Zygnematophyta

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John D. Hall and Richard M. McCourt

Abstract

The Zygnematophyta are among the most diverse green algae, with a variety of thallus types (filaments, unicells, colonies), cell wall structure (one to several layers, with varying degrees of ornamentation), and approximately 4,000 described species. The group lacks flagella at all stages of the life cycle. Several types of asexual spores are produced. Sexual reproduction, when present, involves conjugation or the union of two haploid vegetative protoplasts (individual cells of filaments or unicells) to form a zygospore, which undergoes meiosis to produce a new haploid thallus upon germination. Almost exclusively freshwater, these algae are common in ponds, lakes, and streams, in surface mats, or as phytoplankton or benthic growths. Many, but not all, are found in oligotrophic to mesotrophic waters of moderate to low pH, although the diversity of habitats occupied spans a wide range and may be quite specific for individual species. The fossil record extends at least to the Carboniferous. Recent analyses have placed this group as the sister taxon to land plants, despite the dramatic differences in morphology, life cycles, and reproduction. The group includes the well-known *Spirogyra* and numerous beautiful unicellular forms known as desmids, many of which have elaborate external ornamentations (e.g., spines, granules, large lobes). The conjugating green algae are important as ecological indicator species and for the ecological services they provide.

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Introduction

The Zygnematophyta, here considered a phylum, is equivalent to the class Zygnematophyceae in the botanical literature. The group comprises those freshwater green algae with two unique characteristics: sexual reproduction by conjugation and absence of flagellate (mastigote) cells in the life cycle. The phylum contains some of the most beautiful microscopic organisms known (Fig. 1). The elegance of their microscopic cellular architecture is obscured by their macroscopic appearance as floating pond scums, green planktonic masses, and slimy films on the surfaces of plants and other substrates.

The Zygnematophyta is usually divided into two groups (considered two orders of the class Zygnematophyceae): Desmidiales and Zygnematales (Table 1). The order Zygnematales traditionally included the families Zygnemataceae and Meso-taeniaceae (Mix 1972). The family Zygnemataceae (14 genera, over 800 species) included filamentous algae with cell walls that lack a median incision or pores. The
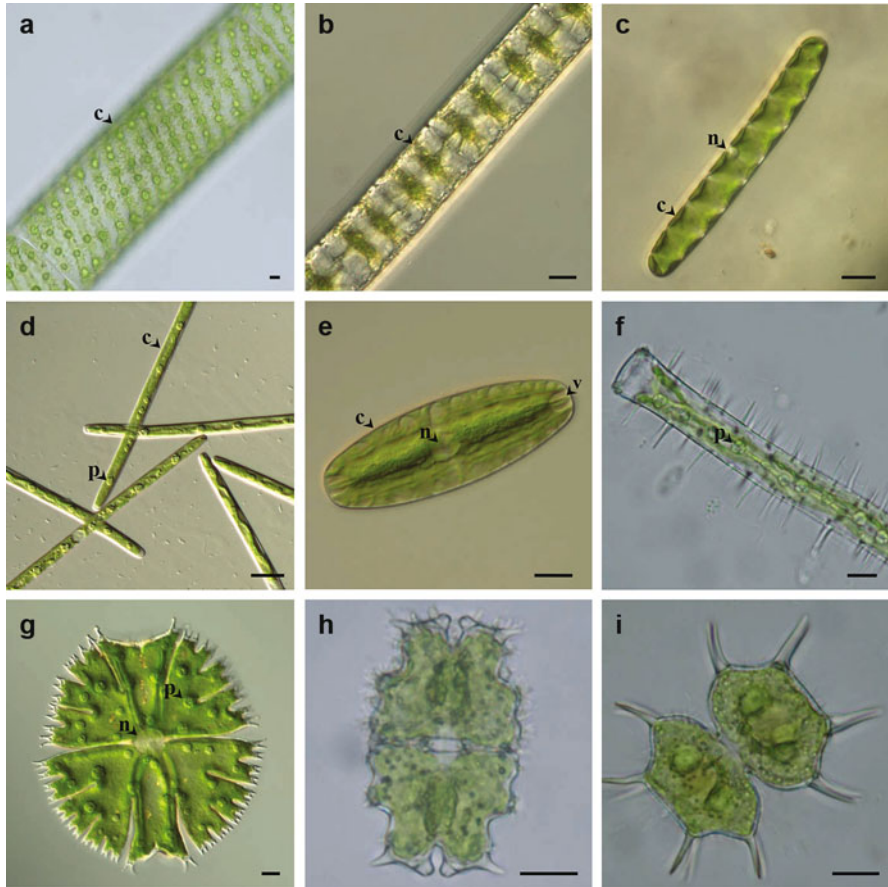
Fig. 1 Structural diversity in the Zygnematophyta. (**a**) *Spirogyra* sp.; (**b**) *Zygnema* sp.; (**c**) *Spirotaenia condensata*; (**d**) *Roya obtusa* var. *montana*; (**e**) *Netrium digitus*; (**f**) *Gonatozygon aculeatum*; (**g**) *Micrasterias rotata*; (**h**) *Euastrum evolutum* var. *glaziovii*; (**i**) *Xanthidium cristatum var. hipparquii*. Structures: *c* chloroplast, *n* nuclear region at site of isthmus between semicells, *p* pyrenoid, *v* apical vacuole. Scale bar = 10 μm in each micrograph

family Mesotaeniaceae contained the saccoderm desmids and was the smallest family (8 genera, approximately 100 species). Molecular phylogenetic studies indicate that the families of the Zygnematales are not monophyletic (McCourt et al. 2000; Gontcharov et al. 2003; Hall et al. 2008a). Zygnematales are generally oblong, rod shaped, or cylindrical, and the smooth cell wall lacks pores; the primary wall is a homogeneous piece, lacking a median constriction. The Desmidiales (41 genera, 3,500+ species) contains the placoderm desmids, which are divided into four families, the Closteriaceae, Gonatozygaceae, Peniaceae, and Desmidiaceae, the latter being the largest of the four families (36 genera, 3,000 species, 12,000 subspecific taxa) (Gerrath 1993; Hall and Delwiche 2007). Most placoderm desmids

Table 1 Classification of Zygnematophyta

Zygnematophyceae	Desmidiales	**Closteriaceae**	*Closterium*	*Spinoclosterium*	
		Desmidiaceae	*Actinotaenium*	*Allorgeia*	*Amscottia*
			Bambusina	*Brachytheca*	*Cosmarium*
			Cosmocladium	*Desmidium*	*Docidium*
			Euastridium	*Euastrum*	*Groenbladia*
			Haplotaenium	*Heimansia*	*Hyalotheca*
			Ichthyocercus	*Ichthyodontum*	*Mateola*
			Micrasterias	*Octacanthium*	*Onychonema*
			Oocardium	*Phymatodocis*	*Pleurotaenium*
			Prescottiella	*Sphaerozosma*	*Spinocosmarium*
			Spondylosium	*Staurastrum*[a]	*Staurodesmus*
			Streptonema	*Teilingia*	*Tetmemorus*
			Triplastrum	*Triploceras*	*Vincularia*
			Xanthidium		
		Gonatozygaceae	*Genicularia*	*Gonatozygon*	
		Peniaceae	*Penium*		
	Zygnematales	**Mesotaeniaceae**	*Ancylonema*	*Cylindrocystis*	*Geniculus*
			Mesotaenium	*Netrium*	*Nucleotaenium*
			Planotaenium	*Roya*	*Spirotaenia*
			Tortitaenia		
		Zygnemataceae	*Hallasia*	*Mougeotia*	*Mougeotiopsis*
			Pleurodiscus	*Sangirellum*[c]	*Sirocladium*
			Sirogonium	*Spirogyra*	*Temnogametum*
			Transeauina (Debarya)	*Trigonum*[c]	*Zygnema*
			Zygnemopsis	*Zygogonium*	

[a]*Staurastrum* in the broad sense, including segregate genera recognized by Palamar-Mordvintseva (2003, 2005)
[b]If Yamagishi's (1963) revision were accepted, we would add two genera: *Mougeotiella* and *Neozygnema*
[c]Dubious genera reported only once from India

are unicellular, but filamentous and colonial species are known. Placoderm cell walls have pores and may be intricately ornamented. Each cell consists of two mirror-image parts called semicells that are joined at a narrow midregion or isthmus where the nucleus is located (Fig. 1g). Chloroplasts and other nonnuclear cell contents are divided equally between semicells. The structure of semicells is often complex, with two, three, or more planes of symmetry. The number of lobes on a semicell on end view determines its degree of radiation, e.g., biradiate semicells have two corners, triradiate have three, and so on.

Because of their intriguing structure and reproduction, the zygnematophytes have been extensively studied since the mid-nineteenth century. Research continues in many areas of zygnematophycean biology. In this review, we focus on seminal works and literature published in the last 30 years. Investigators tend to

specialize on one of the three traditional families, a fact reflected in treatments in monographs and books. Reviews and monographs on filamentous Zygnematales (Zygnemataceae) include Transeau (1951), Randhawa (1959), Hoshaw (1968), Kadlubowska (1972, 1984), and Rundina (1998). Major references on unicellular Zygnematales (Mesotaeniaceae) and Desmidiales are *A Synopsis of North American Desmids* (six volumes, Prescott et al. 1972, 1975, 1977, 1981, 1982; Croasdale et al. 1983), Růžička (1977, 1981), Brook (1981), Förster (1982), Croasdale and Flint (1986, 1988), Croasdale et al. (1994), Palamar-Mordvintseva (2003, 2005), Coesel and Meesters (2007), and Brook and Williamson (2010).

Members of the Zygnematophyta have not been exploited for economic use in any major way. A few species have been used in fish aquaculture, and natural populations are abundant enough that one can infer an important role in natural food webs. Some studies suggest that green algae in general and *Spirogyra* in particular may be useful for the detection and recovery of certain metals from contaminated waterways (Gupta et al. 2001; Singh et al. 2007; Rai et al. 2008). Members of the conjugating green algae, including *Spirogyra*, *Mougeotia*, and the Desmidiales, have been used as indicators of trophic status and water quality (Jarnefelt 1952; Rawson 1956; Brook 1965; Coesel 2001).

Biogeography, Habitats, and Ecology

Zygnematophytes are restricted almost entirely to freshwater, although a few species have been collected from brackish waters. Many conjugating green algae are minute, have large geographic ranges, and are able to survive in many marginal habitats; however, biogeographic patterns do exist. Krieger (1937) proposed that many desmids were part of geographic groups and recognized ten continental-scale geographic areas with distinct desmid floras. Coesel (1996) considered this hypothesis in light of modern distributional data and concluded that there are regional floras, although the Arctic/Alpine flora seems to be mostly determined by a minimum average temperature. The apparent existence of regional desmid floras may be the product of insufficient sampling in some regions. However, many conspicuous species characteristics of a particular area are known only from less-studied regions (such as tropical Asia, Africa, and Australia) and, presumably, would have been noticed in areas with a longer history of investigation (such as Europe and North America) if those species existed there (Tyler 1996). Hundreds of local, regional, and national floras documenting the distribution of zygnematophytes have been published. A search of online databases will reveal many of the most recently contributed books and papers. Older floras are referenced in taxonomic treatments and reviews (e.g., Kadlubowska 1984; Prescott 1984).

Zygnematophytes occur in a wide variety of habitats, ephemeral pools, ponds, lakes, streams, rivers, marshes, and bogs, and on every continent. A considerable number of artificial habitats have been colonized by zygnematophycean species. In fact, the widespread occurrence of reservoirs, cattle tanks, roadside ditches,

irrigation canals, and other water impoundments has probably had a significant but unknown effect on the distribution of many species. Within a given habitat, species often show preference for microhabitats. Planktonic species occupy the water column, either permanently as euplankton or temporarily as tychoplankton after being dislodged from the substrate. Relatively few desmid species are truly plank-tonic. Most conjugating green algae are benthic or periphytic and grow on surfaces or occasionally attached to substrates by means of rhizoids or mucilage. Rhizoids that attach to substrate may be present in all of the filamentous Zygnematales (e.g., *Mougeotia*, *Spirogyra*, and *Zygnema*). Epibiotic species grow on the surfaces of the submerged leaves and stems of plants, especially on plants with highly dissected leaves such as *Utricularia*. Epibionts and species that live in water near plants make up the periphyton.

Some zygnematophytes thrive in habitats subject to extreme physical conditions. For example, some species of saccoderm desmid genera *Ancylonema*, *Cylindrocystis*, and *Mesotaenium* occur on snow and ice. *Cylindrocystis* has also been found in desert crust communities (Lewis and Lewis 2005). Placoderm desmids can be found in Alaskan tundra pools as well as the Antarctic Peninsula and some sub-Antarctic islands, in which freezing temperatures are tolerated for long periods. The filamentous *Zygogonium* is found in very acid pools and rivers (pH < 3) (e.g., Zettler et al. 2002).

Although zygnematophytes occur in a wide variety of habitats, species show distinct preferences for certain habitats characterized by water chemistry and pro-ductivity. A generalization often made is that desmids prefer slightly acidic waters (pH 4–7), such as pools in acid peat bogs. Brook (1981) pointed out that, although this generalization is true, a number of species are common in alkaline waters. Acidic habitats support desmid species with the greatest ornamentation and mor-phological complexity. Generalizations about the distribution of the Zygnemataceae are difficult to make because of the paucity of comparable field measurements. A series of more than 250 collections of *Spirogyra, Zygnema*, and *Mougeotia* from sites across the USA in spring 1982 showed that the temperature and pH of the sites were remarkably uniform at the time of collection (ca. 20 °C and pH 6, in spring season), but this may have been a correlation, rather than a cause (McCourt et al. 1986).

Considerable attention has been devoted to the chemistry and productivity of desmid habitats (Brook 1981). Whereas most placoderm desmids, especially plank-tonic species, are characterized as oligotrophic (occurring in water of low produc-tivity, low pH, high levels of free CO_2, and low levels of bicarbonate), some species commonly occur in eutrophic waters (high productivity, high pH, low levels of free CO_2, and high levels of bicarbonate). Oligotrophic species requiring high levels of free CO_2 for photosynthesis may be outcompeted in eutrophic lakes by eutrophic species that are able to use bicarbonate directly for photosynthesis (Brook 1981). Desmid assemblages have not been definitively linked to chemical properties of water bodies. Factors controlling local distribution of conjugating green algae remain largely speculative.

Nonetheless, the occurrence of distinct assemblages of desmids in particular water types has led to the effective use of assemblages as general indicators of trophic status and water quality (Brook 1981; Coesel 2001, 2003). Researchers have classified desmid communities into associations, i.e., assemblages, characterized by predictable combinations of species. Heimans (1969) noted that, although it is useful to categorize species as oligotrophic or eutrophic, more than one factor such as pH or eutrophy is involved, and a species' response to these multiple factors may change from one region to another. Coesel (1982) studied desmid assemblages in the Netherlands. He used principal components analysis to categorize over 200 species in quaking fen and lowland marsh habitats into ecologically relevant clusters of species. Cluster composition was affected by pH, successional stage of the *Sphagnum* mat in the fen habitat, oligotrophic or eutrophic nature of water, and rapidity with which these factors changed in water-filled depressions in the *Sphagnum* mat.

Coesel (2001) proposed an index for the use of desmids as biological indicators of conservation value of sites in the Netherlands. This index (scaled from 0 to 10) takes into consideration more information about the species than presence or absence. Species richness, the presence of rare taxa, and the presence of taxa indicative of habitat maturity are included in the calculation of the conservation value of a particular habitat. So sensitive are the desmids to environmental parameters that the desmid flora will sometimes change before there are noticeable differences in the macroflora of a degraded habitat (Coesel 2003).

Little is known about the ecology and habitat preferences of filamentous Zygnematales. For a brief review of published work, see Hoshaw (1968). Filamentous Zygnematales are widely distributed but less frequently reported than other green algae because they often occur in ephemeral pools and because species identification relies on characteristics of the infrequently encountered zygospores. Pessoney (1968), in an unpublished dissertation, described reproduction of *Spirogyra*, *Zygnema*, and *Mougeotia* in temporary, semipermanent, and permanent ponds near Austin, Texas, USA. Conjugation occurred mostly in spring and was more frequent in temporary ponds that were flooded and dried in 2 weeks than in more permanent bodies of water. *Spirogyra* species tended to dominate the pools and showed the highest incidence of conjugation of the three genera.

Species of zygnematophytes are often widely distributed. Individual cells, filaments, or zygospores may be dispersed by currents, wind, insects, water birds, or other agents. Vegetative cells and spores may be carried in the gut of water birds or in mud attached to their feet. Several types of desiccation-resistant spores and cells are produced by zygnematophytes (zygospores, parthenospores, and akinetes). Rewetting of dried mud samples as much as 10 or 20 years old is a common means of starting or recovering cultures, which suggests that spore banks may persist in natural habitats over long periods of time.

Little is known of the role of Zygnematophyta in freshwater ecosystems outside of their role as primary producers. The extent and variety of the animals that graze on them are poorly known. Available data suggest that desmids are an important food source for zooplankton and possibly benthic microinvertebrates (Coesel 1997). It has

been demonstrated that grazing induces physical changes in some species of the genus *Staurastrum* (Wiltshire et al. 2003), which implies a complex relationship between these algae and the herbivores that feed on them.

Conjugating green algae are also subject to parasitism. Chytrids, other fungi, and oomycetes are known to parasitize desmids and filamentous Zygnematales and may have significant effects on algal populations (Kadlubowska 1999; see Kagami et al. 2007 for a review on chytrids). Filamentous Zygnematales are also among the preferred hosts of the parasitic dinoflagellate *Cystodinedria* (Carty 2003).

Zygnematophytes are not immune to the adverse impact of human activities. Hoshaw (1968) remarked on the destruction of habitats of the Zygnemataceae. A number of studies have documented long-term declines in natural desmid populations due to pollution and subsequent eutrophication, development of forests that shade out macrophytes that harbor periphyton, and decreased pH of waters subject to acid rain (e.g., Coesel et al. 1978; Van Dam and Buskens 1993).

Characterization and Recognition

General Features

Unicells and unbranched filaments are the most common forms in the Zygnematophyta, but colonial forms are also known. A single nucleus is usually located in the center of the cell. From one to several axile or sometimes parietal chloroplasts occur in a single cell. Chloroplast shapes range from asteroid (*Cylindrocystis* and *Zygnema*, Fig. 1b) to laminate (*Gonatozygon*, *Mesotaenium*, *Mougeotia*, and *Roya*, Fig. 1d, f) to ribbon-like (*Spirogyra* and *Spirotaenia*, Fig. 1a, c). An axile, ridged chloroplast (stellate) is found in many desmids including *Netrium* (Fig. 1e), *Closterium*, and *Penium*. Species of Desmidiaceae contain some of the largest and most elaborate chloroplasts known among the green algae. Their chloroplasts are often ridged, lobed, and highly dissected. Chloroplasts of most species contain thylakoids stacked as in plants; other species lack grana-like structures or have only rudimentary grana. Pigments include those typical of green algae and embryophytes, i.e., the descendants of a common ancestor that includes all green algae and embryophytic plants: chlorophylls *a* and *b*, β-and γ-carotenes, and several xanthophylls including loroxanthin (Donohue and Fawley 1995). Chloroplasts usually contain one or more pyrenoids (Fig. 1d, g) around which starch is stored.

Cell walls of the Zygnematophyta consist of at least three layers: an outer layer of variable composition depending on species (mucus or an amorphous pectic substance), a primary wall composed of irregularly arranged microfibrils beneath the outer layer, and an innermost layer composed of ribbon-like bands of cellulose microfibrils (Fig. 2). Many placoderm desmids shed the primary wall and retain the secondary wall as the outer covering. Ornamentations such as granules, spines, and verrucae may occur in the outer layer only or in the primary and secondary walls.
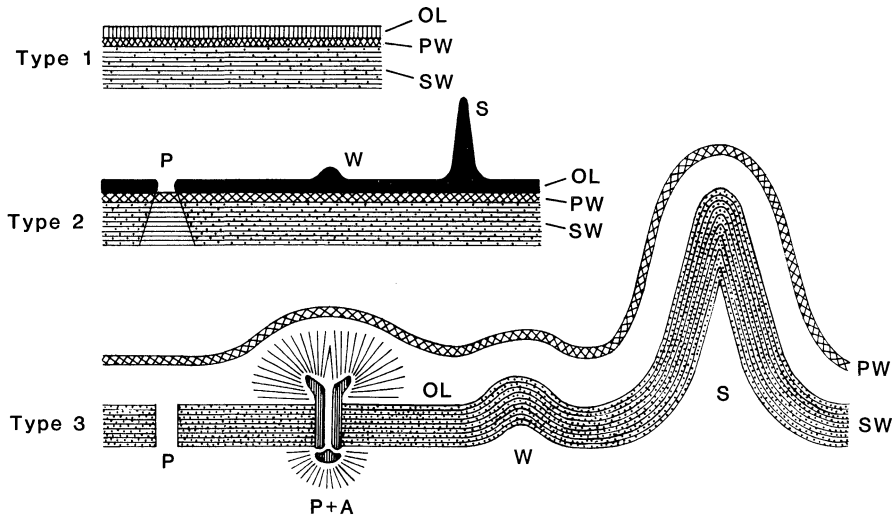
Fig. 2 Cell wall structure of the Conjugaphyta. Wall types *1*, *2*, and *3* are described in Table *2*. Structures: *OL* outer layer, *PW* primary wall, *SW* secondary wall, *P* pore, *P+A* pore apparatus, *S* spine, *W* wart (After Mix 1975)

Cell wall pores are of fundamental importance in classification, although their function is not clear. Pores may be absent (Zygnemataceae, Mesotaeniaceae) or present (Desmidiales). If present, pores may penetrate only the outer layer (Closteriaceae, Gonatozygaceae, Peniaceae) or extend completely through the secondary wall (Desmidiaceae). Pores through the secondary wall in some desmids may be differentiated into a complex pore apparatus, with a lined pore channel and a "spider web" of fibrous material at the inner opening (Neuhaus and Kiermayer 1981). Extrusions of fibrous mucilage from the external openings of the pores are visible under the light microscope.

Classification and Taxonomy

Work on mitosis, cytokinesis, and kinetid (flagellar apparatus) ultrastructure in the green algae resulted in major restructuring of systematics of the green algae at the class level and reinterpretation of their presumed relationship to ancestors of land plants. Mattox and Stewart (1984) proposed the establishment of five classes in the Chlorophyta. They placed the Zygnematales (= Zygnematophyceae) in the class Charophyceae (= Charophyta) on the basis of the presence of a primitive phragmoplast in *Spirogyra*, types of enzymes for glycolate and urea metabolism known for a few genera, and the similarities of the group to *Klebsormidium*, an obviously charophycean genus (Pickett-Heaps 1975). We follow the classification of Lewis and McCourt (2004) for the class-level relationships and the classification of Mix (1972), with some modification, at the family level. The Zygnematophyta are one of several

green algal groups that are monophyletic with embryophytes and one of the major lineages of the Chlorobionta, which itself is one of the main lineages of the Archaeplastida (Adl et al. 2012; McCourt 2016). Although recent studies have changed our view of the green algal sister taxon to embryophytes (see discussion below), the working classification proposed by Lewis and McCourt (2004) is still a reasonable one.

The Zygnematophyta are part of a lineage of green algae, known as Charophyta (Karol et al. 2001), equivalent to Streptophyta of other authors (e.g., Wickett et al. 2014), that contains some green algae plus all land plants (McCourt et al. 2004; Leliaert et al. 2012). Early molecular studies with broad taxon sampling and several genes showed the conjugating green algae to be more distantly related to land plants than either the Charophyceae or Coleochaetophyceae (Karol et al. 2001). However, later work with many genes, albeit with fewer taxa, supported zygnematophytes as the sister group (Turmel et al. 2006, 2007). Most recently, the latter hypothesis has garnered strong support from several recent phylogenomic analyses so that the strongly supported consensus is that a filamentous zygnematophyte-like ancestor gave rise to the Zygnematophyta and its sister taxon, all embryophytes (Wickett et al. 2014; Ruhfel et al. 2014). The implications of the latter relationship are profound in regard to the origin of a land flora and the likely morphological and physiological traits of early land-colonizing green algae (Delwiche and Cooper 2015; Davis et al. 2014; deVries et al. 2016). Taxon sampling in larger phylogenomic analyses has been, however, limited (only two zygnematophytes are included in Wickett et al. 2014). Delwiche and Cooper (2015) noted this problem of taxon sampling and suggested that further studies might provide alternative topologies. Davis et al. (2014) also commented that "In some cases, however, high support for relationships should be interpreted cautiously because conflicting topologies are supported by other data. Key examples include the previously mentioned sister groups of land plants . . ." Thus, although the consensus currently is that the zygnematophytes are the sister group to land plants, the issue is not quite a settled question.

Within the zygnematophytes, relationships among the numerous genera are becoming clear, but traditional taxonomy has not been supported by molecular work (Gontcharov et al. 2003, 2004; Hall et al. 2008a). For example, it is not clear if the Zygnematales is a monophyletic group and there is some evidence that the Desmidiales may include *Netrium* and *Roya*, genera not previously recognized as part of this lineage (Gontcharov et al. 2003, 2004; Hall et al. 2008a). There is some evidence that species of the genus *Spirotaenia* may not belong to the conjugating green algae (Gontcharov and Melkonian 2004). Although phylogenetic position of this genus is uncertain, it shares many of the characteristics of other zygnematophytes including reproduction by conjugation and will be treated as a member of the group.

Classification within the conjugating green algae is somewhat unsettled, and structural synapomorphies have not been found for many of the lineages discovered in molecular phylogenetic studies (e.g., Gontcharov and Melkonian 2008). There is some evidence that chloroplast shape may be an important phylogenetic

Table 2 Cell-wall characteristics of the Zygnematophyta

Zygnematales	Type 1. Mesotaeniaceae and Zygnemataceae	1. Cell wall one homogeneous piece
		2. Primary wall not shed
		3. Outer hyaline (mucus) layer smooth
		4. Ornamentations weak or absent
		5. Pores absent
Desmidiales	Type 2. Closteriaceae, Gonatozygaceae and Peniaceae	1. Cell wall may be formed of several segments, which are divided by very slight constrictions
		2. Primary wall not shed
		3. Compact, structured outer layer (warts, spines, and ridges originating from outer layer)
		4. Ornamentations strong and variable
		5. Pores or pore-like gaps only in outer layer
	Type 3. Desmidiaceae	1. Cell wall formed of two segments divided by a marked constriction (isthmus)
		2. Primary wall shed
		3. No continuous outer layer but mucilaginous envelope originating from pore organs from secondary wall
		4. Pores in secondary wall

After Brook (1981)

characteristic (McCourt et al. 1995; Hall et al. 2008a), but groups within the Zygnematales have not been formally circumscribed.

Before differences in cell wall characteristics were revealed, three families of conjugating green algae were recognized: Desmidiaceae, Mesotaeniaceae, and Zygnemataceae. The traditional Desmidiaceae is equivalent to the Desmidiales in this treatment, and species in this group are often referred to in general literature as the "placoderm desmids." Conversely, the Mesotaeniaceae in earlier literature were called the "saccoderm desmids." It is important to recognize that the saccoderm desmids and Zygnemataceae are not natural groups, but rather groupings based on growth habitat (saccoderms are unicellular and zygnemataceans are filamentous). The order Zygnematales as defined here includes those species formerly assigned to the families Zygnemataceae and Mesotaeniaceae. They share the trait of a simple cell wall, but this is likely a primitive trait that does not diagnose a monophyletic group. Nevertheless, the current classification is based primarily on characteristics of the cell walls. These characteristics are outlined in Table 2.

The taxonomy of the Zygnematophyta has involved the descriptions of numerous morphological species. Among the characters distinguishing species in this diverse group of algae are radiation, cell wall ornamentation, cell dimensions, chloroplast number and form, details of cell division and the conjugation process, as well as zygospore color and ornamentation. Intraspecific polymorphisms and aberrant forms are numerous, especially among placoderm desmids (Brook 1981).

The correlation between morphological species and biological species is not clear. Watanabe and Ichimura (1982), working with *Closterium*, have shown the existence

of several ecologically and sexually isolated mating groups within a single morphological species or species complex. This and other studies (Ichimura 1983) suggest that, although a species of *Closterium* may appear to be widely distributed, crossing experiments reveal that reproductively isolated mating groups are restricted to much smaller areas, and several biological species may be involved. In our own studies of the Gonatozygaceae, we have found that some morphological species constitute distinct phylogenetic lineages although reproductive isolation has not been demonstrated (Hall unpub.).

Although the *Closterium* studies imply the existence of more biological species than are presently described, research on polyploidy in desmids and *Spirogyra* has indicated that perhaps too many species have been described because of morphological variation within a polyploid species complex (polyploid derivatives from a single clone). Polyploidy has been reviewed by Nichols (1980). Placoderm desmids display changes in cell volume and degree of radiation associated with ploidy changes (Pickett-Heaps 1983). Clonal cultures of *Spirogyra* have been reported to develop filaments of three or more distinct widths that are the phenotypic expression of euploid increases in chromosome number (Allen 1958; Hoshaw et al. 1985, 1987). Variations in ploidy of *Spirogyra* and the morphological changes associated with them have undoubtedly led to excessive numbers of species descriptions (over 400 species). At the same time, it is likely that cryptic genetic variation may have led to underestimates of species diversity in other genera. The nature of diversity in species of these algae remains a fertile area of research.

Reproduction

A cardinal feature distinguishing the Zygnematophyta from other chlorophytes is sexual reproduction by conjugation involving the fusion of non-flagellate gametes. Asexual reproduction is by fragmentation, cell division, akinetes, and parthenospores. These types of reproduction have been observed either in natural populations or in laboratory cultures.

Conjugation was first observed nearly 200 years ago, but only during the twentieth century were the events adequately interpreted (Fritsch 1935; Randhawa 1959). Investigations of cultures have provided data on the events of entire sexual cycles. Observations have been made of stages from cell or filament pairing to zygospore germination.

Sexual cycles (Figs. 3 and 4) consist of conjugation (the physical joining of cells or filaments and subsequent union of gametes to form a zygote), formation of a thick-walled zygospore, a period of zygospore dormancy, and germination of the zygospore to produce vegetative cells. Sexual cycles of the conjugating algae are haplobiontic. They display zygotic meiosis; growing cells are haploid; and meiosis occurs in the zygote, the only diploid cell in the sexual cycle. Strains of species may be homothallic (conjugation is intraclonal) or heterothallic (conjugation is interclonal between plus and minus mating types). A great deal of work has focused on molecular mechanisms involved in mating-type determination in *Closterium* (Hirano et al. 2015; Sekimoto et al. 2014).
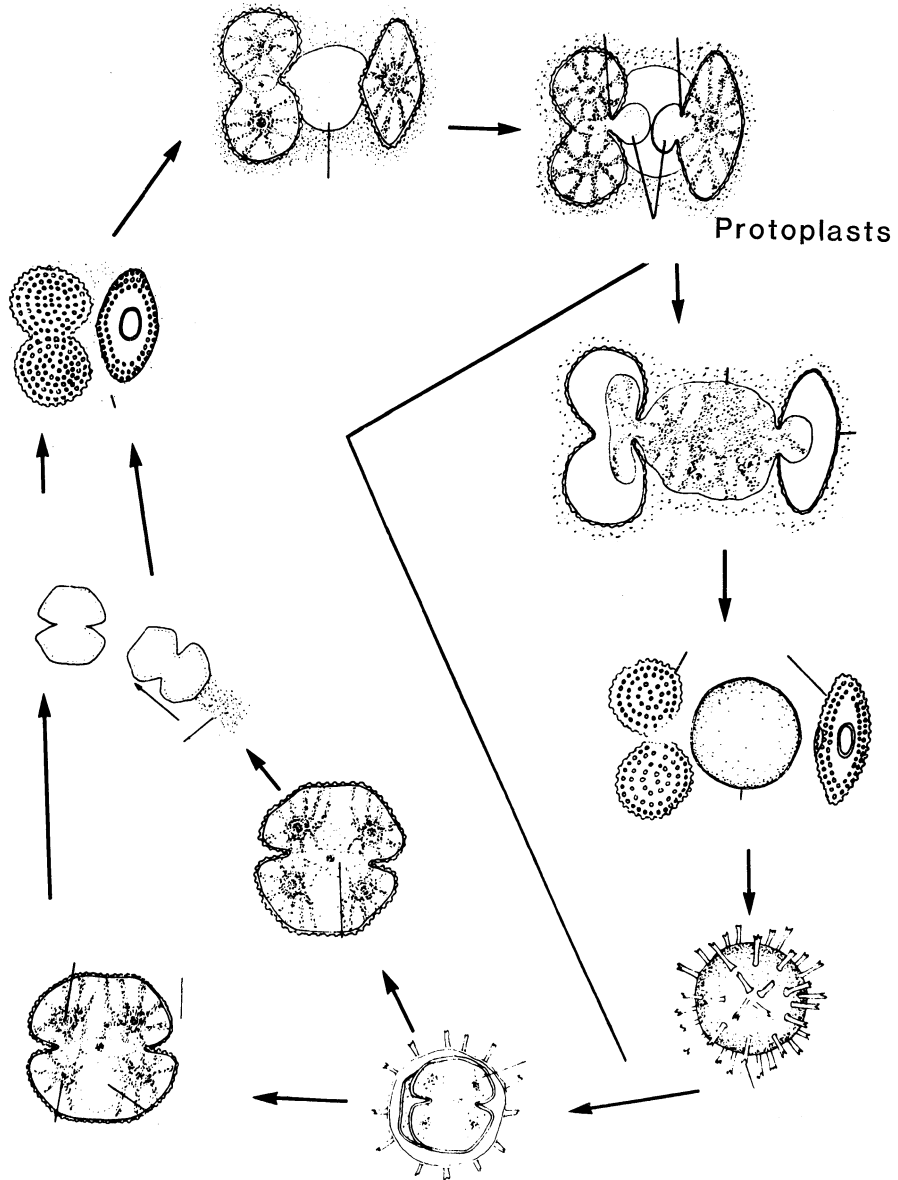
Fig. 3 *Cosmarium* life cycle (After Lee 1980)

Laboratory strains of placoderm and saccoderm desmids have been induced to conjugate by increasing carbon dioxide in the atmosphere surrounding cells (Starr 1964) and by suspending cells in a low-nitrogen medium (Biebel 1973). Brandham (1967) described the movement of *Cosmarium botrytis* cells to form
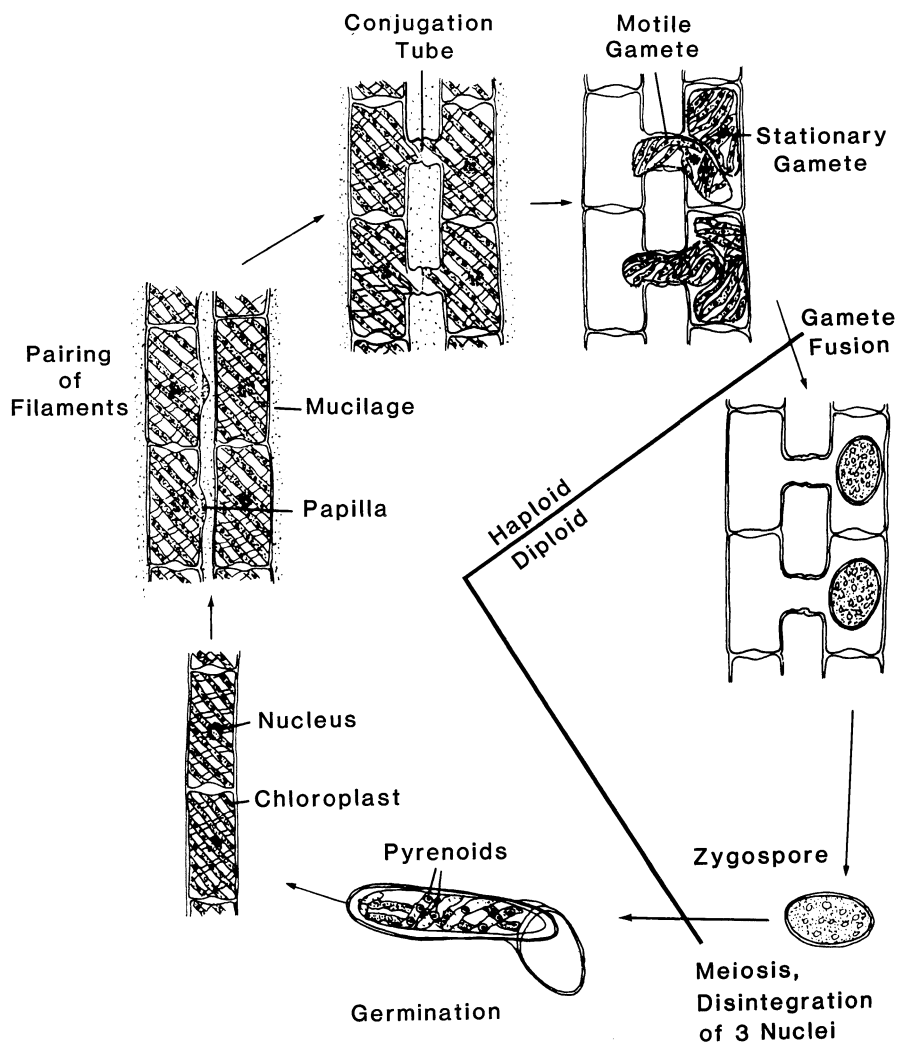
Fig. 4 *Spirogyra* life cycle (After Lee 1980)

pairs; this movement suggests the action of a sexual pheromone. Pheromones were long thought to be involved in initiating sexual reproduction. Hoshaw (1968) reported evidence that a sexual pheromone produced by one mating type of *Zygnema circumcarinatum* facilitated conjugation. A fragment of a gene encoding a putative pheromone thought to be involved in the induction of sexual reproduction has been identified in *Closterium* (Tsuchikane et al. 2003; Fukumoto et al. 2003).

It seems that optimal conditions for conjugation vary from species to species. Filamentous Zygnematales often conjugate when filaments are transferred to nutrient-poor conditions, such as the surface of a 1.2% tap water agar. We have found that freshly collected filaments spread on agar conjugate more readily than filaments from cultures. Scalariform conjugation involves the movement of filaments into a paired ladder-like arrangement after which outgrowths from adjacent cells (papillae) join to form a conjugation tube. In Zygnemataceae with lateral conjugation, a short curved tube joins adjacent cells in a single filament.

Many different mating systems are known among the conjugating green algae. Gametes of saccoderm and placoderm desmids usually leave parent cells and meet in a conjugation vesicle or tube. In conjugation of filamentous Zygnematales, cells of the filament function as gametangia and form isogametes or anisogametes (see glossary in Hoshaw 1968). One gamete may move to the opposite cell, or both gametes may move into and fuse in the conjugation tube. Physiological anisogamy occurs when one isogamete moves and the other isogamete remains stationary, as happens in most species of *Spirogyra* (Hoshaw 1968). Gamete directionality is not fixed. That is, filaments may contain some cells that act as a gamete donor and others that act as a gamete recipient.

The zygospore wall is composed of three major wall layers: exospore (outermost), mesospore (middle), and endospore (innermost). These layers may themselves be multilayered. DeVries et al. (1983) reported the presence of sporopollenin in the two mesospore layers of *Spirogyra hassallii* along with cellulose and/or pectin in exospore and endospore layers. Walls of zygospores are smooth or ornamented and may be black or shades of yellow, brown, or blue.

Meiosis in the placoderm desmid *Pleurotaenium* occurs in the zygospore immediately before germination (Blackburn and Tyler 1981). In contrast, meiosis in *Spirogyra crassa* occurs early in zygospore formation, even before the wall matures (Godward 1966). Zygospore germination involves the rupture of the exospore and mesospore with the emergence of one to four germlings (single cells or the first cell of a filament). The endospore becomes the cell wall of germlings, or cleavage of the single remaining protoplast from meiosis may occur within the endospore during the formation of two, three, or four germlings (Biebel 1973). Chloroplasts from only one gamete survive in germlings, even in homothallic isogamous species where the gametes are genetically identical. In all species of *Spirogyra* studied and some species of *Zygnema*, in which only one gamete moves, chloroplasts persisting in the zygospore and resulting progeny are those of the stationary gamete (Biebel 1976).

Conjugation has infrequently been observed in natural populations of placoderm and saccoderm desmids. In some species, asexual spores are known. Brook (1981) described two types of akinetes formed asexually by placoderm desmids. The protoplast either emerges from the cell to form a spore or secretes a thick wall inside the original cell wall. Akinetes form in many filamentous zygnematalean species by

the second method. A parthenospore is similar to an akinete but forms when gametangial cells form spores without gamete fusion. Less commonly observed are akinetes, thick-walled vegetative cells, which in *Zygnema* form most readily under conditions of high light (6,500 lux) and low nitrogen (Pessoney 1968).

Photomovement of Chloroplasts and Cells

Mougeotia and *Mesotaenium* are useful experimental organisms in the study of phytochrome-mediated movements of chloroplasts (Haupt 1982). The laminate chloroplast moves within the cell to display its maximum surface area or face toward low-intensity white light, whereas in high-intensity white light, the chloroplast aligns itself with the edge profile toward the light. Presumably these motions optimize photosynthetic performance and minimize damage to the photosynthetic apparatus. *Mougeotia* has advantageous features for such research, with its cells each containing a single large chloroplast that can be partially irradiated with microbeam illumination. Unlike chloroplast movements in other organisms, chloroplast orientation in *Mougeotia* proceeds to completion in darkness after exposure to brief light flashes, allowing the processes of light perception and response to be studied separately (Haupt 1982, 1983).

Phytochrome in the peripheral cytoplasm appears to be the photoreceptor pigment in both the low and high light-intensity responses, but the action spectra are very different. Low-intensity white light (or red light) striking the cell from one side transforms red-absorbing phytochrome (P_r) to the far-red-absorbing active form (P_{fr}) more effectively on front and rear cell surfaces than on the flanks. A higher concentration of P_{fr} builds up in the front and rear cortical cytoplasm, and the chloroplast edges move as if repelled by high P_{fr} levels so that the flat surface of the chloroplast turns toward the light. In high-intensity light, the same P_f-P_{fr} gradient is set up, but an additional blue-light photoreceptor in the cortical cytoplasm somehow acts as a switch in the reaction chain; the chloroplast edges, instead of being repelled by high P_{fr} levels, are attracted to them, whereby the edge profile of the chloroplast is displayed to the light. Actin microfilaments are believed to move the chloroplast edge touching the cortical cytoplasm, and calcium apparently plays a key role in the transduction chain. Many details of this process remain unknown, but progress has been made toward a greater understanding of phytochrome signaling in *Mougeotia* and *Mesotaenium* (see Wagner 2001, for details). In particular, full-length phytochrome-coding sequences have been cloned from *Mesotaenium* and *Mougeotia*. In addition, a new photoreceptor, neochrome (similar to the *Adiantum* phy3), has been identified from *Mougeotia scalaris* (Suetsugu et al. 2005). This molecule seems to be the product of the fusion of portions of phytochrome and phototropin genes.

Placoderm and saccoderm desmid cells have long been known to be capable of gliding or somersaulting movements. It has been shown that gliding movement is effected by the localized excretion of mucilage, usually from the apices (Oertel et al.

2004; Rogers-Domozich et al. 1993). Some filamentous Zygnematales, such as *Spirogyra*, are also capable of a gliding motility (Kim et al. 2005).

Cytomorphogenesis

Placoderm desmids, particularly members of the genus *Micrasterias*, have been valuable in research on cytomorphogenesis, the process of growth and differentiation of their elaborately structured cell walls following cell division. Several features make *Micrasterias* especially useful in this research (Kiermayer 1981; Meindl 1993). The cells are relatively large (200–300 μm in diameter), easily centrifuged to produce enucleate semicells, and easy to observe without staining. Experimental production of enucleate, polyploid, or aneuploid cells has shown effects of the nucleus and cytoplasm on differentiation.

Micrasterias cells are typically biradiate, each semicell comprising a polar lobe flanked by two wings (lateral lobes), which are incised and differentiated at the edges in a species-specific pattern. In *M. denticulata*, following mitosis and septum formation at the isthmus, each semicell generates an adjoining mirror image of itself in about 16 h (Kiermayer 1981; Meindl 1993). The septum swells outward under the influence of turgor pressure, the polar lobe and wings begin to form, and the chloroplast flows into the forming semicell and eventually divides into two, but the two halves stay joined as semicells at the isthmus (Fig. 5).

Although all conjugating green algae are thought to use some variation of centripetal cell division, members of the group display a number of interesting and unusual cell division characteristics. In the placoderm desmid *Staurastrum* (*Pleurenterium*), after cytokinesis, the nucleus moves out of the isthmus into the developing semicell. The nucleus is then moved by means of a microtubular system in a circular motion. After most of the semicell has formed, the nucleus then returns to its place in the isthmus of the cell, a process termed nuclear migration (Meindl

Fig. 5 Early stage in semicell regeneration following cell division in *Micrasterias denticulata.* ×
(Photomicrograph courtesy of Dr. J. D. Pickett-Heaps, University of Colorado)

1986). Many of the molecular players in this process have been identified (e.g., Holzinger and Lütz-Meindl 2002).

In addition to cytological differences in cell division, some filamentous placoderm desmids display different patterns of cell division. The existence of replicate folds on the cross wall of species of *Desmidium* and *Bambusina* have long been known (Hauptfleich 1888). It was also discovered that *Onychonema* had an unusual mode of cell division that involved the delay of mitosis and the formation of a division vesicle (Krupp and Lang 1985). Additional differences in cell division among filamentous desmids are now known. Hall et al. (2008b) proposed that characteristics of cell division may be a valuable systematic character in filamentous Desmidiaceae.

It is worth noting that among charophytes (=streptophytes), the Zygnematophyta are the only group for which transformation protocols have been developed (Abe et al. 2011 for *Closterium*; Sorensen et al. 2014 for *Penium*; and Vannerum et al. 2010, 2011 *Micrasterias*). These protocols may prove valuable in using zygnematophytes as model systems.

Nuclear Cytology and Genetics

The most significant features of nuclear cytology in Zygnematophyta are (1) the presence during mitosis of stainable material derived from the nucleolus and known as nucleolar substance, (2) the presence of a complex interphase nucleolus, and (3) the absence of a localized centromere on the chromosomes of some species. In *Spirogyra*, large, complex nucleoli have been observed at interphase or prophase (Godward 1966). In *Spirogyra*, *Sirogonium* (Hoshaw and Wells 1982), and *Zygnema* (Harada and Yamagishi 1980), new nucleoli are organized by nucleolar-organizing chromosomes, which possess long secondary constrictions called nucleolar-organizing regions. Placoderm and saccoderm desmids also possess nucleolar-organizing chromosomes and nucleolar substance (Godward 1966; Brook 1981).

According to Godward (1966), *Spirogyra* metaphase chromosomes are of three types: (1) minute dot-like chromosomes in which the centromere position cannot be observed, (2) chromosomes with localized centromeres, and (3) polycentric chromosomes. In some genera such as *Zygnema* and *Mougeotia*, only dot-like chromosomes have been reported. Chromosomes in most saccoderm and placoderm desmids are small (less than 1.5 μm long). The parallel separation of chromatids at anaphase in most species suggests the presence of polycentric chromosomes.

Godward (1966) summarized data on chromosome numbers in the Zygnematophyta. Chromosome numbers in growing cells of saccoderm desmids range from 20 to 592, in placoderm desmids from 9 to 220, and in Zygnemataceae from 4 to 94. Considerable intraspecific variation in chromosome numbers has been reported (Brook 1981).

The genetics of the group was reviewed by Biebel (1976). He noted that their potential for genetic studies has rarely been exploited. Inheritance of genes for mating type and zygospore structure has been demonstrated. Selective abortion of

meiotic products in a zygospore results in incomplete, unordered tetrads. Recessive lethal alleles have been found in *Cosmarium turpinii* that are expressed in the homozygous condition in diploid zygospores but not in haploid vegetative cells.

Maintenance and Cultivation

Laboratory investigations of the conjugating green algae often begin with cultures from one or more of the world's culture collections, thereby bypassing the steps of collection and isolation of experimental organisms. If organisms directly from nature are desired, plankton and periphyton samples frequently contain desmid species, and filaments of the Zygnemataceae occur suspended in submerged masses or as surface scums that permit easy collection by hand. Once collected, cells or filaments can be isolated into unialgal culture by one of the methods described in Stein (1973) and Andersen (2005).

Many growth media are available; formulae and suggestions have been provided by Hoshaw (1968), Stein (1973), Starr (1978), and Andersen (2005). Strains obtained from culture collections often have the growth medium designated. For isolation and early growth, biphasic soil-water medium (Starr 1978) is often preferred; addition of a small quantity of peat is necessary to lower the pH for some species. For organisms requiring soil extract, we have found that we can grow many of these species in axenic conditions by adding 40 mL/L of filter-sterilized soil extract to sterile Bold's basal medium (see Stein 1973, for formula). Most placoderm desmids grow well in general defined medium with the addition of B vitamins. After a suitable growth medium is selected, cultures of Zygnematophyta will often produce luxuriant growth after 2–4 weeks in stationary culture under 40-watt cool-white fluorescent lamps of 50 $\mu Em^{-2}\,s^{-1}$ intensity on a 16:8 h light-dark cycle at 15–20 °C.

Fossil Record

Until the late twentieth century, fossil zygospores of conjugating green algae were largely ignored or identified as form taxa of non-zygnematalean spores (Van Geel 1979). Although fossil zygospores have been used to reconstruct ancient climates and aquatic habitats, a major hindrance to their use in reconstructing past climates is the general lack of knowledge of the ecology of extant species (Ellis and Van Geel 1978). Nevertheless, fossil zygospores provide information independent of pollen records (Van Geel and Van der Hammen 1978). The presence of these algal zygospores (usually *Mougeotia* and *Spirogyra*) at a site implies the past presence of shallow, stagnant, mesotrophic habitats subject to warming temperatures in spring that induce conjugation (Van Geel 1978; Van Geel and Van der Hammen 1978; Jarzen 1979). The presence of fossil desmids in deposits suggests the former presence of slightly acid swamp waters (Tappan 1980). An exception to this is the presence of *Oocardium*, one of the few desmids that prefer limy waters, in which it forms calcareous tubules called tufa (Bradley 1974).

Extant ally

Devonian | Carboniferous | Permian | Triassic | Jurassic | Cretaceous | Tertiary | Quaternary

Cosmarium cf. *botrytis*[3,4]
Cosmarium cf. *controversum*[4]
Cosmarium protractum[4]
Cosmarium cf. *sportella*[4]
Cosmarium turpinii var. *eximium*[3,4]
Euastrum insulare var. *lacustre*[3,4]
Bacinellula cosmarioides[5]

Cosmarium
Oocardium
Staurastrum

Stenexis cosmarioides[1]
Staurastrum enteroxenon[1]
Desmidiaceaesporites cosmarioides[6]
Cosmarium[1]
Oocardium[2]

Cosmarium-like[7]

Closterium idiosporum[3]
Closterium cf. *rostratum*[3]
Closterium cf. *kuetzingii*[9]

Closterium

cf. *Closterium*[8]

Paleoclosterium[1]

Mougeotia cf. *punctata*[11]
Mougeotia cf. *gracillima*[12]

Mougeotia

Tetraporidites
Tetrangulodinium conspicuum[13]
Mougeotia laetivirens[10,14]

Tetraporina[10]

Lecaniella irregularis[14]*

Zygnema

Lacundites[10]

Singranulentites[10]

Cylindrocystis brebisonii[12]

Cylindrocystis

Schizosporis[10]

Brazilea[10]

Tympanocysta[18]
Kagulubeites[10]

Spirogyra cf. *scrobiculata*[16]

Spirogyra

Spirogyra wyomingia[17]

Ovoidites[10,14,15]
Schizocystia[14]

Schizocystia[14]

Aletoverrucosisora[10]
Peltacystia[10]
Lecaniella[10]

?

Debarya

Fig. 6 (continued)

One of the most commonly reported fossil zygospores is that of *Debarya glyptosperma* (Van Geel and Van der Hammen 1978; Ellis and Van Geel 1978), reported originally as the form taxon *Peltacystia* (Van Geel 1979). The commonness of these distinctly keeled zygospores in deposits would not be predicted based on current distribution and abundance of this species, which, although found on every continent, is quite rare. The explanation for this is all the more elusive because, being rare, *Debarya* is not well known ecologically. Based on fossil evidence, *D. glyptosperma* in the Colombian Andes was restricted to cold to cool high-mountain climates (Van Geel and Van der Hammen 1978).

The fossil record of the Zygnematophyta is incomplete but extends to the middle Devonian (Fig. 6; Table 3). Because zygnematalean vegetative cells are fragile, most fossils are those of zygospores, which are usually necessary to identify living as well as fossil Zygnematophyta to species. The oldest zygnematalean fossils are Carboniferous zygospores of *Mougeotia* (reported as the form taxon *Tetraporina*), *Brazilea*, and *Lacunulites*. The phylogenetic placement of some fossil Zygnematophyta is less certain. *Paleozygnema spiralis* has been reported from Cretaceous amber in Germany (Dörfelt and Schäfer 2000). Both vegetative filaments and spores were preserved in the amber. The spore has a superficial spiral pattern similar to early stages of spore formation in *Spirogyra*. Placement of this taxon is uncertain. Based on the supposed phylogenetic affinities of the fossils to modern taxa, it can be deduced that all major lineages of the Zygnematales had diverged before the Carboniferous (Fig. 6; Table 3).

The oldest reported desmid fossils are vegetative cells of *Paleoclosterium leptum* from the middle Devonian. Fossils with obvious affinities to the Desmidiaceae do not appear until the Triassic. Extant genera of the Desmidiaceae (sensu stricto) do not appear until the Tertiary. This late appearance of Desmidiaceae in the fossil record is congruent with the derived placement of the group in molecular phylogenies (McCourt et al. 2000; Gontcharov et al. 2003; Hall et al. 2008a). Similarly, the filamentous nature of the oldest zygnematophycean fossils is congruent with the proposal of Delwiche and Cooper (2015) that the ancestor of charophyte (=streptophyte) algae and embryophytes was a filamentous taxon, most likely a branched one (see their Fig. 3).

Fig. 6 Fossil record superimposed on phylogenetic tree (After Hall et al. 2008a). Branching order based on Hall et al. 2008a. Phylogenetic position of *Debarya* is not known; however, it is thought to be closely related to *Mougeotia*. The placement of the *Debarya* lineage is unknown. *Debarya* is thought to be most closely related to *Mougeotia*. A "?" indicates the point of uncertainty. Sources used for fossil dates are indicated by the *superscript*. *1* Tappan 1980; *2* Bradley 1974; *3* Van Geel et al. 1981; *4* Van Geel et al. 1989; *5* Weyland 1963; *6* Hunger 1953; *7* Schmidt et al. 2006; *8* Waggoner 1994; *9* Van Geel and Van der Hammen 1978; *10* Van Geel 1996; *11* Van Geel 1976; *12* Van Geel 1978; *13* Schrank 2005; *14* Zippi 1998; *15* Hofman and Zetter 2005; *16* Van Geel et al. 1989; *17* Bradley 1970; *18* Afonin et al. 2001. Only new or particularly interesting fossils are referenced; many more fossils including many species within the form genera are known. * There is some disagreement as to the placement of *Lecaniella*, but Zippi (1998) indicates that this fossil belongs to the Zygnemoid lineage rather than the *Debarya* lineage

Table 3 Oldest fossil record of select Zygnematophyta

Taxon	Fossil type	Age	Location	References
Desmidiales				
Baccinellula cosmarioides	Cells	Pliocene	Italy	Weyland (1963)
Closterium sp.	Zygospores	Pleistocene	Colombian Andes	Van Geel and Van der Hammen (1978)
Cosmarium – like	Cells	Triassic	Italian Dolomites, Italy	Schmidt et al. (2006)
Cosmarium sp.	Conjugating cells	Eocene	Wyoming, USA	Tappan (1980)
Oocardium sp.	Layered tufa	Eocene	Wyoming, USA	Bradley (1974)
Desmidiaceaesporites cosmarioformis	Zygospore	Early Miocene	Poland	Hunger (1953)
Paleoclosterium leptum	Cells	Mid-Devonian	New York, USA	Tappan (1980)
Staurastrum enteroxenum	Conjugating cells and zygospores	Eocene	Wyoming, USA	Tappan (1980)
Stenixis cosmarioides	Cells	Late Triassic	Location not given	Tappan (1980)
Zygnematales				
Cylindrocystis brebissonii	Zygospores	Holocene	Germany and The Netherlands	Van Geel (1978)
Debarya glyptosperma (= *Lacunulites*?)	Zygospores	Permian	Western Australia	Van Geel (1979)
Mougeotia sp. (= *Tetraporina*? ?)	Zygospores	Carboniferous	Moscow, USSR	Van Geel (1979)
Spirogyra spp. (= *Brazilea*?)	Zygospores, aplanospores	Carboniferous	Moscow, USSR	Van Geel (1979)
Tympancysta stoschiana	Filaments with chloroplasts	Early Triassic	Russia	Afonin et al. (2001)

The affinities of fossils to extant taxa are rarely known with certainty. Mapping the reported fossils on the phylogeny of extant lineages in light of our incomplete understanding of affinities is somewhat bold, but if taken at face value, it suggests that most of the lineages of extant Zygnematales are very ancient.

Our understanding of evolution in the group has been much affected by molecular phylogenetic studies. It is clear that the traditional families Mesotaeniaceae and Zygnemataceae are not natural groups and that the order Zygnematales may be paraphyletic with respect to the Desmidiales. We now hypothesize that the

Desmidiales are a monophyletic group unto themselves that share a common ancestor with some unicellular and filamentous Zygnematales (as opposed to being derived from one or more unicellular zygnematalean lineages as was often proposed). Among the Desmidiales, most colonial and filamentous forms seem to belong to one or two major lineages, implying few transitions between these two growth habits (McCourt et al. 1995; Gontcharov et al. 2003; Hall et al. 2008a). Among the Zygnematales, however, there have been several transitions between the unicellular and filamentous forms. Although the direction of such transitions is not certain, lineages related to the conjugating green algae (Klebsormidiophyceae and Coleochaetophyceae) comprise filamentous or multicellular organisms, which is the inferred growth habit of the ancestor of conjugating green algae. Continued studies of evolution in the conjugating green algae and related lineages will provide greater insight into the origin and early diversification events in this group.

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