REVIEW

Phylogeny and classification of Zygnematophyceae (Streptophyta): current state of affairs

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Abstract: Conjugates (Zygnematophyceae) represent the most species-rich (ca. 4000 spp) algal lineage within the Streptophyta. Studies using various molecular markers have placed the class as one of the crown groups in Streptophyta and even suggested a close relationship between the zygnematophycean algae and land plants. Phenotypic diversity complicates systematics of the class and the relationships between its taxa remain poorly understood. Molecular phylogenetic analyses have established a number of clades in the class and revealed a disagreement between the traditional taxonomic structure of Zygnematophyceae and the pattern of phylogenetic relationships between the class members. In this paper, an overview of recent advances in our understanding of the phylogeny and classification of conjugating green algae is presented, with emphasis on the major clades established in the class, the clades' structure and polyphyly of some genera. We describe the areas of conflict and agreement between molecular phylogeny and traditional morphology-based taxonomy and stress that phenotypic characters provide little evidence for inferring relationships between taxa even at low taxonomic levels.

Key words: conjugates, phylogenetic relationship, polyphyly, Streptophyta, Zygnematophyceae

Introduction

Green conjugating algae comprise coccoid, filamentous and colonial forms that are distinct in a unique type of sexual reproduction (conjugation, first recognized by Vaucher 1803), the complete absence of flagellate reproductive stages, and centriolar centrosomes through the life cycle. The conjugates 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. This attraction resulted in the description of more than 4000 species that are classified in ca. 60 genera (Hoshaw & McCourt 1988, Gerrath 1993, Appendix). It is not really known whether this number reflects natural diversity in the group or not because other estimates range from 1500 to 12000 spp (Cranwell et al. 1990, Hoshaw et al. 1990).

The distinctness of the conjugates and their alliance with the green algae has never been in doubt (Brook 1981). However, the relationships between the families, genera and species of the conjugates as 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 algae previously known as the Charophyceae (sensu Mattox & Stewart 1984) and the embryophyte land plants (Pickett-Heaps 1975, Mattox & Stewart 1984, recent reviews by Lewis & McCourt, 2004, McCourt et al. 2004). In a cladistic framework (Bremer 1985, Bremer et al. 1987, Kenrick & Crane 1997) the conjugating green algae lineage is designated as the class Zygnematophyceae within the division Streptophyta [Streptobionta (Kenrick & Crane 1997)], which, together with the Chlorophyta, form the green plants [Viridiplantae or Chlorobionta (Cavalier-Smith 1981, Kenrick & Crane 1997)]. It appears that the name of the class, Zygnematophyceae Round (1971), is an illegitimate substitute for the Conjugatophyceae Engler (1892) because *Conjugata* (*Spirogyra*) is not a synonym for *Zygnema* (see Silva 1980). Also, the class was described without a Latin diagnosis and therefore it is not a validly described taxon.

Classification schemes of the Zygnematophyceae have generally been based on morphology, emphasizing cell wall ultrastructure, cellular organization (unicells, filaments), and chloroplast structure (MIX 1972, PRESCOTT et al. 1972, 1975, HOSHAW & MCCOURT 1988, GERRATH 1993). Two orders based on cell wall ultrastructure are recognized within the Zygnematophyceae, namely, the Zygnematales and the Desmidiales (MIX 1972, RŮŽIČKA 1977). The Zygnematales are characterized by smooth cell walls consisting of a single piece lacking pores and other ornamentations. Zygnematalean taxa forming filaments are distinguished as the family Zygnemataceae from the unicellular algae classified as the Mesotaeniaceae, formerly known as "saccoderm desmids" (TRANSEAU 1951, PRESCOTT 1972, GERRATH 1993, KADLUBOWSKA 1984). The Desmidiales ("placoderm desmids") have cell walls consisting of two or more pieces having ornamentations and pores (Mix 1972). Based on cellular organization and cell wall features the Desmidiales are subdivided into three or four families (MIX 1972, KOUWETS & COESEL 1984, BROOK 1981, GERRATH 1993), the Peniaceae, Gonatozygaceae, Closteriaceae, and Desmidiaceae. Genera and species of the Zygnematophyceae 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.

Generally, evolution of morphological traits in the class is not well understood. There was a number of hypotheses regarding origin and evolution of certain phenotypical character but these were largely logical constructions that often contradicted each other in very basic assumptions. Particularly this refers to the ancestral growth habit (filamentous or unicellular), chloroplast type and degree of the cell radiation (WEST & WEST 1904, WEST & FRITSCH 1927, PRESCOTT 1948, TEILING 1950, 1952, RANDHAWA 1959, YAMAGISHI 1963, PRESCOTT et al. 1977, HOSHAW & MCCOURT 1988).

In the last two decades molecular phylogenetic analyses have advanced our understanding of the relationships between plants at different levels, have challenged many long-standing hypotheses on plants evolution and significantly affected their systematics. The conjugates are not an exception in this respect, however, apart from the fact that the first zygnematophycean nucleotide sequence was obtained as early as in 1985 (HORI et al. 1985), phylogenetic relationships in the group are far from being well established. Here my attempt is to summarize recent studies that have addressed the evolution and taxonomy of the Zygnematophyceae from a molecular phylogenetic perspective. This paper briefly discusses the class closest relatives, the types of molecular data used to analyse relationships within the Zygnematophyceae, the major clades established in the class and point out discordance between the class phylogeny and taxonomy, arguing that considerable work remains to be done before a convincing picture emerges.

Phylogylogenetic position within Streptophyta

Conjugating green algae have been always considered as members of the green plant lineage because of their pigments and starch as a reserve product (BROOK 1981), althought in some classification schemes conjugates have been regarded as a class distinct from the other green algae (FOTT 1971, ROUND 1971, BOLD & WYNNE 1985). Even when classified within Chlorophyta, the conjugates have been seen as a group with rather unclear relationships due to the uniqueness of their defining characters. Ultrastructural studies in the conjugates revealed cytokinesis by a phragmoplast allying them with the Charales, *Coleochaete*, a few other algal taxa and embryophyte plants (FOWKE & PICKETT-HEAPS 1969a, b, PICKETT-HEAPS 1975, MATTOX & STEWART 1984, GROLIG 1992). Further biochemical and molecular data has shown an advanced position of the class Zygnematophyceae among streptophyte algae but were not conclusive regarding its exact placement there because their results were affected by insufficient taxon sampling, weak resolving power of the markers used or long-branch attraction artefact (KRANZ & HUSS 1996, BHATTACHARYA & MEDLIN 1998, CHAPMAN et al. 1998, QIU & PALMER 1999, MCCOURT et al. 2000).

A phylogeny based on four genes from three genomes (the nuclear 18S rRNA, the chloroplast *atp*B and *rbc*L and the mitochondrial *nad*5) suggested that the conjugates diverged after *Mesostigma*, *Chlorokybus* and *Klebsormidium* but before *Coleochaete* and *Chara*. The latter was resolved as a sister to the land plants with high support (KAROL et al. 2001, MCCOURT et al. 2004). Generally this sistership is in agreement with the hypotheses on evolution of morphological, ultrastructural and biochemical characters that accompanied the emergence of streptophyte algae from an aquatic habitat and the colonization of the land (Graham 1993, 1996, Lewis & McCourt 2004, McCourt et al. 2004, Fig. 1). However, the following analyses of chloroplast small and large subunit rRNA contended the sister relationship between Charales and embryophytes (Turmel et al. 2002). As a further development in this controversial issue, analysis of 76 cpDNA-encoded genes resolved the conjugates as sister to land plants with high support. This novel sistership was further corroborated by the chloroplast gene order, gene and intron content, and synapomorphic indels (insertions and deletions) in the coding regions (Turmel et al. 2005, 2007, Adam et al. 2007). Large data sets, comprehensive taxon sampling and sophisticated analyses provided solid evidence for the advanced position of the Zygnematophyceae but it is still hardly explainable from the traditional point of view on the course of evolution in the Streptophyta. *Coleochaete* and *Chara* are more complex than the conjugates in thallus organization, patterns of cell division, and share with land plants many phenotypical traits (McCourt et al. 2004). We can expect that the sistership between the embryophytes and zygnematophycean algae will be challenged with more genes and data sets but not necessarily this intriguing issue will not necessarily be fully resolved soon.

Types of molecular data

The first insights into the conjugates phylogeny were obtained with nuclear-encoded SSU rRNA and chloroplast protein-coding *rbc*L genes and these markers remain to be the major source of information in the class (Bhattacharya et al. 1994, McCourt et al. 1995, 2000, Besendahl & Bhattacharya 1999, Gontcharov et al. 2003, 2004, Gontcharov 2005). In early phylogenetic analyses these molecular markers independently confirmed monophyly of the class and the order Desmidiales but also rejected monophyly of the order Zygnematales and its two families Zygnemataceae and Mesotaeniaceae. It was found that in all (see however Gontcharov et al. 2003, Gontcharov & Melkonian 2004) zygnematophycean taxa nuclear SSU rDNA contains 1506 group I intron and it was seen as another evidence for monophyletic origin of the conjugates. Likely, the intron entered SSU rDNA of the conjugates ancestor and was then vertically inherited by its descendants unlike many other groups of organisms where introns generally have patchy distribution (Dujon 1989, Bhattacharya et al. 1996). Analyses of the intron secondary structure and self-splicing capacity in the class revealed that it has undergone significant changes after the enter event dated ca. 350-400 Mya (Bhattacharya et al. 1994, 1996, Besendahl & Bhattacharya 1999).

A growing amount of new SSU rDNA and *rbc*L sequences soon revealed a limited power of their individual use, particularly at the deep and shallow branches of the conjugates tree. It would be logical to expect that a larger number of characters (analyses of several genes) may improve phylogenetic accuracy and resolution (Hillis 1996) and an advantage of the combined analyses of SSU rDNA and *rbc*L was explored by Gontcharov et al. (2004). Concatenation of these genes generally increased phylogenetic resolution and could overcome a problem of accelerated evolutionary rates in some taxa in the individual data sets. As a further development of the multi-gene approach, Hall et al. (2008) combined chloroplast *rbc*L, *psa*A and mitochondrial *cox*III genes (ca. 4050 nt) to analyse phylogenetic relationships in the conjugating green algae. Their data set produced a tree that confirmed the results of previous single- and two-gene studies. The support for many branches was not much higher than that obtained with SSU rDNA along or in combination with *rbc*L (Gontcharov et al. 2003, 2004). According to Hall and his co-authors *rbc*L and *psa*A genes are more informative markers compared to *cox*III that has a limited resolving power.

These analyses were mostly focused on studying relationships between orders and families of the conjugates. Gontcharov & Melkonian (in press) used the same markers to address the genus concept in the family Desmidiaceae. Their relatively large data set (SSU rDNA+1506 group I intron+*rbc*L, 3260 nt) and an extensive taxon sampling (97 sequences) recovered a number of novel strongly supported clades that combined representatives of several traditional desmid genera. Within the family these markers also successfully complemented each other and provided high support for most of the clades established. Still, the phylogenetic signal was limited at the deeper branches of the tree leaving the relationships between the clades largely unresolved. To increase resolution further, the slow-evolving chloroplast-encoded LSU rRNA gene was added to the data set (5509 nt, 40 taxa). That resulted in increased resolution at some branches but not yet fully resolved phylogeny. Reduced taxon sampling in this analysis could be partly responsible for still unsatisfactory result.

At the species level, non-coding sequences from nuclear ribosomal operon (1506 group I intron, ITS1 and ITS2) were used to address relationships in the genera *Closterium* (DENBOH et al. 2003), *Staurastrum*, its putative relative *Staurodesmus* and *Cosmarium* (GONTCHAROV & MELKONIAN 2005). Despite warnings about potential flaws of the internal transcribed spacers, which may ultimately lead to incorrect phylogeny, these markers continue to be the most popular non-plastid region for species-level phylogenetic studies in plants (FELINER & ROSSELLO 2007).

The ribosomal ITS and introns are subjected to evolutionary constraints related to the maintenance of specific secondary structures necessary for the correct processing of mature RNAs (CECH 1988, LAMBOWITZ & BELFORT 1993, SALDANHA 1993, MAI & COLEMAN 1997, COLEMAN et al. 1998). In most cases this structure is relatively conserved and once it has been established, it can serve as a guidance to align divergent sequences (COLEMAN 2003). Secondary structure of the 1506 group I intron in the Zygnematophyceae is well known (BHATTACHARYA et al. 1994, 1996 ,BESENDAHL & BHATTACHARYA 1999) but for ITS1 and ITS2 it was proposed only recently (DENBOH et al. 2003, GONTCHAROV & MELKONIAN 2005). In both studies, analyses of the secondary structure-based alignments were very informative and resulted in the largely resolved trees. We anticipate that ribosomal non-coding sequences will be more broadly used in analyses aiming phylogenetic relationships at and below the genus level. Chloroplast intergenic spacers are also widely used to analyse relationships in many groups of land plants and algae (SHAW et al. 2005, 2007), but their utility for the zygnematophycean phylogeny is yet to be tested.

It is almost inevitable that the spectrum of molecular markers in analyses addressing the deeper branches of the Zygnematophyceae should be also extended. Putatively slow-evolving sequences of the large subunits of nuclear and chloroplast ribosomal operons are logical candidates for these studies. Concatenation of two operons will produce an alignment of ca. 10000 nt, however, it is an open question whether this fairly large data set is enough to resolve phylogeny of the Zygnematophyceae. The protein-coding genes could be also useful in this respect and may add the signal needed (MOON & LEE 2003, 2007, MOON et al. 2005, HALL et al. 2008).

Monophyly of the class

Conjugating green algae were always considered as a natural group, only affiliation of the genus *Entransia* HUGHES to it was somewhat questionable (KADLUBOWSKA 1984, MCCOURT et al. 2000). Not

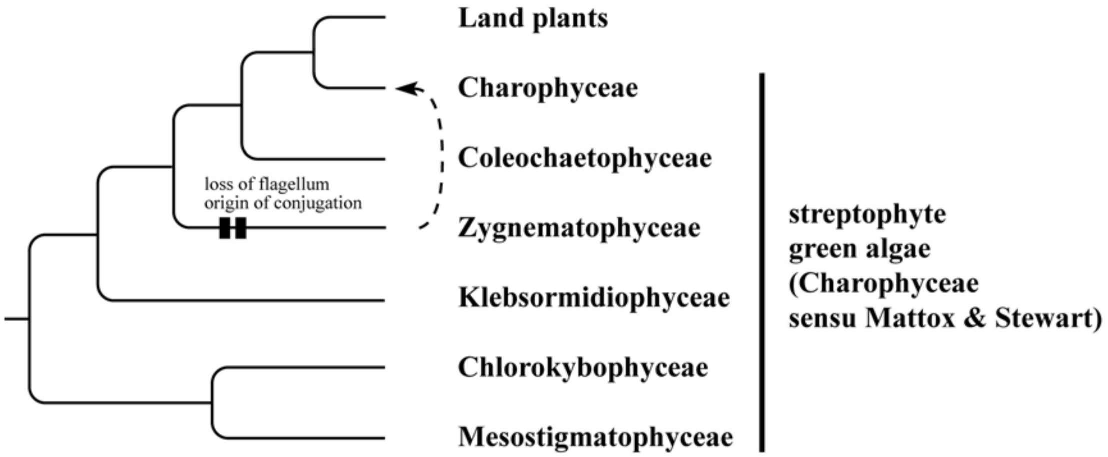

Fig. 1. Summary of the phylogenetic relationships among the major lineages of streptohytes based on analyses of DNA sequence data. Dotted line indicates the possible placement of the Zygnematophyceae supported by 76 cpDNA-encoded genes phylogeny, chloroplast gene order, gene and intron content, and synapomorphic indels in the coding regions (ADAM et al. 2007,TURMEL et al. 2007).

surprisingly, the first assessment with the molecular tools confirmed the anticipated monophyly of the class (Bhattacharya et al. 1994, 1996, Besendahl & Bhattacharya 1999, McCourt at al. 2000, Gontcharov et al. 2003). However, later it was found that one genus of the conjugates, *Spirotaenia*, may be only distantly related to the rest of the class or even not monophyletic with it (Gontcharov & Melkonian 2004, Gontcharov 2005). This hypothesis contended McCourt at al. (1995, 2000) a conclusion on the close relationships between *Spirotaenia condensata* and *Spirogyra*, expected because of their similar spiral chloroplasts (Hoshaw & McCourt 1988, McCourt et al. 1995). The data obtained by Gontcharov & Melkonian suggested that McCourt et al. (1995, 2000) result was based on a mix up of the strains or DNA samples.

Analyses of SSU rDNA and *rbc*L sequences positioned three *Spirotaenia* species apart from the other members of the class Zygnematophyceae among streptophyte algae in a clade with the genus *Chlorokybus* (Gontcharov & Melkonian 2004, Fig. 2). The distant relationship between *Spirotaenia* and the rest of the class attained high support and was further substantiated by the lack of 1506 group I intron in SSU rDNA, typical of the conjugates, in all studied *Spirotaenia* species. In analyses without *Chlorokybus*, *Spirotaenia* was placed as a sister to the Zygnematopyceae but without any support for their short common branch (Gontcharov & Melkonian 2004). Thus, it is not clear yet, whether *Spirotaenia* is the first branch of the conjugates tree or an independent lineage within Streptophyta, or a sister to *Chlorokybus*.

Sexual reproduction by conjugation, synapomorphic feature of the class, has been documented in four species of *Spirotaenia*, and two of them were analysed by Gontcharov & Melkonian (2004). However, the process of conjugation is rather diverse among the Zygnematophyceae (Brook 1981), and *Spirotaenia* differs from all the other conjugating green algae in producing no conjugation tube or vesicle. Instead, in members of this genus paired cell gelatinize cell walls completely prior to gamete fusion (Biebel 1975, Hoshaw & Hilton 1966). Another peculiar character of sexual reproduction in *Spirotaenia* is a production of two gametes per cell that fuse with the respective gametes of the opposite cell to yield a pair of zygospores (Hoshaw & Hilton 1966, Haga & Ehara 1977). It is likely that the differences mentioned above reflect different fine machinery of the conjugation process in two lineages and may support deep genetic divergence between the Zygnematophyceae and *Spirotaenia*. Virtually, nothing is known about cytokinesis in *Spirotaenia* and very little about the cell wall ultrastructure in this genus and we can expect new distinctive features to be found. It is obvious that it deserves further attention.

Zygnematophyceae classification and phylogeny: the order Zygnematales

Earlier molecular phylogenetic analyses resolved the order Zygnematales as not a monophyletic taxon because the root of the Zygnematophyceae was placed within this order, and, thus, revealed it as paraphyletic (McCourt et al. 2000, Gontcharov et al. 2003). Molecular data also rejected monophyly of two zygnematalean families, Zygnemataceae and Mesotaeniaceae, intermixing their representatives. It was found that *Netrium* and *Roya* have more affinity to the Desmidiales than to the rest of Zygnematales. Zygnematalean algae (both filamentous and unicellular) are still poorly sampled and phylogenetic structure of this group is tentative. Many genera are not represented in the culture collections and their affiliation remains unknown.

Currently seven lineages were identified within the traditional Zygnematales, namely *Roya*, *Netrium digitus* and allied species, *Spirogyra*, the "crown Zygnematales" that includes most members of the order (see below), and three individual taxa, *Mesotaenium endlicherianum*, *Netrium interruptum*, and *N. oblongum* SVCK 255 (Gontcharov et al. 2004, Hall et al. 2008). Of these, *Roya* and *Netrium* taxa showed more close relationships to the Desmidiales rather than to the Zygnematales suggesting that the Desmidiales likely originated from a paraphyletic stock of derived unicellular Zygnematales (i.e., *Roya* and *Netrium*, Fig. 3). 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, West & West 1904, Yamagishi 1963, Brook 1981) and the molecular data support this view. However, it is not yet clear what structural character(s) separates these genera from the other Zygnematales and whether

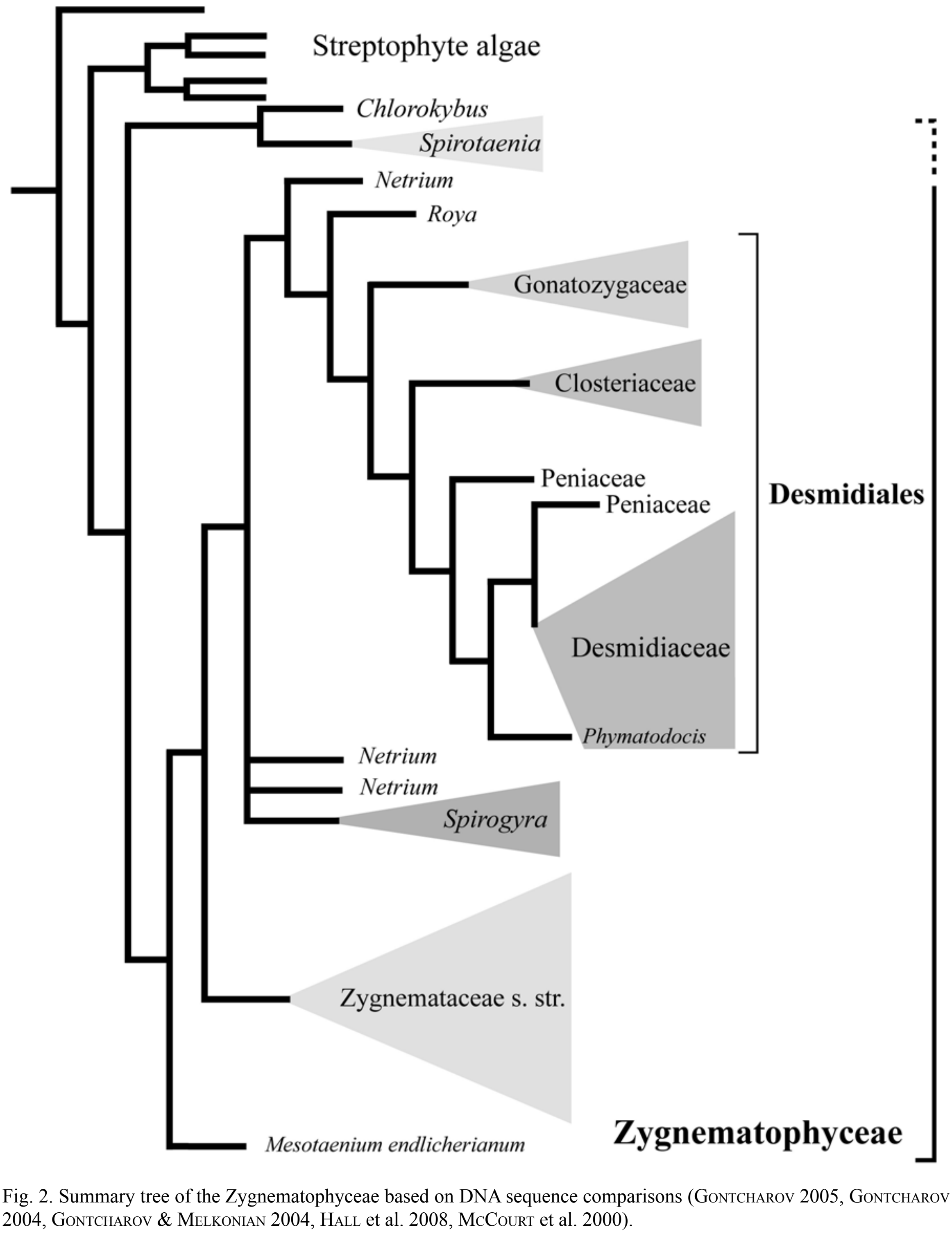

Fig. 2. Summary tree of the Zygnematophyceae based on DNA sequence comparisons (GONTCHAROV 2005, GONTCHAROV et al. 2004, GONTCHAROV & MELKONIAN 2004, HALL et al. 2008, MCCOURT et al. 2000).

any morphological synapomorphies for *Netrium*, *Roya*, and the Desmidiales exist. Paraphyly of relatively small genus *Netrium* (10 species; Appendix) further complicated the picture.

Pattern of relationships revealed in the Zygnematales strongly suggests that an importance of morphological characters such as the growth habit (unicellular or filamentous) and chloroplast shape (ribbonlike, stellate and laminate) above the genus and in some cases even above the species levels (see below) was greatly overestimated. Similarity in chloroplast morphology between unicellular and filamentous taxa was seen as an indication of their possible common ancestry (PALLA 1894, RANDHAWA 1959, Yamagishi 1963) and these hypotheses gained some support in early molecular phylogenetic analyses (McCourt et al. 1995, 2000). However, more comprehensive taxon sampling revealed rampant distribution of the stellate and laminate chloroplasts and unicellular/filamentous habit in the Zygnematales rejecting their monophyletic origin here (Gontcharov et al. 2003, 2004). It should be noted that there were no detailed study on chloroplast morphology in the Zygnematales and the whole class Zygnematophyceae and it well could be that chloroplasts attributed to the same morphological type are in fact distinct in their fine structure and organisation. Polyphyletic nature of zygnematalean genera based on chloroplast morphology (e. g. *Cylindrocystis, Mesotaenium, Netrium*; see below) provides good evidence for that.

Although the traditional order Zygnematales requires further phylogenetic studies before it could be reshaped taxonomically, we can make some conclusion on the composition and structure of its family Zygnemataceae. The type genus of the family, *Zygnema*, was resolved as a member of the clade "crown Zygnematales" (Fig. 2) binding the family name to this clade. It is likely that the clade/family will accommodate all filamentous zygnematalean genera except *Spirogyra* and most unicellular saccoderms except *Roya*, *Netrium*, and perhaps *Mesotaenium endlicherianum* and its possible relatives (McCourt et al. 2000, Gontcharov et al. 2003, 2004, Hall et al. 2008). It is obvious that the diagnosis of the family Zygnemataceae should be emended to accommodate the unicellular forms but also to discriminate the family members from the other taxa having similar morphology but showing little affinity to the Zygnemataceae. It is quite unlikely that the known characters of the cell-wall ultrastructure and/or gross cell and chloroplast morphology may serve this purpose.

The order Desmidiales

In contrast to the Zygnematales, monophyly of the order Desmidiales, defined by the complex cell wall ultrastructure and pores, received strong support in most phylogenetic analyses (Denboh et al. 2001, Gontcharov et al. 2003, 2004). Affiliation of the mesotaeniacean genus *Roya* to the Desmidiales challenging the order concept has been hypothesised (McCourt et al. 2000, Hall et al. 2008). However, the sisteship of *Roya* and Gonatozygaceae received support only from chloroplast protein-coding genes and it could be explained by any phenotypic traits (Gontcharov et al. 2004). *Roya* shares the cell wall features with other Zygnematales (Mix 1972) and it is rather unlikely that they are a result of the secondary simplification in this genus or that of the cell wall ornamentation and segmentation arose in the Gonatozygaceae independently from the rest of the Desmidiales (possible evolutionary scenarios implied by this sistership). Thus, homoplasy of molecular characters is a more parsimonious explanation for the affiliation of *Roya* with the Desmidiales.

Molecular data confirmed monophyly of the desmid families Gonatozygaceae and Closteriaceae but questioned concepts of the families Peniaceae and Desmidiaceae, forming a crown clade of the tree. Gonatozygaceae was resolved as a basal branch in the order clade but support for its early divergence is not very strong (McCourt et al. 2000, Denboh et al. 2001, Gontcharov et al. 2003, 2004, Hall et al. 2008, Fig. 2). The branching pattern within the Desmidiales generally corresponds the traditional hypotheses of relationships between desmid families (Mix 1972) and reflects the increasing complexity of the cell wall ultrastructure from more primitive cell wall and pores (cell wall consisting of several segments separated by shallow groove(s); simple pores perforating only the outer cell wall layer, Gonatozygaceae, Closteriaceae, and Peniaceae) towards derived character state (constricted cells composed of two semicells with complex cell wall pores) in the Desmidiaceae. Also molecular data clearly showed that the features of the cell wall ultrastructure in the Gonatozygaceae, Closteriaceae, and Peniaceae have plesiomorphic character state rather than synapomorphic (Fig. 4). Not surprisingly, phylogenetic analyses rejected monophyly of the suborder Archidesmidiineae based on these features (McCourt et al. 2000, Gontcharov et al. 2003, Hall et al. 2008). It was also found that the families Gonatozygaceae and Peniaceae, indistinct in their cell wall ultrastructure characters and therefore merged into one family (Kouwets & Coesel 1984), are only distantly related.

Molecular data resolved *Penium* as a paraphyletic genus and allied one of its lineages, composed by small-celled species (e. g. *P. cylindrus*, *P. exiguum*) with the family Desmidiaceae

(GONTCHAROV et al. 2004, see however HALL et al. 2008). Close relationships between some *Penium* spp. and in other cases spp. *Phymatodocis* having semicells typical of the Desmidiaceae but primitive pores and not shading primary cell wall (ENGELS & LORCH 1981) suggest that they may also share character states of the cell wall features transitional between the two families (Table 1). Further studies, both molecular and ultrastructural, are necessary to resolve major lineages in the Desmidiaceae/Peniaceae assemblage and define them taxonomically. To my mind, combination of the cell wall ultrastructure character states in *Phymatodocis* is distinct enough to recognise this genus as a subfamily within the Desmidiaceae or even an independent family. However, there was no support in molecular phylogenies so far for the separate position of *Phymatodocis* (GONTCHAROV et al. 2004, HALL et al. 2008). Moreover, the most recent analyses revealed alliance of two more desmid taxa, *Actinotaenium cruciferum* and *Cosmarium depressum*, with small-celled *Penium* spp. (GONTCHAROV & MELKONIAN, in press, Fig. 5).

Table 1. Character states of the cell wall ultrastructure features in the families Peniaceae, Desmidiaceae and the genus *Phymatodocis*.

Taxon/character	Complex pores	Semicells
Peniaceae	-	-
Phymatodocis	-	+
Desmidiaceae	+	+

Sequencing of multiple strains of *Actinotaenium cruciferum* rejects strain/sequence confusion as a possible explanation for such an unexpected relationship that requires further attention.

The sister relationship between Desmidiaceae and Peniaceae generally supports TEILING's, (1950, 1952) hypothesis on origin of the current diversity of morphologies in the Desmidiaceae from rather simple omniradiate ancestor with the stellate chloroplasts (*Penium* morphology). However, an emerging pattern of relationships within this crown assemblage suggests a more complex evolutionary scenario in the Desmidiaceae than it was expected. Already one of branches of the desmidiacean clade, *Phymatodocis nordstedtiana*, is characterised by 4-radiate cells with furcoid chloroplasts, derived features according to TEILING (1950, 1952). As a result, this disputes an inherited nature of the omniradiate cell morphology and the stellate chloroplasts occurring in many desmid species (see also below).

Challenge for the genus concept

According to the traditional morphology-based taxonomy, conjugating green algae are classified into 55-60 genera (Appendix). Most of these were described more than 100 years ago and their concepts have not change much since then. In the Zygnematophyceae, generic diagnoses rely mostly on the characters of gross morphology such as habit, cell and semicell shape, type of the cell wall ornamentation and chloroplast shape. Only some zygnematacean genera were based on distinct features of the sexual reproduction (KADLUBOWSKA 1984).

Most characters defining the genera have a wide variability range and/or occur in different combinations across the class therefore many zygnematophycean generic diagnoses are rather indistinct and often overlap with each other. As a result, it is hardly possible to find any genus of the conjugates whose original concept has never been disputed. Several exceptionally species-rich and morphologically diverse desmid genera such as *Cosmarium*, *Staurastrum* and some of their putative relatives, *Xanthidium*, *Staurodesmus*, and *Eustrum*, are particularly problematic in this respect (PALAMAR-MORDVINTSEVA 1976a, b, BROOK 1981, PRESCOTT et al. 1982, CROASDALE et al. 1994, GERRATH 1993). There were numerous attempts to split them into smaller, more natural (morphologically uniform) taxa that, however, also could not withstand the same criticism and were finally abandoned.

The first assessment of the generic concepts in the Zygnematophyceae with the molecular tools confirmed that many genera of the conjugates are not monophyletic indeed and require a revision (GONTCHAROV et al. 2003, 2004, MOON & LEE 2003). It was also found that a small number of species and their relatively uniform appearance is not a warranty for the genus monophyly (e.g. *Spondylosium*, *Desmidiaum*, *Hyalotheca*, *Netrium*, *Cylindrocystis*, *Mesotaenium*). These preliminary results were indicative but not yet conclusive because the analyses suffered from the limited taxon sampling, presence of long branches and limited resolving power of the markers used. Studies specifically addressing the generic concepts in *Staurastrum*, *Staurodesmus* (Gontcharov & Melkonian 2005, Moon et al. 2005, Moon & Lee 2007), *Cosmarium* and *Actinotaenium* (Gontcharov & Melkonian, in press), based on large data sets and many strains, fully confirmed polyphyletic nature of these traditional genera. In most cases their representatives were found in several distantly related clades of the tree, often together with representatives of other genera. *Cosmarium* is particularly notorious in this respect – its species were distributed between 10 generally highly supported clades, of these 6 included also *Staurodesmus*, *Eustrum, Micrasterias* members and some filamentous desmid genera (Gontcharov & Melkonian, in press, Fig. 5). Polyphyly of *Staurodesmus* and *Staurastrum* seems to be less conspicuous; *Staurodesmus* was split into tree independent lineages (two of them included *Cosmarium* spp. as well), while most (20 of its 23 species analysed) *Staurastrum* members formed

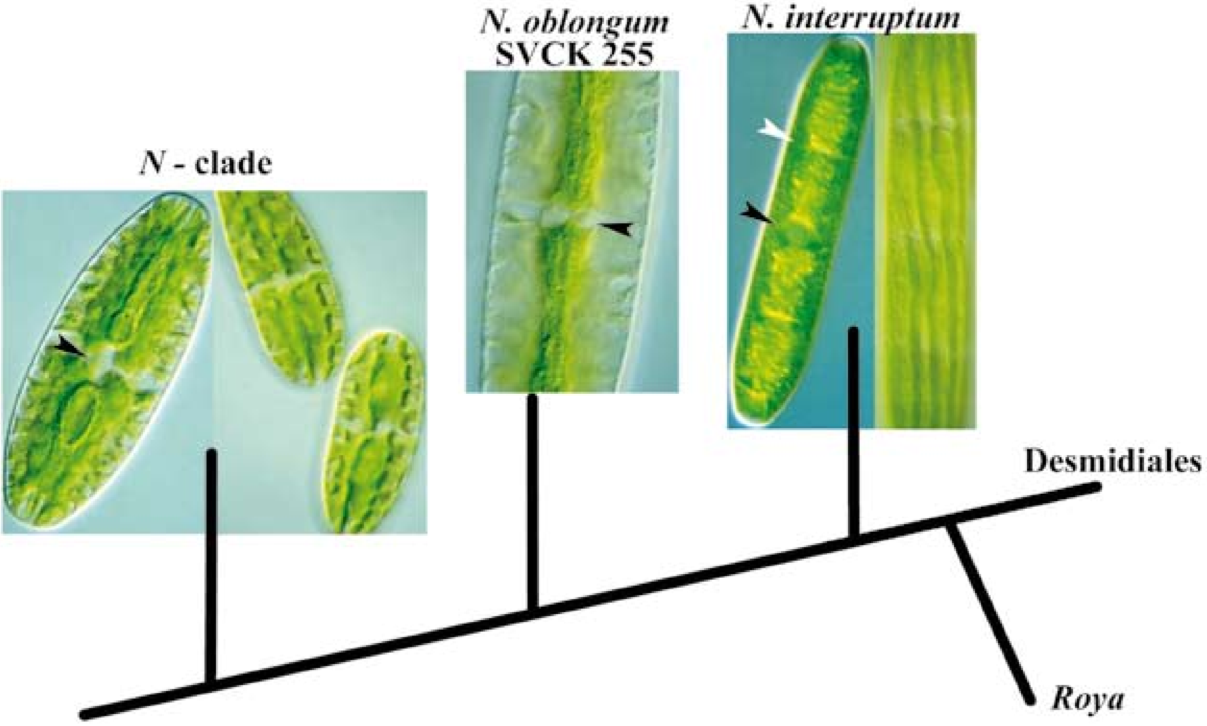

Fig. 3. Paraphyletic divergence of three *Netrium* branches and *Roya* at the base of the Desmidiales clade according to Gontcharov et al. (2004). Each *Netrium* lineage is distinct in the number of chloroplasts per cell (2, 1 and 4, respectively) and, consequently, the positions of the nucleus in the cell (black arrowheads) and/or nuclear behaviour during cytokinesis (Jarman & Pickett-Heaps 1990, Pickett-Heaps 1975). White arrowhead indicates the large vacuole separating two chloroplasts that harbours the daughter nucleus soon after cytokinesis in *N. interruptum*.

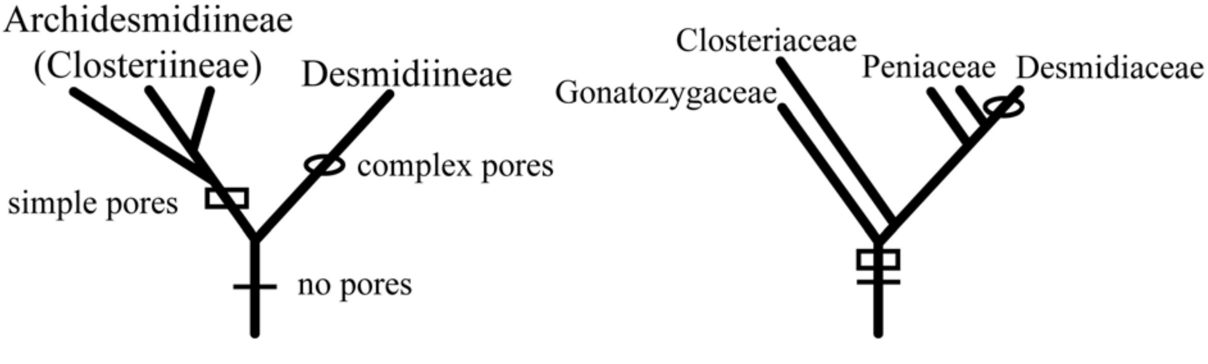

Fig. 4. Taxonomic (a, Mix 1972) and phylogenetic (b; summary based on DNA sequence data) structures of the order Desmidiales with the characters of the cell wall ultrastructure mapped.

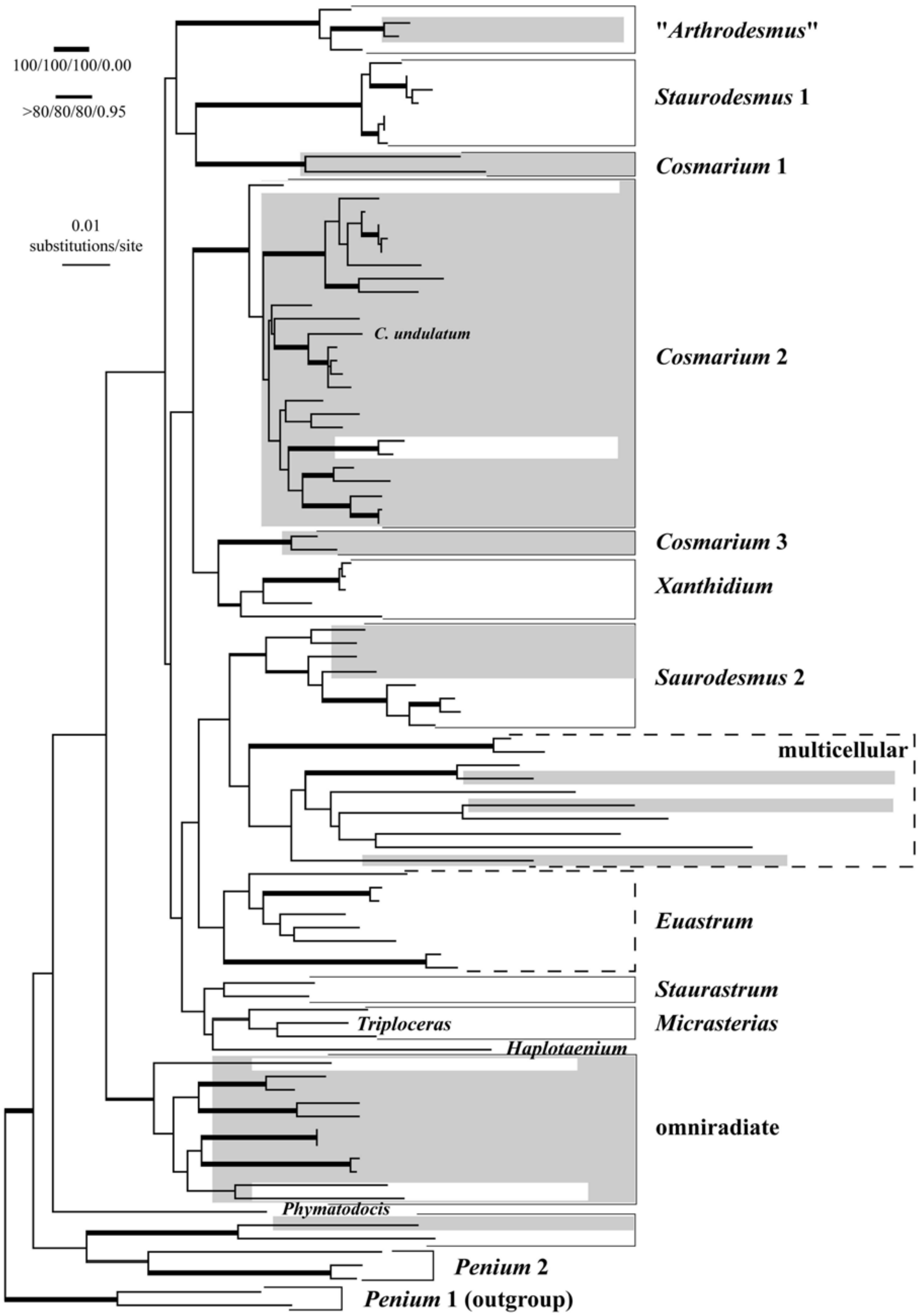

Fig. 6. Major clades of Desmidiaceae established in the combined analyses of nuclear SSU rDNA, 1506 group I intron and *rbc*L sequences (97 taxa, 3260 nt, ML topology). The tree was rooted with two *Penium* spp. *Cosmarium* species are shaded with grey. Clade *Cosmarium* 2 includes *C. undulatum*, the type species of the genus (SILVA 1952).

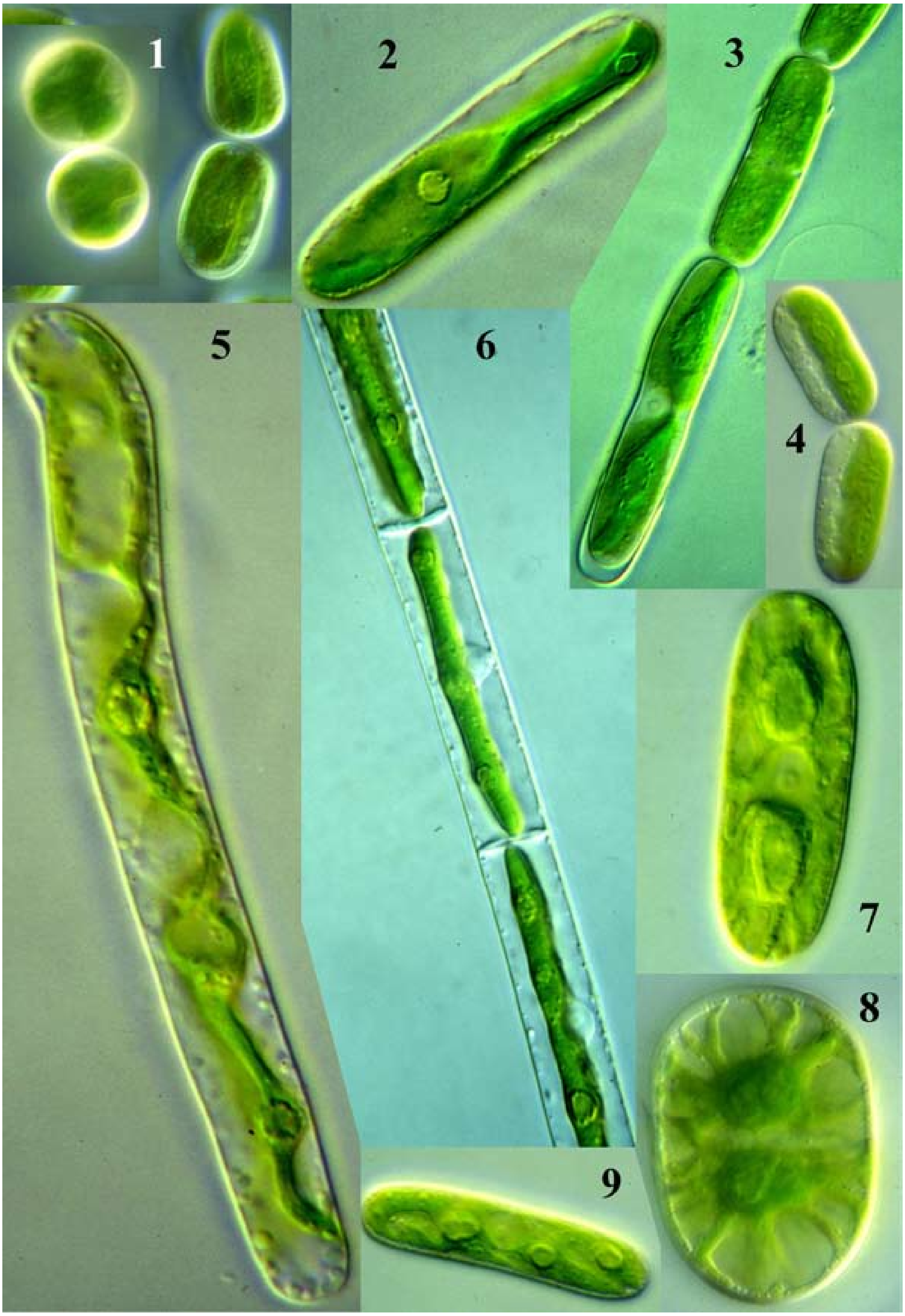

Fig. 5. Representatives of distinct lineages found in the genera *Mesotaenium* and *Cylindrocystis*. Note diversity of chloroplast morphology in *Mesotaenium* species and their difference with chloroplasts in putatively related *Mougeotia*. 1 – *Mesotaenium macrococcum* (Kütz.) Roy et Bisset M 2214; 2 – *M. kramstai* Lemmerm. UTEX 1024; 3 – (?) *M. endlicherianum* Näg. SAG 12.97; 4- *M.* cf. *chlamydosporum* De Bary M 2155; 5 – *M. caldariorum* (Lagerh.) Hansg. ACOI 127; 6 – *Mougeotia* sp.; 7 – *Cylindrocystis* cf. *brebissonii* De Bary M 2213; 8 - *C. crassa* De Bary M 2328; 9 – *C. cushleckae* Brook M 2158.

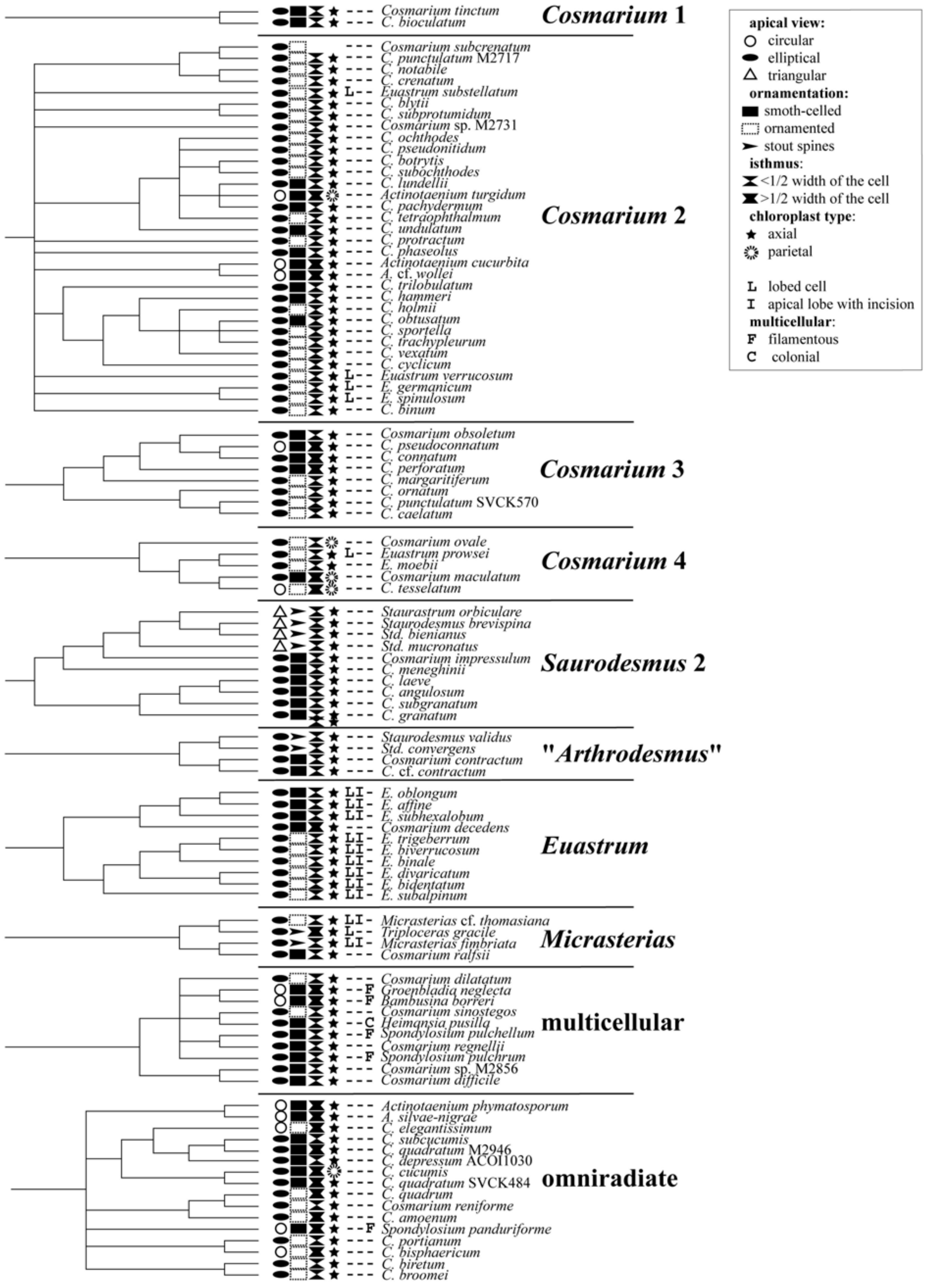

Fig. 7. Bootstrap consensus topology of the major clades of desmids (Desmidiaceae) established in NJ(ML) analyses of *rbc*L sequence data (127 strains, GONTCHAROV & MELKONIAN, in press) with mapped features of cell and chloroplast morphology traditionally used to define genera. The data matrix was drawn up from the strains used to infer phylogeny.

a strongly supported generic clade (Gontcharov & Melkonian 2005, see also Moon et al. 2005, Moon & Lee 2007). A deep divergence between most of the clades accommodating *Cosmarium* and *Staurodesmus* species leave little chance that they may eventually form the respective generic assemblages.

Artificial nature of many morphologically heterogeneous and species rich desmid genera was evident for a long time and discussed in the literature (Palamar-Mordvintseva 1976a, b, Brook 1981, Prescott et al. 1982, Croasdale et al. 1994, Gerrath 1993, 2003). The generic concept in the saccoderm desmids (Mesotaeniaceae) was much less debated, however, largely due to the fact that this group was very poorly studied. Not surprisingly, all three major mesotaeniacean genera, *Netrium*, *Cylindrocystic* and *Mesotaenium*, were also resolved as polyphyletic, each split into three or more lineages (Gontcharov et al. 2004). In case of *Netrium*, distant relationships between its species could be explained by differences in their morphological traits. Each lineage is distinct in the number of chloroplasts per cell (1, 2, and 4, Fig. 3), differing positions of the nucleus in the cell, and varying nuclear behaviour during cytokinesis (Jarman & Pickett-Heaps 1990, Pickett-Heaps 1975). What features differentiate *Cylindrocystic* and *Mesotaenium* branches remains unknown (Fig. 6). As it has been noted above, phylogenetic structure of the order Zygnematales and its clades remains tentative mostly due to the limited taxon sampling and requires further scrutiny. Only a when it will be firmly established comparative analyses of phenotypic traits within and between the clades could shed a light on this issue.

Thus, molecular data suggest that the generic structure of the class Zygnematophyceae should be significantly altered towards recognition of the new monophyletic lineages, mostly in the family Desmidiaceae and in saccoderm desmids (traditional Mesotaeniaceae), as genera. In some cases existing genera should be abandoned because they do not represent independent lineages but are embedded into their "mother" genera (e.g. *Genicularia* is a member of *Gonatozygon* clade, *Sirogonium* is a part of *Spirogyra*, *Spinoclosterium* is a part of *Closterium* (Gontcharov et al. 2004, 2004, Hall et al. 2008, see however Drummond et al. 2005). We anticipate, however, that most existing zygnematophycean genera will be retained but it is very likely that their species number will be altered, mostly reduced.

A great obstacle to this scenario is a process of taxonomic circumscription of the new genera. In order to be validly described a taxon should be provided with a diagnosis that states the characters distinguishing the taxon from other taxa (Art. 32, ICBN). In most plant groups, and conjugating green algae are not exceptional in this respect, these characters relate to the morphology, rarely to biological properties or fine structures of the organisms. However, in the Zygnematophyceae the results of phylogenetic analyses strongly suggest the inadequacy of the morphological features traditionally used to distinguish genera. Mapping of semicell shape, degree of radiation, cell-wall features (smooth vs. ornamented), and chloroplast morphology on the phylogenetic tree showed that each of these morphological characters has a mosaic distribution in the tree and does not characterise a specific clade (Gontcharov & Melkonian, in press, Fig. 7). It is obvious that these characters evolved several times in the class and do not reflect common origin of the species they occur in.

Morphological heterogeneity of most novel clades and lack of information on other phenotypical features currently do not allow conclusions to be drawn on the synapomorphies that may unite their members or, more importantly, that differentiate the clades (potential genera). Hopefully, the phylogenetic structuring of the class Zygnematophyceae, yet preliminary, will stimulate comparative studies on the conjugates morphology, biology and ultrastructure that will eventually discover characters suitable for purposes of genera and higher taxa diagnosing.

Conclusion

Our understanding of phylogenetic relationships in the class Zygnematophyceae is still incomplete after almost 20 years of studies with molecular tools. Further investigations are still needed to clarify monophyly of the class, structure of the order Zygnematales, some families and most genera. Molecular data have highlighted the inadequacy of morphological features such as habit, chloroplast morphology, cell shape, degree of radiation and ornamentation of the cell wall, traditionally used to discriminate zygnematophycean taxa and revealed numerous cases of their homoplasy. Many clades established in the phylogenetic analyses deserve to be recognized as new genera but cannot be formally described in the framework of the current morphology-based taxonomic concept either because their phenotypical features are insufficiently known or not different from those in other clades and genera. Although many strains of the conjugates are available in culture collections, this still represents only a minute fraction of the taxa described and the taxon sampling limits further progress in constructing robust phylogeny of the class Zygnematophyceae. Many more strains, refined analyses of morphological traits and additional molecular markers are needed before we will fully understand the complex morphology evolution and diversification of the conjugates and produce a classification system that adequately reflects relationships in the group.

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Appendix. Genera of the Zygnematophyceae, their type species and estimated number of species (combined with changes from Gerrath 1993, Hoshaw & McCourt 1988).

Genus	Type species	Species
Family Zygnemataceae		
Debarya (Wittr.) Transeau 1934	*Debarya glyptosperma* (De Bary) Wittr.	10
Hallasia Rosenv. 1924	*H. reticulata* (Hallas) Rosenv.	1
Mougeotia Agardh	*M. genuflexa* (Dillwyn) Agardh	140
Mougeotiopsis Palla 1894	*M. calospora* Palla	1
Pleurodiscus Lagerh. 1895	?*P. borinquinae* Tiffany	1
Temnogametum W. et G.S. West 1897	*T. heterosporum* W. et G.S. West	16
Sirocladium Randhawa 1941	*S. kumaoense* Randhawa	5
Spirogyra Link 1820	*S. porticalis* (O.F. Müller) Cleve	>400
Zygnema Agardh 1817	*Z. cruciatum* (Vaucher) Agardh	139
Zygnemopsis (Skuja) Transeau 1934	*Z. desmidoides* (W. et G. S. West) Transeau	43
Zygogonium Kütz. 1843	*Z. ericetorum* Kütz.	29
Family Mesotaeniaceae		
Ancylonema Berggren 1871	*A. nordenskioeldii* Berggern	1
Cylindrocystis Menegh. ex De Bary 1858	*C. brebissonii* (Menegh. ex Ralfs) De Bary	12
Geniculus Prescott 1967	*G. gatunensis* Prescott	1
Mesotaenium Nägeli 1849	*M. endlicherianum* Nägeli	12
Netrium (Nägeli) Itzigs. et Rothe in Rabenh. 1856	*N. digitus* (Nägeli) Itzigs. et Rothe in Rabenh.	10
Roya W. et G.S. West 1896	*R. obtusa* (Bréb.) W. et G. S. West	4
Spirotaenia Bréb. ex Ralfs 1848	*S. condensata* Bréb. ex Ralfs	23
Family Gonatozygaceae		
Gonatozygon De Bary 1856	*G. monotaenium* De Bary	11
Family Closteriaceae		
Closterium Nitzsch ex Ralfs 1848	*C. lunula* Nitzsch ex Ralfs	190
Spinoclosterium Bernard 1909	S. *curvatum* Bernard	2
Family Peniaceae		
Penium Bréb. ex Ralfs 1848	*P. margaritaceum* Bréb. ex Ralfs	16
Family Desmidiaceae		
Actinotaenium (Nägeli) Teiling 1954	*A. curtum* (Bréb. ex Ralfs) Teiling ex Růžička et Pouzar	50
Allorgeia Gauth.-Lièvre 1958	*A. valiae* Gauth.-Lièvre	2
Amscottia Grönblad 1954	*A. mira* (Grönblad) Grönblad	2
Bambusina Kütz. ex Kütz. 1849	*B. borreri* Delponte	6
Bourrellyodesmus Compére 1976	*B. heimii* Compére	5
Brachytheca Gontcharov et Watanabe 1999	*B. sulcata* Gontcharov et Watanabe	2
Cosmarium Corda ex Ralfs 1848	*C. undulatum* Corda ex Ralfs	>1100
Cosmocladium Bréb. 1856	*C. pulchellum* Bréb.	6
Desmidium Agardh ex Ralfs 1848	*D. swartzii* Agardh ex Ralfs	20
Docidium Bréb. ex Ralfs 1848	*D. baculum* Bréb. ex Ralfs	8
Euastrum Ehrenb. ex Ralfs 1848	? *E. ansatum* Ehrenb. ex Ralfs	265
Groenbladia Teiling 1952	G. *neglecta* (Racib.) Teiling	5
Haplotaenium Bando 1988	*H. minutum* (Ralfs) Bando	3
Heimansia Coesel 1993	*H. pusilla* (L. Hilse) Coesel	1
Hyalotheca Ehrenb. ex Ralfs 1848	*H. mucosa* (Mert.) Ehrenb. ex Ralfs	6
Ichthyocercus W. et G. S. West 1897	*I. anglensis* W. et G. S. West	6
Ichthyodontium Scott et Prescott 1956	*I. sachlanii* Scott et Prescott	1
Micrasterias Agard ex Ralfs 1848	*M. furcata* Agard ex Ralfs	75
Oocardium Nägeli 1849	*O. stratum* Nägeli	1
Phymatodocis Nordst. 1877	*P. alternanans* Nordst.	3-4
Pleurotaenium Nägeli 1849	*P. trabecula* (Ehrenb.) Nägeli	50
Prescotiella Bicudo 1976	*P. sudanensis* Bicudo	1
Sphaerozosma Corda ex Ralfs 1848	*S. vertebrtum* Bréb. ex Ralfs	13
Spinocosmarium Prescott et Scott 1942	*S. quadridens* (Wood) Prescott et Scott	2
Spondylosium Bréb. ex Kütz. 1849	S. *depressum* Bréb. ex Kütz.	34
Staurastrum Meyen ex Ralfs 1848	? *S. gracile* Ralfs	800
Staurodesmus Teiling 1948	*S. triangularis* Teiling	100

Appendix Cont.

Streptonema WALL. 1860	*S. trilobatum* WALL.	2
Teilingia BOURR. 1954	*T. excavata* (RALFS) BOURR.	7
Tetmemorus RALFS 1848	*T. granulatus* BRÉB. ex RALFS	6
Triplastrum IYENGAR et RAMANATHAN 1942	*T. abbreviatum* (W. B. TURNER) IYENGAR et RAMANATHAN	3
Triploceras (BAILEY ex RALFS) BAILEY 1851	*T. verticillatum* (BAILEY ex RALFS) BAILEY	3
Xanthidium EHRENB. ex RALFS 1848	*X. aculeatum* EHRENB. ex RALFS	115

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