon sampling within the genus *Spirogyra* will lead to final clarification of its position within the Zygnemophyceae, especially if sequences with slower rates of evolution can be found. In addition, we found the typical zygnemophycean 1506 group I intron (Bhattacharya et al. 1994) in three of five strains of *Spirogyra/Sirogonium* analyzed, and thus, our study clearly reveals the unusual absence of this intron in two strains of *Spirogyra* (SVCK 253 and 261) as a secondary loss within the *Spirogyra/Sirogonium* lineage. We also demonstrate that *Sirogonium sticticum* cannot be separated at the genus level from *Spirogyra*, thus, the genus *Sirogonium* must be rejected [*S. sticticium* is the type species of the genus (Silva 1980)], and, as already proposed by Wille (1884), *Sirogonium sticticum* is considered here as a species of *Spirogyra*, *S. stictica*. The taxonomic identity of other described species of *Sirogonium* (ca. 15) requires further study.

As in previous analyses (Bhattacharya et al. 1994; Besendahl and Bhattacharya 1999; McCourt et al. 1995, 2000), a separation of the Zygnematales into two families, the filamentous Zygnemataceae and the coccoid Mesotaeniaceae, is not supported even when the anomalous *Spirogyra* lineage is disregarded (Fig. 2). These two families, though considered as closely related by Lütkemüller (1902), are traditionally treated as separate entities (e.g., Bold and Wynne 1985; Brook 1981; Gerrath 1993; Prescott et al. 1972; Transeau 1951). Although in some evolutionary schemes (Randhawa 1959; West and Fritsch 1927; Yamagishi 1963) the Zygnemataceae are rooted within the (para- or polyphyletic) Mesotaeniaceae [sometimes even with independent origins of different filamentous genera as by Yamagishi (1963)], no formal attempt to merge the two families was made until recently. McCourt et al. (2000), based on their *rbc*L analyses, proposed to abandon the Mesotaeniaceae in favor of a single family Zygnemataceae (which has taxonomic priority) within the order Zygnematales. While we agree, based on our results, that the two families cannot be recovered, we also fail (as did McCourt et al. 2000) to positively identify a single monophyletic lineage encompassing either the Zygnematales or the Zygnematales minus the *Netrium* and *Roya* clades. Unfortunately, our SSU rDNA analyses currently do not resolve natural lineages within the Zygnematales with significance. This problem presumably relates to different evolutionary rates among the SSU rDNA sequences in question, not only concerning the extreme case of *Spirogyra* (e.g., consider the faster rates of *Mougeotia* and *Zygnema*), and to the still insufficient taxon sampling. Tentatively, our data suggest that the Zygnematales may form at least four monophyletic lineages diverging in a paraphyletic succession, namely, the "*Mesotaenium* lineage," *Spirogyra* (nonpositioned long-branch lineage), and the *Netrium* and *Roya* clades. It should be noted that McCourt et al. (2000), using the *rbc*L gene, arrived at a similar but again tentative conclusion regarding the "unorthodox positions" of both *Netrium* and *Roya* (although the placement of *Roya* differs; see below).

In their *rbc*L phylogeny of the Zygnemophyceae, McCourt et al. (1995, 2000) found that some genera of the Zygnematales with similar chloroplast morphology grouped together as clades, i.e., *Mesotaenium* and *Mougeotia* (laminate chloroplasts), *Cylindrocystis* and *Zygnemopsis* (stellate chloroplasts), and *Spirotaenia*, *Spirogyra*, and *Sirogonium* (ribbon-shaped chloroplasts). This result was not unequivocal, however, because two further taxa with stellate chloroplasts (*Zygnema* and *Zygogonium*) were not significantly placed within the clade containing *Cylindrocystis* and *Zygnemopsis* (McCourt et al. 2000). Previous phylogenetic studies based on SSU rDNA sequence comparisons did not support clades based on chloroplast morphology (Besendahl and Bhattacharya 1999). In addition, there was preliminary evidence that one genus (*Cylindrocystis*) was not monophyletic (Besendahl and Bhattacharya 1999). Unfortunately, McCourt et al. (2000) studied only a single representative of each genus and thus could not address the monophyly of genera. Here we present evidence, based on relative rate and KH tests, that two genera, *Cylindrocystis* and *Mesotaenium* (induding *M. endlicherianum*) cannot be considered monophyletic. However, strain SAG 12.97, designated *Mesotaenium endlicherianum*, is presumably misidentified and might even belong to another (new?) genus (based on cell and chloroplast structure; unpublished observations). Nevertheless, the unexpected nonmonophyly of *Cylindrocystis* (always considered as a "good" genus) introduces an additional level of complexity to the phylogeny of the Zygnemophyceae because it suggests (see also below for taxa in the Desmidiaceae) that several genera require taxonomic revision and further morphological investigation.

Netrium and Roya. The position of the *Netrium* and *Roya* clades in our phylogenetic trees deserves some comment. Based on their plastid morphology (axial laminate chloroplasts as in many Desmidiales) the two genera have often been considered as transitional forms between saccoderm (Mesotaeniaceae) and placoderm desmids [= Desmidiales (Brook 1981; West and West 1904; Yamagishi 1963)] and the molecular data tend to support this view. However, it is not yet clear what other structural character(s) (if any) separates these genera from other Zygnematales and whether any morphological synapomorphies of *Netrium*, *Roya*, and the Desmidiales exist. For *Netrium*, several distinctive (autapomorphic) features in the mode of cell division (Jarman and Pickett-Heaps 1990), cell wall ultrastructure [8–10 cellulose microfibrils in one band versus 3 or 4 in the other Mesotaeniaceae (Mix 1972)], and sexual reproduction (Biebel 1964) are known, but neither *Roya* nor the Desmidiales share these characters. SSU rDNA and *rbc*L phylogenies are basically in agreement regarding the position of *Netrium* but are in conflict with respect to *Roya*. The sister-group relationship found between *Roya* and the Gonatozygaceae by McCourt et al. (2000) was explicitly rejected in our ML analyses (the sequences revealed similar evolutionary rates; Table 2). A clade containing *Roya* and *Gonatozygon* is difficult to reconcile with the pronounced differences in cell wall ultrastructure between the two taxa as shown by Mix (1972) and would postulate either a complete loss of cell wall pores/ornamentation in *Roya* or the independent (convergent) evolution of the same cell wall type (ornamented with pores) in the Gonatozygaceae and in the other placoderm desmids. Obviously, the conflicting results between the *rbc*L and the SSU rDNA phylogenies concerning the placement of *Roya* demand a closer inspection of this genus in both morphological and molecular terms.

Phylogeny of the Desmidiales. The order Desmidiales, established as a robust, late-diverging lineage in the Zygnemophyceae and characterized by structural synapomorphies as the presence of cell wall pores, is composed of three (perhaps four) clades, corresponding to currently recognized families. In general agreement with the *rbc*L phylogeny (McCourt et al. 2000), the long-branch clades Gonatozygaceae and Closteriaceae represent independent divergences within the Desmidiales, whereas *Penium* (= Peniaceae) and the Desmidiaceae are combined within a third lineage (see Results). This topology demands a comparison with the cell wall-based subdivision of the Desmidiales into two groups, "Archidesmidiineae," with simple pores (through the outer layer only) and ornamentations formed by the "outer cell wall layer," and "Desmidiineae," characterized by pores penetrating the whole cell wall and by ornamentations formed by the secondary wall (Mix 1972). Both molecular phylogenies (here and in McCourt et al. 2000) recover the "Archidesmidiineae" comprising the families Closteriaceae, Gonatozygaceae, and Peniaceae, as a paraphyletic entity and reveal the defining characters (see above) as plesiomorphies within the order Desmidiales. As another example of a "noncladistic" use of plesiomorphic characters, Kouwets and Coesel (1984) included the Gonatozygaceae as a part of their larger family Peniaceae, an association clearly rejected by molecular phylogenies. The "Desmidiineae," equivalent to the family Desmidiaceae, probably form a monophyletic, although weakly supported group. Surprisingly, the apparently profound differences between the two cell wall morphologies are not reflected by SSU rDNA distances, especially concerning the Desmidiaceae and the Peniaceae: the branch separating *Penium margaritaceum* from the Desmidiaceae as well as the common branch of the *Penium*/Desmidiaceae clade is relatively short and unstable in KH tests (see Results). Although recently reduced in number of species (Kouwets and Coesel 1984), *Penium* still contains more than a dozen species and analysis of additional taxa at the molecular level may better resolve the Peniaceae within the Desmidiales.

Gonatozygon and Genicularia. Genicularia spirotaenia emerges firmly nested within *Gonatozygon* as a sister to *G. aculeatum*. The genus *Genicularia* (de Bary 1858) is characterized by two or three helical, parietal chloroplasts per cell, whereas in *Gonatozygon* the two (rarely one) chloroplasts are said to be axial, usually band-shaped, and often slightly undulate. These apparently gradual differences in chloroplast morphology [*Genicularia elegans*, the second species in the genus, is said to have "later spirals" (West and Fritsch 1927) than *G. spirotaenia*] already led Lütkemüller (1902) to reunite the two genera as *Gonatozygon*, a conclusion which was not accepted by most taxonomists (Gerrath 1993; Kossinskaja 1952; Prescott et al. 1972; West and West 1904; Ruzicka 1977). Here, the SSU rDNA phylogeny fully confirms Lütkemüller's (1902) view. Therefore, *Genicularia spirotaenia* is considered here as *Gonatozygon spirotaenium*, the name originally given to this taxon by de Bary himself (1856) before proposing the new genus *Genicularia* (de Bary 1858).

Phylogenetic Status of Desmidiacean Genera. The approach to analyze genera (20 of the 32 genera studied here) by several species clearly revealed the artificial nature (polyphyly) of at least half of the genera studied (see also discussion for *Genicularia and Sirogonium* above). Two genera are particularly problematic because they exhibit a great variety of cell shapes and chloroplast types and together contain >2000 described species, namely, *Cosmarium* and *Staurastrum*. Boundaries between both genera have been blurred by "intermediate taxa" (e.g., some unusual *Cosmarium* species, which are triangular in end view, resemble species of *Staurastrum*) and they are further linked with other genera in the Desmidiaceae through morphological transitions [e.g., *Xanthidium*, *Staurodesmus*, *Euastrum* (Brook 1981; Croasdale et al. 1994; Prescott et al. 1982)]. Numerous attempts have been made to split the two genera into several supposedly monophyletic (sub-) genera, but none of the proposals received general recognition [sec summaries by Croasdale et al. (1994) and Prescott et al. (1982)]. In our analysis, neither genus could be either recovered or constrained (KH tests) into monophyletic entities. Six strains of *Cosmarium* analyzed form several individual branches [in only one case is a species of *Cosmarium* (*C. contractum*) significantly related to any other taxon (*Staurodesmus convergens*)], whereas seven of nine species of *Staurastrum* studied are resolved within two (closely related) lineages. In *Cosmarium* the resolution in sequence analyses is further hampered by vastly accelerated rates of sequence evolution in two species (*C. ovale*, *C. lundellii*), but in *Staurastrum* this explanation could be ruled out by relative rate tests (e.g., in *S. tumidum*). One lineage of *Staurastrum* contains three species, namely, S. cf. *granulosum*, *S. hirsutum*, and *S. teliferum*. These species are similar in morphology and have even been transferred into a separate genus, *Cosmoastrum*, as defined by variously ornamented, roughly elliptical semicells without processes (Palamar-Mordvintseva 1976, 1982). The second, morphologically more diverse lineage contains four species of *Staurastrum* from three "sections" of the genus [(West and West 1912); one species, *S. lunatum*, was subsequently transferred to the genus *Raphidiastrum* (Palamar-Mordvintseva 1976, 1982)]. This lineage is tentatively associated with one of the single branches, *Staurastrum pingue*. The other individual species of *Staurastrum*, *S. tumidum*, is characterized by a distinctive cell shape (large cells with a weak median constriction [isthmus]) and chloroplast type (several parietal, ribbon-like chloroplasts with numerous pyrenoids) and therefore was classified within the subgenus *Pleurenterium* by Lundell (1871; later raised to the genus level by Lagerheim 1888). *Pleurenterium* was not generally accepted and *S. timidum* is either classified within *Staurastrum* (Prescott et al. 1982; West and West 1912) or *Staurodesmus* (Croasdale et al. 1994; Teiling 1967). Studies on cytomorphogenesis in desmids revealed different modes of nuclear migration during and after morphogenesis in different taxa (Kiermayer and Meindl 1989). In *Micrasterias*, *Cosmarium*, and *Euastrum*, the nucleus moves from its central position into the developing semicell and then back to the isthmus region. In *S. tumidum*, however, the nucleus remains central and circulates within the broad isthmus [(Meindl 1986); unfortunately, no other *Staurastrum* species has been examined]. The same type of nuclear circulation has been recorded so far only in *Xanthidium* (Höftberger and Meindl 1993). Indeed, our phylogenetic analyses provided some (albeit weak) support for a relationship between *S. tumidum* and two *Xanthidium* species (Fig. 1; see Results); however, *X. armatum* [the species studied by Höftberger and Meindl (1993)] is a long-branch taxon in our phylogenetic analyses and could not be positioned.

Interestingly, of nine Desmidiaceae (including *X. armatum*) with parietal chloroplasts (often correlated with large-sized cells) sequenced during this study, six were revealed as long-branch taxa (except *S. tumidum* and *Euastrum* spp.). This observation raises the question whether in the Desmidiaceae an evolutionary transition from the more common axial to parietal chloroplasts was perhaps accompanied by an acceleration in mutational rates of their SSU rDNA and, if so, whether this occurred independently in different lineages. Analysis of additional taxa and perhaps other molecular markers may shed light on this intriguing question.

The phylogenetic and taxonomic status of *Staurodesmus*, a problematic genus putatively related to *Staurastrum*, remains unsettled following our molecular phylogenetic analyses of four *Staurodesmus* strains, which were found scattered among other taxa of the Desmidiaceae (Fig. 1; Results). Only one taxon, *S. convergens*, forms a robust clade with *Cosmarium contractum* and indeed, cells of *S. convergens* can often be found without spines and thus resemble *Cosmarium*. A single spine at the lateral angles of semicells is, however, the main diagnostic feature of *Staurodesmus* (in addition to the lack of warts or protuberances on the cell surface). The variability in the presence of spines in *S. convergens* thus undermines the genus concept of *Staurodesmus*, although this character is more stable in other species. Surprisingly, *S. bulnheimii*, which is morphologically very similar to *S. convergens* (when spines are present), is not closely related to this taxon in the molecular phylogeny, and KH tests (not shown) rejected their monophyly. Furthermore, the SSU rDNA sequence of *Staurodesmus dickiei* turned out to be very similar to that of *Micrasterias crux-melitensis* (only 15 nucleotide differences in the alignment used for Fig. 1) and forms a robust lineage with this taxon as characterized by a long common branch of 30 synapomorphic base changes in an analysis using MP (tree not shown). In consequence, the genus *Micrasterias*, represented here by only two species, appears paraphyletic. These results are unexpected because of substantial morphological differences between *Micrasterias* spp. (flattened cells with a distinctly lobed and ornamented outline) and *Staurodesmus dickiei* with nonflattened, three-radiate cells ornamented by simple spines. Since the well-known genus *Micrasterias* is always considered to be monophyletic, the position of *S. dickiei* raises the provocative question whether this taxon could represent a generic *Micrasterias*, which became extremely reduced in its morphological complexity. The opposite explanation would assume independent (convergent) evolution toward extremely similar SSU rDNA sequences in two unrelated organisms.

Despite the considerable number of sequences representing the genus *Cosmarium*, a monophyletic origin of this genus was not established. This result either can be attributed to the occurrence of two long-branch taxa or may also reflect true polyphyly of the genus and real phylogenetic distances among species. In contrast to elliptical (in apical view) species, omniradiate *Cosmarium* species (*C. isthmium* and *C. elegantissimum*) are positioned in a clade with taxa of similar morphology [*Spondylosium* (formerly *Cosmarium*) *panduriforme* and *Actinotaenium* (*Penium*) *phymatosporum*; resolved by all methods, but without bootstrap support]. Although other omniradiate taxa studied here (i.e., two additional *Actinotaenium* species) are not included in this cluster, this result deserves further scrutiny.

Like *Staurastrum*, *Staurodesmus*, and *Cosmarium*, the filamentous genera *Spondylosium* (five strains), *Desmidium* (two strains), and *Hyalotheca* (two strains analyzed) are not monophyletic. Within a well-supported lineage comprising colonies (*Cosmocladium saxonicum* and *C. constrictum*) and filaments (*Spondylosium planum*, *S. secedens*, and *Teilingia granulata*) of *Cosmariurn*-like cells, filamentous taxa form a robust subclade (*Teilingia* clade), suggesting that filament formation evolved here by the same mechanism. However, another filamentous taxon (*Onychonema laeve*), also characterized by *Cosmarium*-like cells but with peculiar protrusions, is significantly positioned outside the *Teilingia* clade. The presumed convergent origin of these filaments might be reflected by different modes of cell connections (see Kirk et al. 1976).

Another three clades of filamentous desmids were recovered across genene boundaries — *Desmidium swartzii*/*Spondylosium pulchrum*, *Desmidium grevillii*/*Bambusina*, and *Groenbladia*/*Hyalotheca dissiliens*; all these clades combine taxa without obvious synapomorphies but instead with profound differences in cell and chloroplast morphology (e.g., triangular versus laterally compressed cells in *D. swartzii* and *S. pulchrum*), cell morphogenesis [*Bambusina* (see Gerrath 1975)], and/or mode of conjugation (isogamy versus anisogamy). In summary, at least four independent origins of filamentous forms within the Desmidiaceae [two comprehensive lineages, *Spondylosium* (*Cosmarium*) *panduriforme* and *S. pulchellum*] likely (Fig. 1). The *rbc*L phylogeny of the Zygnemophyceae (McCourt et al. 2000) revealed a lineage of four filamentous taxa (*Spondylosium pulchellum*, *Desmidium* sp., *Onychonema* sp., and *Hyalotheca dissiliens*), which in our analysis using a noncongruent taxon sampling was recovered only tentatively (*Desmidium* clade) and to the exclusion of *S. pulchellum* [same strain as studied by McCourt et al.(2000)]; the SSU rDNA sequence of this strain, however, has a relatively fast rate of evolution and thus could not be positioned in the tree. McCourt et al. (2000) recovered a second clade of colonial/filamentous Desmidiaceae containing the genera *Cosmocladium* (*C. perissum*), *Sphaerozosma* (sp.), and *Groenbladia* (*G. undulata*), which in our analysis tentatively corresponds to the *Teilingia* clade plus *Cosmocladium* (see above). We note, however, that the *Groenbladia* species studied by us (*G. neglecta*; originally described as *Hyalotheca*) does not belong to this clade and is instead resolved as sister of *Hyalotheca dissiliens*.

Finally, our data support some recent taxonomic rearrangements in the genera *Cosmocladium* [separation of the genus *Heimansia* (Coesel 1993)] and *Pleurotanium* [separation of the genus *Haplotaenium* (Bando 1988)].

Acknowledgments. We wish to thank Matthias Rüsing for providing the sequence of *Sirogonium sticticum* and Barbara Surek for help with cultures and critical comments on the manuscript. We also thank Dr. Hervé Philippe and one anonymous reviewer for their helpful comments on the manuscript. This study was supported by a fellowship from the Humboldt-Stiftung to A.A.G.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Contr 19:716–723

Bando T (1988) A revision of the genera *Docidium, Haplotaenium* and *Pleurotaenium* (Desmidiaceae, Chlorophyta) of Japan. J Sci Hiroshima Univ Ser B Div 2 22:147–160

Besendahl A, Bhattacharya D (1999) Evolutionary analyses of small-subunit rDNA coding regions and the 1506 group I introns of Zygnematales (Charophyceae, Streptophyta). J Phycol 35:560–569

Bhattacharya D, Surek B, Rüsing M, Damberger S, Melkonian M (1994) Group I introns are inherited through common ancestry in the nuclear-encoded rRNA of Zygnematales (Chlorophyta). Proc Natl Acad Sci USA 91:9916–9920

Bhattacharya D, Damberger S, Surek B, Melkonian M (1996) Primary and secondary structure analysis of the rDNA group I introns of the Zygnematales. Curr Genet 29:282–286

Biebel P (1964) The sexual cycle of *Netrium digitus*. Am J Bot 51:697–704

Bold HC, Wynne MJ (1985) Introduction to phycology, 2nd ed. Prentice Hall, Englewood Cliffs, NJ

Bremer K (1985) Summary of green plant phylogeny and classification. Cladistics 1:369–385

Bremer K, Humphries CJ, Mishle BD, Churchill SP (1987) On cladistic relationships in green plants. Taxon 36:339–349

Brook AJ (1981) The biology of desmids. Botanical monographs, 16. Blackwell Scientific, Boston, Melbourne

Cavalier-Smith T (1981) Eukaryote kingdoms: Seven or nine? BioSystems 14:461–481

Chapman RL, Buchheim MA, Delwiche CF, Friedl T, Huss VAR, Karol KG, Lewis LA, Manhart J, McCourt RM, Olsen JL, Waters DA (1998) Molecular systematics of the green algae. In: Soltis DE, Soltis PS, Doyle JJ (eds) Molecular systematics of plants II. Kluwer Academic, Boston, pp 508–540

Coesel PFM (1993) Taxonomic notes on Dutch desmids II. Cryptogamie Algol 14:105–114

Croasdale HT, Flint EA, Racine MM (1994) Flora of New Zealand. Freshwater algae, chlorophyta, desmids. IlII. Caxton Press, Christchurch

de Bary A (1856) Zu *Gonatozygon monotaenium*. Hedwigia 1:105–106

de Bary A (1858) Untersuchungen über die Familie der Conjugaten (Zygnemeen und Desmidieen), Förstner, Leipzig

Denboh T, Hendrayanti D, Ichimura T (2001) Monophyly of the genus *Closterium* and the order Desmidiales (Charophyceae, Chlorophyta) inferred from nuclear small subunit rDNA data. J Phycol 37:1063–1072

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Engels M, Mix M (1980) Nachtrag zur Liste der Sammlung von Conjugaten-Kulturen im Institut für Allgemeine Botanik der Universität Hamburg. Mitt Inst Allg Bot Hamburg 17:165–171

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

Gerrath JF (1975) Notes on desmid ultrastructure. I. Cell wall and zygote wall of *Cylindrocystis brebissonii* Meneghini. II. The replicate division septum of *Bambusina brebissonii* Küzing. Beih Nova Hedwigia 42:103–114

Gerrath JF (1993) The biology of desmids: A decade of progress. In: Round FE, Chapman DJ (eds) Progress in phycological research 9. Biopress, Bristol, pp 79–192

Höftberger M, Meindl U (1993) Cell differentiation, ultrastructure and nuclear migration in the desmid *Xanthidium armatum*. Nova Hedwigia 56:75–88

Hoshaw RW, McCourt RM (1988) The Zygnemataceae (Chlorophyta): A twenty-year update of research. Phycologia 27:511–548

Jarman M, Pickett-Heaps J (1990) Cell division and nuclear movement in the saccoderm desmid *Netrium interruptum*. Protoplasma 157:136–143

Kadlubowska JZ (1984) Süsswasserflora von Mitteleuropa. Bd. 16, Chlorophyta VIII, Conjugatophyceae I, Zygnematales. Gustav Fischer Verlag, Stuttgart

Kenrick P, Crane PR (1997) The origin and early diversification of land Plants. A cladistic study. Smithsonian Institute Press, Washington, DC, London

Kiermayer O, Meindl U (1989) Cellular morphogenesis: The desmid (Chlorophyceae) system. In: Coleman AW, Goff LJ, Stein-Taylor JR (eds) Algae as experimental systems. Alan R. Liss, New York, pp 149–167

Kies L (1967) Über Zellteilung und Zygotenbildung bei *Roya obtusa* (Bréb.) W. et G.S. West. Mitt Inst Allg Bot Hamburg 12:35–42

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

Kirk WL, Postek MT, Cox ER (1976) The desmid genera *Sphaerozosma, Onychonema* and *Teilingia*: A historical appraisal. J Phycol 12:5–9

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of the Hominoidea. J Mol Evol 29:170–179

Kossinskaja EK (1952) Flora plantarum cryptogamarum URSS. Vol. II Conjugatae 1. Mesotaeniales et Gonatozygales. Nauka, Moscow, Leningrad

Kouwets FAC, Coesel PFM (1984) Taxonomic revision of the conjugatophycean family Peniaceae on the basis of cell wall ultrastructure. J Phycol 20:555–562

Kranz HD, Miks D, Siegler ML, Capesius I, Sensen CW, Huss VAR (1995) The origin of land plants: phylogenetic relationships among charophytes, bryophytes, and vascular plants inferred from complete small-subunit ribosomal RNA gene sequences. J Mol Evol 41:74–84

Kusel-Fetzmann E, Schagerl M (1992) Verzeichnis der Sammlung von Algen-Kulturen an der Abteilung für Hydrobotanik am Institut für Pflanzenphysiologie der Universität Wien. Phyton 32:209–234

Lagerheim G (1888) Über Desmidiaceen aus Bengalen nebst Bemerkungen über die geographische Verbreitung der Desmidiaceen in Asien. Bih Kongl Svenska Vet-Akad Handl 13:1–12

Lundell PM (1871) De Desmidiaceis quae in Suecia inventae sunt, observationes criticae. Nova Acta Reg Soc Sci Uppsaliensis III 8:1–100

Lütkemüller J (1902) Die Zellmembran der Desmidiaceen. Beitr Biol Pflanz 8:347–414

Marin B, Melkonian M (1999) Mesostigmatophyceae, a new class of streptophyte green algae revealed by SSU rRNA sequence comparisons. Protist 150:399–417

Marin B, Klingberg M, Melkonian M (1998) Phylogenetic relationships among the Cryptophyta: Analysis of nuclear-encoded SSU rRNA sequences support the monophyly of extant plastid-containing lineages. Protist 149:265–276

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

McCourt RM, Karol KG, Kaplan S, Hoshaw RW (1995) Using *rbc*L sequences to test hypotheses of chloroplast and thallus evolution in conjugating green algae (Zygnematales, Charophyceae). J Phycol 31:989–995

McCourt RM, Karol KG, Bell J, Helm-Bychowski KM, Grajewska A, Wojciechowski MF, Hoshaw RW (2000) Phylogeny of the conjugating green algae (Zygnemophyceae) based on rbcL sequences. J Phycol 36:747–758

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

Meindl U (1986) Autonomous circular and radial motions of the nucleus in *Pleurenterium tumidum* and their relation to cytoskeletal elements and the plasma membrane. Protoplasma 135:50–66

Mix M (1972) Die Feinstruktur der Zellwände bei Mesotaeniaceae und Gonatozygaceae mit einer vergleichenden Betrachtung der verschiedenen Wandtypen der Conjugatophyceae und über deren systematischen Wert. Arch Mikrobiol 81:197–220

Mix M (1975) Die Feinstruktur der Zellwände der Conjugaten und ihre systematische Bedeutung. Beih Nova Hedwigia 42:179–194

Nakayama T, Marin B, Kranz HD, Surek B, Huss VAR, Inouye I, Melkonian M (1998) The basal position of scaly green flagellates among the green algae (Chlorophyta) is revealed by analyses of nuclear-encoded SSU rRNA sequences. Protist 149:367–380

Ohtani S (1990) A taxonomic revision of the genus *Netrium* (Zygnematales, Chlorophyceae). J Sci Hiroshima Univ Ser B Div 2 23:1–51

Olsen GJ (1990) Sequence editor and analysis program. University of Illinois, Urbana

Page RDM (1996) TreeView: An application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Palamar-Mordvintseva GM (1976) Novi rodi Desmidiales. Ukr Bot Zhurn 33:396–398

Palamar-Mordvintseva GM (1982) Desmid algae of the Ukrainian SSR. Naukova Dumka, Kiev

Park NE, Karol KG, Hoshaw RW, McCourt RM (1996) Phylogeny of *Gonatozygon* and *Genicularia* (Gonatozygaceae, Desmidiales) based on *rbc*L sequences. Eur J Phycol 31:309–313

Pickett-Heaps JD (1975) Green algae: Structure, reproduction and evolution in selected genera. Sinauer Associates, Sunderland, MA

Posada D, Crandal KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818

Prescott GW, Croasdale HT, Vinyard WC (1972) A synopsis of North American desmids. Part I: Desmidiales: Saccodermae, Mesotaeniaceae. North American Flora Series II(6). New York Botanical Garden, Bronx

Prescott GW, Croasdale HT, Vinyard WC (1975) A synopsis of North American desmids. Part II: Desmidiaceae: Placodermae. Section 1. University of Nebraska Press, Lincoln, London

Prescott GW, Bicudo CE, Vinyard WC (1982) A synopsis of North American desmids. Part II. Desmidiaceae: Placodermae. Section 4. University of Nebraska Press, Lincoln, London

Qiu YL, Palmer JD (1999) Phylogeny of early land plants: insights from genes and genomes. Trends Plant Sci 4:26–30

Randhawa MS (1959) Zygnemaceae. Indian Council of Agriculture Research, New Delhi

Robinson M, Gouy M, Gautier C, Mouchiroud D (1998) Sensitivity of the relative-rate test to taxonomic sampling. Mol Biol Evol 15:1091–1098

Ruzicka J (1977) Die Desmidiaceen Mitteleuropas. Band 1, 1 Lieferung. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart

Saiki RK, Gelfand DH, Stoffen S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with thermo-stable DNA polymerase. Science 239:487–491

Schlösser UG (1994) SAG-Sammlung von Algenkulturen at the University of Göttingen. Catalogue of strains 1994. Bot Acta 107:113–186

Silva PC (1980) Remarks on algal nomenclature VI. Taxon 29:121–145

Starr RC, Zeikus JA (1993) UTEX—The culture collection of algae at the University of Texas at Austin. J Phycol Suppl 29:1–106

Surek B, Sengbusch P (1981) The localization of galactosyl residues and lectin receptors in the mucilage and the cell walls of *Cosmocladium saxonicum* (Desmidiaceae) by means of fluorescent probes. Protoplasma 108:149–161

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 (1998) PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4. Sinauer Associates, Sunderland MA

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Teiling E (1967) The genus *Staurodesmus*. Ark Bot Ser 2 6:467–629

Transeau EN (1951) The Zygnemataceae. Ohio State University Press, Columbus

van den Hoek C, Mann D.G, Jahns HM (1995) Algae: An introduction to phycology. Cambridge University Press, Cambridge

Watanabe MM, Kawachi M, Hiroki M, Kasai F (2000) NIES collection. List of strain. Microalgae and protozoa, 6th ed. NIES, Tsukuba

West GS, Fritsch FE (1927) A treatise on the British freshwater algae, 2nd ed. Cambridge University Press, Cambridge

West W, West GS (1904) A monograph of the British Desmidiaceae. Vol 1. Ray Society, London

West W, West GS (1912) A monograph of the British Desmidiaceae, Vol 4. Ray Society, London

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

Wille N (1884) Bidrag til Sydamerikas Algflora. I–III. Bih till Kongl Svenska Vet-Akad Handl 8:1–64

Yamagishi T (1963) Classification of the Zygnemataceae. Sci Rep Tokyo Kyoiku Daigaku B 11:191–210