Algae

Third Edition

Graham | Graham | Wilcox | Cook

20

Green Algae V

Streptophyte Algae (Charophyte Algae, Charophyceans)

Spirogyra filaments—L. W. Wilcox

More than 500 million years ago certain green algae dramatically changed planet Earth by colonizing the land surface and giving rise to embryophytes, also known as land plants. By colonizing land, green algae and their plant descendants made it possible for animal and fungal life to also move onto land and diversify in response to terrestrial selection pressures. All modern terrestrial ecosystems that include plants, fungi, and animals are based on this pivotal event.

Together, land plants and their ancient and modern green algal relatives are known as streptophytes, a term based on the Greek word *streptos*, meaning pliant or twisted. Consequently, modern green algae that are closely related to land plants are called streptophyte algae. A wide range of ultrastructural, biochemical, and molecular evidence derived from the study of modern species reveals that land plants evolved from streptophyte algae. From this evolutionary perspective, the embryophytes can be viewed as a particular branch of green algae. Because of their close relationship to plants, the panoply of modern streptophyte algal species provides essential information about the evolution of fundamental land plant traits. In addition, some streptophyte algae are very abundant in nature, sometimes forming conspicuous blooms.

20.1 General Features and Classification of Streptophyte Algae

Streptophyte algae include several early-diverging lineages of flagellate unicells, colonies, and unbranched filaments and several later-diverging lineages having more complex structure and reproduction (Graham et al. 2014). The Zygnematales, Desmidiales, Coleochaetales, and Charales are the more complex orders of streptophyte algae, and include such common and familiar genera as *Spirogyra* and *Chara*.

Streptophyte algae share traits with land plants. Some traits occur widely among streptophytes, but others occur only in the more complex streptophyte algae and land plants. Traits generally present in streptophytes include asymmetric flagellar roots with a multilayered structure (MLS) in flagellate cells, glycolate metabolism enzymes located within a peroxisome, and an open and persistent spindle (see Table 16.1). A cell wall that includes a distinctive type of cellulose is another feature that most streptophyte algae share with land plants.

Most streptophyte algae and land plants produce cellulose microfibrils at the cell membrane by membrane particles arrayed as rosettes (Figure 20.1) (Kiermayer and Sleytr 1979; Hotchkiss and Brown 1987; Giddings and Staehelin 1991; Okuda and Brown 1992). Such rosettes contain cellulose synthase, an enzyme complex that generates cellulose from precursor UDP-glucose molecules. In contrast, the cellulose-synthesizing complexes of other green algae and bacteria occur in linear arrays (see Figure 20.1), as do those of stramenopile or red algae (see Figure 15.7). As a consequence of this difference, the cellulose produced by plants and streptophyte algae occurs as smaller-diameter microfibrils in which less-crystalline (amorphous) regions are relatively common. Streptophyte cellulose is also richer in a form of cellulose known as the I_β allomorph. By contrast, other algal celluloses occur as larger-diameter, more-crystalline microfibrils having fewer amorphous regions (see Figure 20.1). Non-streptophyte algal celluloses are typically richer in cellulose allomorph I_α. These differences in biochemical structure cause the celluloses of plants and streptophyte algae to have physical properties distinct from those of other algae. Differences in algal cellulose structure have influenced the ecology, evolution, and fossil record of algae (Graham et al. 2013), and also impact industrial applications (Hoover et al. 2011; Zulkifly et al. 2013) (Chapter 4). The pectic polysaccharides of representative streptophyte algae exhibit both similarities to and differences from early-diverging land plants (O'Rourke et al. 2015).

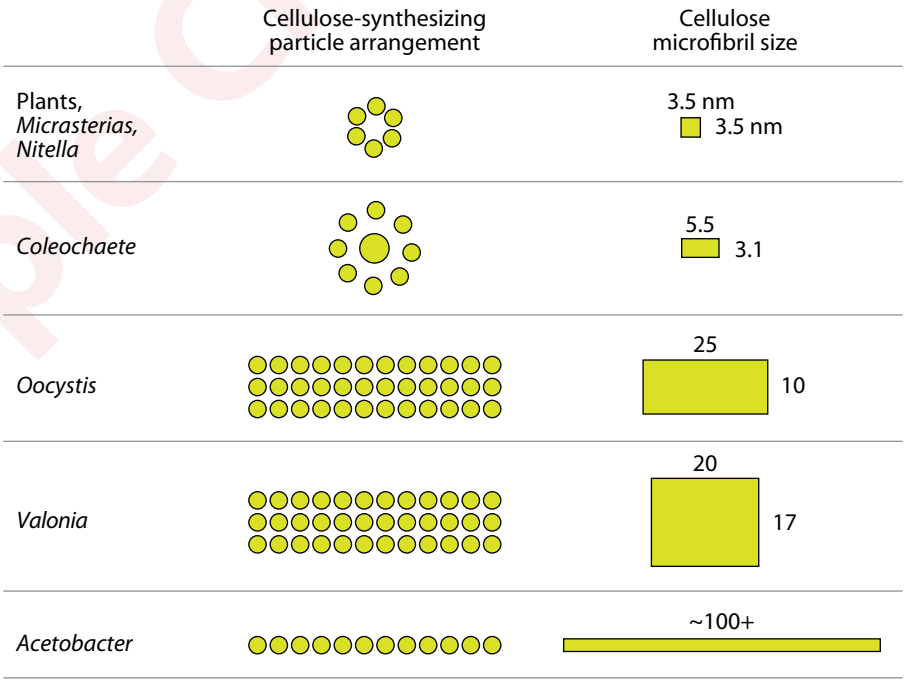

Figure 20.1 Diversity of cellulose-synthesizing complexes within the green algae. Rosettes similar to those of land plants occur in charophycean algae. These rosettes generate relatively thin microfibrils (shaded boxes). In contrast, cellulose-synthesizing complexes of other green algae are linear and are composed of three rows of particles that generate thicker microfibrils. The bacterium *Acetobacter* is regarded as having the ancestral type of linear cellulose-synthesizing complex. (Drawing modified from Okuda and Mizuta. 1993. Diversity and evolution of putative cellulose-synthesizing enzyme complexes in green plants. *Japanese Journal of Phycology* 41:151–173)

An important plant feature that originated during the diversification of the streptophyte algae is a system that protects photosynthetic light harvesting systems from excess light. Although light is essential for photosynthesis, too much light inhibits photosynthesis

in a process known as photoinhibition. In response to this challenge, photosynthetic organisms have acquired mechanisms for dissipating excess light energy as heat, by the process called non-photochemical quenching (NPQ) (see Chapter 1). To stimulate NPQ, many eukaryotic algae use a protein called LHCSR (Lhc-like protein Stress Related), formerly known as LI818 (Light Induced protein 818). By contrast, land plants generally rely on a different protein, PSBS (photosystem II subunit S). A recent analysis of NPQ-related proteins in streptophyte algae revealed evidence for PSBS-linked NPQ in Charales, Coleochaetales, and Zygnematales + Desmidiales, but not earlier-diverging streptophyte algae (Gerotto and Morosinotto 2013).

Acquisition of the PSBS system for protecting photosynthesis from damage by excess light might first have arisen in response to bright conditions in shallow freshwaters, where many species of Charales, Coleochaetales, and Zygnematales + Desmidiales can be found today. This change and other features of modern, later-diverging streptophyte algae (Graham et al. 2012) can be viewed as preadaptations that fostered ancestral relatives' ability to colonize the land. Inheriting the new NPQ system was likely valuable for early embryophytes, which were exposed to even brighter conditions in the terrestrial habitat, and thus has been retained by modern lineages of land plants. The value of streptophyte algae in understanding the evolution of land plant features has led many researchers to focus closely on classification. Emphasis on the phylogenetic relationships of streptophyte algae raises the prospect of identifying the modern algae most closely related to land plants.

Classification of Streptophyte Algae

Because streptophyte algae form a paraphyletic group, Bremer (1985) recommended that they be clustered with land plants to form the monophyletic phylum, Streptophyta (informally streptophytes) (Figure 20.2). This classification distinguishes the streptophytes from prasinophyte green algae (Chapter 16) and Chlorophyta (ulvophyceans, chlorophyceans, and trebouxiophyceans) (Chapters 17-19). Lewis and McCourt (2004) and others have proposed an alternate formal classification scheme that groups streptophyte algae with embryophytes to form a phylum Charophyta with several classes, one being the land plants. In this alternative classification, the informal term "charophyte algae" would correspond with "streptophyte algae," and the informal term "**charophytes**" would refer to land plants plus the charophyte algae. Because other modern authors have used the term "charophytes" to refer to different groups of organisms (e.g., the order Charales, informally known as stoneworts), we have chosen to use Bremer's terminology, as have some others (e.g., Becker and Marin 2009). Streptophyte algae are subdivided into several orders (see Figure 2.1), and the ordinal names used here are consistent with the recommendations of Lewis and McCourt (2004).

Streptophyte Algal Orders and Their Evolutionary Significance

Several lines of molecular evidence indicate that the Mesostigmales, represented by the unicellular flagellate *Mesostigma viride* is the earliest-diverging modern charophycean known to date (Kim et al. 2006; Nedelcu et al. 2006; Simon et al. 2006; Rodríguez-Ezpeleta et

al. 2007) (see Figure 20.2). The unicellular or colonial order Chlorokybales diverges next in most phylogenies, though *Mesostigma* plus *Chlorokybus* formed an early-diverging clade in a study based on chloroplast genomes (Lemieux et al. 2007). Unbranched filaments that form the order Klebsormidiales diverge next. A clade that includes both Zygnematales and Desmidiales diverges later, and includes unicells and unbranched filaments that display isogamous sexual reproduction. Because these two orders together form a monophyletic group, here we refer to the larger group as Zygnematales + Desmidiales. Also relatively late-diverging are Coleochaetales and Charales, composed of branched filaments that display oogamous sexual reproduction.

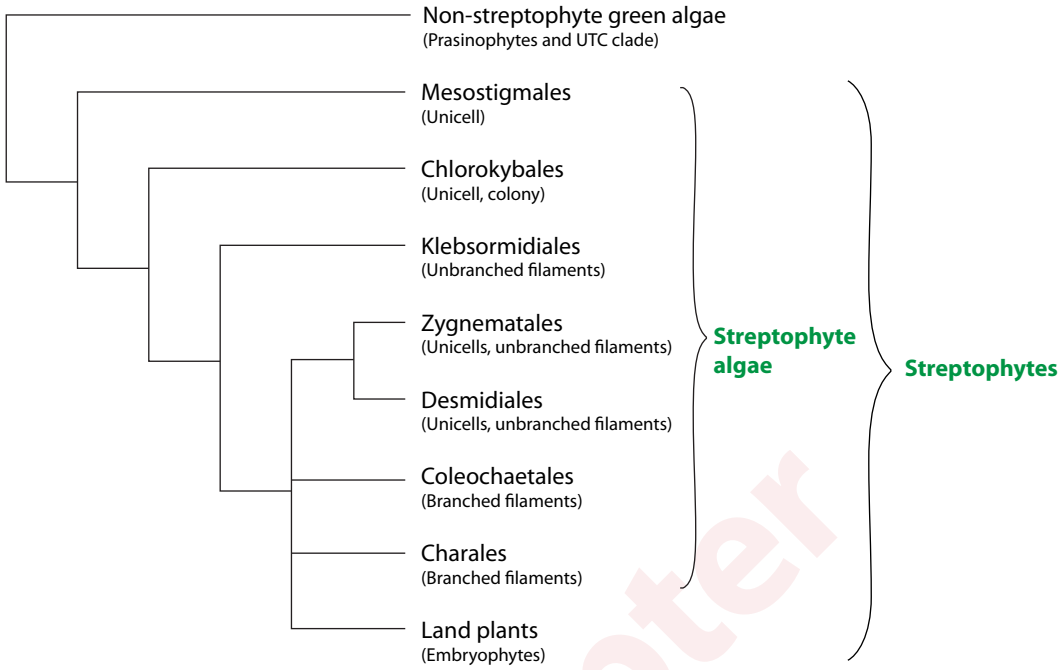

Figure 20.2 Relationships of the orders of streptophyte algae

Recent phylogenetic analyses, described below, do not agree about the order in which Zygnematales + Desmidiales, Coleochaetales, Charales, and land plants diverged. For this reason, Figure 20.2 displays a polytomy, rather than a dichotomous branching pattern, to reflect relationships among these orders and embryophytes. Although Charales (informally charaleans) are more closely linked to land plants in some cases (Karol et al. 2001; Turmel et al. 2003; Luo and Hall 2007; Qiu et al. 2007), other studies suggest that the sister group to land plants might be Zygnematales + Desmidiales (Timme et al. 2012; Zhong et al. 2013; Wickett et al. 2014), or a clade composed of Coleochaetales and Zygnematales + Desmidiales (Finet et al. 2010, 2012; Wodniok et al. 2011; Laurin-Lemay et al. 2012). Analyses based on chloroplast or mitochondrial genome sequences generally indicate that Charales diverged earlier than Coleochaetales and/or Zygnematales + Desmidiales (Turmel et al. 2007, 2013). Work remains to be done in resolving charophycean divergence patterns, knowledge of which is important because it suggests the order in which traits first appeared. Such work may be fostered by the acquisition of whole genome sequences for representative streptophyte algae and by new approaches to the analysis of genomic sequence data. In view of phylogenetic uncertainty, in this chapter the orders of streptophyte algae are arranged in ascending order of body complexity, though this arrangement may not reflect evolutionary pattern.

The identity of the particular modern streptophyte algae most closely related to embryophytes has been considered important because it is assumed that such protists would best mirror the traits of the pivotal common ancestor. However, it is important to note that hundreds of millions of years have passed since modern streptophyte algae diverged from embryophytes (Clarke et al. 2011). Modern

streptophyte algae likely display many traits that have appeared in the meantime and thus would not reflect the ancestral type. For example, fossil evidence covering hundreds of millions of years indicates that during that time charaleans have undergone structural and reproductive evolution in response to aquatic selection regimes (Kelman et al. 2003; Feist et al. 2005). During their evolution, the genomes of charaleans and some zygnemataleans have expanded to the point of "genomic obesity," likely in conjunction with the evolution of large cells. By contrast, *Mesostigma*, Klebsormidiales, and Coleochaetales have smaller genome sizes within the range typical for haploid bryophytes and thus would be inferred as the ancestral plant type (Kapraun 2007).

Yet another issue to consider in the quest for a genomic profile of the common ancestor is raised by evidence for horizontal gene transfer (HGT). Charophycean mitochondrial genomes are known to resemble those of land plants. So, if charophycean mitochondria also have active DNA uptake systems and a propensity to fuse, HGT might have influenced charophycean genomes, as well as those of early-diverging land plants (Turmel et al. 2006). The possibility of HGT means that genomic sequences that are used for comparative analyses of streptophyte algae and bryophytes should be routinely tested for vertical descent.

For the reasons detailed above, even if one particular order of streptophyte algae is ultimately identified as the sister group of embryophytes, the traits of this order might not always accurately reflect the traits of an ancestor shared with land plants. Investigating the traits of diverse representatives is probably necessary to achieve a full understanding of the evolutionary steps that gave rise to the first land plants. For example, TCP-type transcription factors characteristic of land plants are inferred to have appeared after divergence of Klebsormidiales, but prior to the divergence of Desmidiales (Navaud et al. 2007). Other important examples can be found in the following discussion of charophycean diversity.

20.2 Streptophyte Algal Diversity

Mesostigmales

MESOSTIGMA (Gr. *mesos*, middle + Gr. *stigma*, mark). The freshwater biflagellate *Mesostigma viride* (Figure 20.3) was originally recognized as a possible streptophyte based on presence in zoospores of an MLS flagellar root (Rogers et al. 1981; Melkonian 1989) (see Chapter 16). As noted earlier, several recent molecular studies have concluded that *Mesostigma* branches at the base of the streptophytes. The observation that *Mesostigma* and most other streptophyte algae live primarily in freshwaters supports a current concept that early streptophyte radiation primarily occurred in freshwaters rather than marine waters (Graham 1993; Melkonian et al. 1995; Becker and Marin 2009).

Mesostigma is disk shaped and has a central, or sometimes lateral, flagellar pit opening that penetrates quite deeply into the cell. A layer of small, flat, polygonal scales covers the flagella, and similar scales serve as the lowermost layer of body scales. *Mesostigma* has two additional layers of distinctive scales: The middle layer is composed

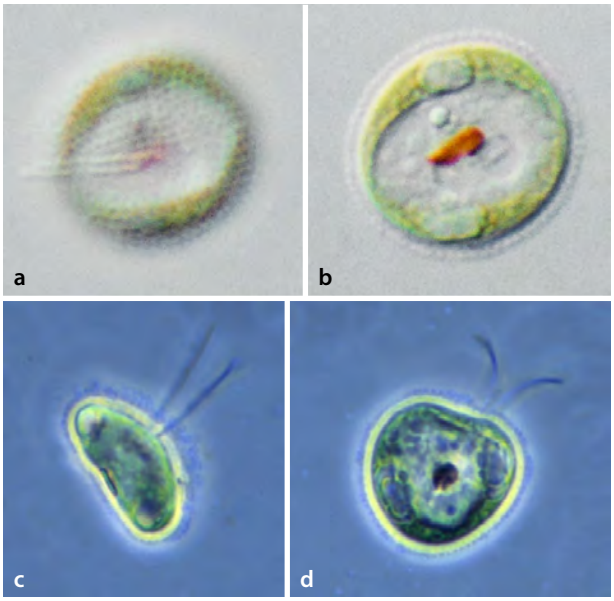

Figure 20.3 ***Mesostigma.*** This genus has two flagella and a single flat chloroplast that contains pyrenoids and an eyespot in the region near the flagellar basal bodies. Scales cover both the flagella and cell proper. In (a) and (b) a cell viewed with DIC optics is shown in two planes of focus—with flagella and scales apparent in (a) and the chloroplast with two pyrenoids plus eyespot in (b). In (c) and (d) cells were viewed with phase-contrast optics in which the same features take on a somewhat different appearance. (a, b: M. E. Cook; c, d: L. W. Wilcox)

of larger, flattened, oval scales that are ornamented with small pits; and the outer layer consists of large, distinctive basket scales (Manton and Ettl 1965) (Figure 20.4). However, there is no evidence for a cellulose-rich cell wall, a distinctive trait of other streptophytes. Consequently, Mesostigma is important in tracing the evolutionary history of the cellulose-rich plant cell wall.

There is a single platelike chloroplast, which is thickened at the edges and contains several pyrenoids (see Figure 20.4). A study of plastid pigments in six strains of *Mesostigma viride* identified lycopene, lutein, siphonaxanthin, siphonaxanthin C12:0 ester, siphonaxanthin C14:0 ester, γ-carotene, β-carotene, antheraxanthin, violaxanthin, all-*trans* neoxanthin, and chlorophylls *a* and *b* (Yoshii et al. 2003). In apparently lacking 9'-cis neoxanthin, *Mesostigma* differs from other green algae and land plants. Siphonaxanthin may function as an antenna pigment in blue-green light absorption, suggesting adaptation to relatively deep water. Extensions of the chloroplast appear to connect to the flagellar basal bodies, which is unusual.

An eyespot composed of two or three layers of pigmented globules lies within the plastid near the basal bodies. The occurrence of a mutant having a colorless eyespot allowed Matsunaga and associates (2003) to detect positive phototaxis of *Mesostigma* in blue light and to observe diaphototactic behavior, swimming perpendicularly to the direction of incident light in response to green light. Eyespots have not been reported in flagellate cells of streptophytes other than *Mesostigma*, suggesting that the eyespot must have been lost early in streptophyte diversification.

A large, lobed peroxisome lies between the chloroplast and the basal bodies and is attached to the latter. This association is believed to facilitate division and distribution of the peroxisome to daughter cells at cell division. *Mesostigma* has a plastidic GAPDH ($NADP^+$-specific glyceraldehyde-3-phosphate dehydrogenase) subunit B that seems to be unique to streptophytes (Simon et al. 2006). Glycolate oxidase is a part of the photorespiratory pathway of *Mesostigma*, a trait shared with other streptophytes (Iwamoto and Ikawa 2000). As in other streptophytes, *Mesostigma* uses both the plastidic DOXP/MEP and cytosolic mevalonate (MVP) pathways for isoprene biosynthesis, whereas all tested chlorophytes use only the plastidic pathway (Schwender et al. 2001). These examples indicate that many of the metabolic traits typical of streptophytes originated prior to the divergence of *Mesostigma*.

Fibrous connecting bands similar to those of other green algae link basal bodies, and each basal body is associated with a single flagellar root containing five to seven microtubules. The proximal part of each root is a multilayered structure (MLS); thus there are two MLSs per cell. As noted in Chapter 16, MLSs mark the flagellate cells of streptophytes, though they are occasionally observed in a number of other algae.

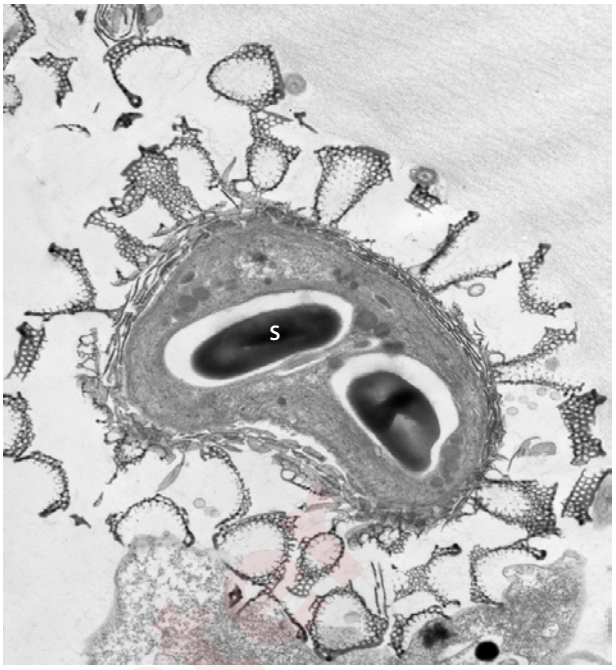

Figure 20.4 *Mesostigma* scale ultrastructure. TEM view of *Mesostigma* cell showing the multiple layers of scales as well as starch (S) within the chloroplast. (Micrograph: E. Kim and L. E. Graham)

Chlorokybales

The order Chlorokybales includes *Chlorokybus atmophyticus* and perhaps also *Spirotaenia*, a unicellular genus historically classified with Zygnematales but suggested to be allied with *Chlorokybus* based on phylogenetic analysis (Gontcharov and Melkonian 2004).

CHLOROKYBUS (Gr. *chloros*, green + Gr. *kybos*, cube) is a rarely encountered terrestrial or freshwater green alga (Geitler 1955). The vegetative body is a packet of rounded cells (Figure 20.5) surrounded by a thick extracellular matrix that includes cellulose (Figure 20.6). This observation suggests that cellulose biosynthesis and extracellular deposition originated in streptophytes prior to the divergence of *Chlorokybus*. Cytokinesis is followed by deposition of cell wall material only at the new cross-wall, as in filamentous and more complex streptophyte algae and plants. However, *Chlorokybus* is not considered to be either filamentous or parenchymatous (composed of true tissue) and is regarded as the simplest charophycean alga having a nonmotile vegetative stage. *Mesostigma* and *Chlorokybus* plastid genomes encode seven genes that are absent from all other green algae whose plastid genomes have been completely sequenced (Lemieux et al. 2007).

Sexual reproduction has not been observed. *Chlorokybus* cells can be induced to produce a single biflagellate zoospore during asexual reproduction. The zoospore flagella emerge laterally and are associated with a groove, in these respects resembling *Mesostigma*. Zoospore release in *Chlorokybus* is by disintegration of the parental cell wall, a process that is regarded as unspecialized compared to zoospore release mechanisms of other streptophyte algae (Rogers et al. 1980). The body and flagella of the zoospores are covered with small flat scales resembling those of *Mesostigma*. The flagella also possess hairs, as do those of some other streptophyte algae. No eyespot is present in the zoospores, suggesting that eyespots were lost from the streptophyte lineages prior to divergence of *Chlorokybus*. There is a single peroxisome, which, like that of *Mesostigma* but not other streptophyte algae, is attached to the flagellar apparatus. The MLS and associated microtubular extension form the only known flagellar root. This MLS root contains fewer microtubules (10 or 11) than do those of other streptophyte algae (or land plants), and its microtubules do not extend as far down into the cell (Rogers et al. 1980). After the zoospores swim for about an hour, they become round, retract their flagella, and begin to deposit a cell wall beneath the body scales (Rogers et al. 1980). As the cell wall becomes well developed, the scale layer is lost. Each cell of *Chlorokybus* possesses a single cup-shaped chloroplast resembling those of some other streptophyte algae but, atypically, there are two distinct types of pyrenoids—one embedded within the plastid and containing numerous traversing thylakoids, and another located at the periphery of the plastid and lacking thylakoids. The pyrenoids of other streptophyte algae (and hornworts among land plants) are embedded and traversed by thylakoids; the simpler peripheral pyrenoid type is lacking (Lokhorst et al. 1988). The physiological and evolutionary relevance of these differing pyrenoids in *Chlorokybus* is not known.

At the initiation of mitosis in *Chlorokybus*, centrioles are positioned at the plane of cell division, which is indicated by a precocious (early developing) cleavage furrow. Centrioles then duplicate and move apart, forming the poles of the mitotic spindle; they are associated with material that serves as a microtubule organizing center (MTOC). The centrioles are also associated with an array of astral microtubules. By metaphase, the nuclear envelope has completely broken down; mitosis is thus described as "open." The spindle persists until it is disrupted by the formation of a cross-wall (septum) as the cleavage furrow is completed. Microtubules are arrayed in the plane of the developing furrow (Lokhorst et al. 1988).

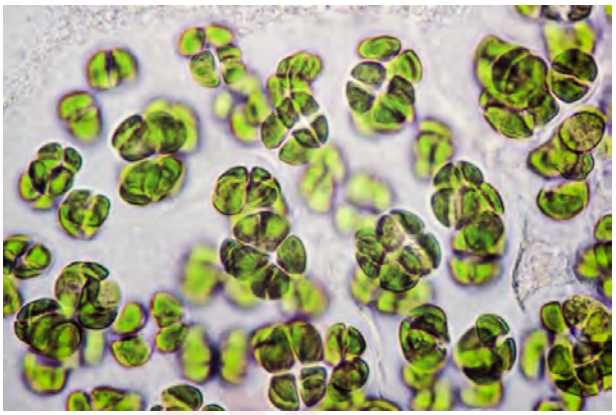

Figure 20.5 *Chlorokybus*. This genus is sarcinoid, meaning that the cells are arranged in packets of variable number, held together by mucilage. (Photo: L. E. Graham)

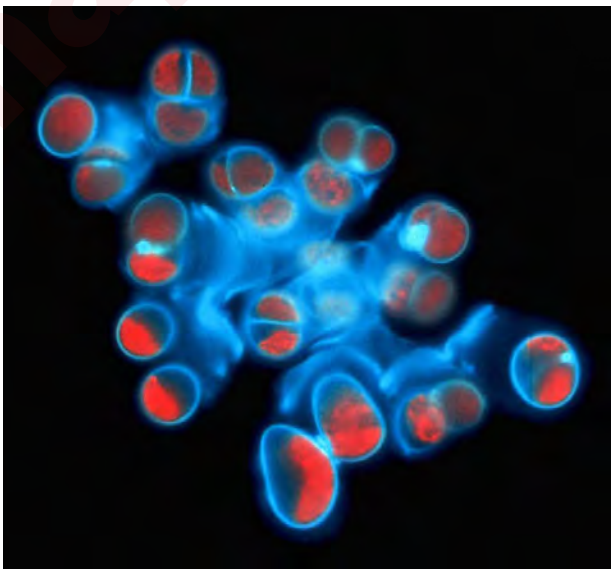

Figure 20.6 *Chlorokybus* extracellular matrix containing cellulose. These cells were treated with a compound that binds to cellulose and fluoresces bright blue-white. (Photo: L. E. Graham)

Klebsormidiales

This order includes freshwater and terrestrial genera such as *Klebsormidium* and *Entransia* that primarily occur as unbranched filaments having a single, parietal chloroplast. However, the genus *Interfilum*, which groups into Klebsormidiales, occurs as unicells, packetlike aggregations (as in *Chlorokybus*), or biseriate (two-rowed), branched filaments (Rindi et al. 2011). It has been pointed out that some culture collection isolates labeled *Klebsormidium* group with Trebouxiophyceae (Sluiman et al. 2008), but that when authentic strains of *Klebsormidium* are included in phylogenetic analyses, the order forms a well-supported clade. Sexual reproduction is not known to occur in *Klebsormidiales*, but asexual reproduction occurs via flagellate zoospores.

KLEBSORMIDIUM (named for Georg Albrecht Klebs, a German phycologist, + *Hormidium*, a genus of green algae [Gr. *hormidion*, small chain]) (Figure 20.7) occurs widely in terrestrial habitats such as soil and on tree trunks, as well as in splash zones of water bodies (Sluiman et al. 2008). Noting that the cells produced only a single zoospore, and that this zoospore lacked an eyespot and had two laterally emergent flagella, Marchant et al. (1973) predicted that *Klebsormidium* zoospores would possess an MLS-type flagellar root, and subsequent ultrastructural examination confirmed this prediction. *Klebsormidium* is regarded as more specialized than *Chlorokybus* because zoospores are discharged through differentiated pores in the cell wall. Production of the pore involves protrusion of the cytoplasm from a specific wall site and then deposition of presumed cell wall lytic vesicles rather than the generalized dissociation of the entire zoosporangial wall, as occurs in *Chlorokybus*. The ovoid zoospores are devoid of body scales, and the flagella also lack scales and hairs. It is presumed that scales and hairs were lost sometime after the divergence of *Klebsormidium* from the main line of streptophyte evolution. After swimming for about an hour, the zoospores become round, retract their flagella, and form a cell wall without attaching to a substrate or forming a holdfast, as do some other green filamentous algae.

Klebsormidium vegetative cells and zoospores each contain a single parietal chloroplast with a pyrenoid surrounded by starch grains, but no eyespot. Each cell contains a single, land plantlike peroxisome, but this organelle is not attached to the basal bodies as it is in *Chlorokybus* and *Mesostigma*. Rather, the peroxisome lies appressed to the midpoint of the chloroplast and is segregated at mitosis along with the plastid. Mitosis is open. Centrioles are present at the spindle poles; they are believed to be associated with spindle microtubule organizing material. An unusual large vacuole that forms in the interzonal region about halfway through anaphase appears to help complete chromosomal separation. Cytokinesis occurs by the development of a constricting furrow from the periphery (Floyd et al. 1972).

Klebsormidium may be difficult to distinguish at the light microscopic level from several other unbranched filamentous green algae that are not allied to the streptophyte algae. However, if it can be observed that only a single eyespot-less zoospore is produced per cell, the filament is probably *Klebsormidium*. Comparative taxonomic studies of European species of *Klebsormidium* were done by Lokhorst (1996). Some terrestrial isolates have the ability to survive desiccation for several weeks (Kaplan et al. 2012, Karsten and Holzinger 2012). Genome sequencing has been accomplished for *K. flaccidum* (Hori et al. 2014).

Figure 20.7 *Klebsormidium*, an unbranched filament. Cells have a single platelike, parietal plastid. (Photo: L. W. Wilcox)

ENTRANSIA (named for E. N. Transeau, an American phycologist) has distinctive plastids that have multiple pyrenoids and are lobed at the edges in a way that resembles dripping paint (Figure 20.8). A tapering spine occurs at some filament tips. The unbranched filaments may spiral around each other and attach to substrates by means of basal adhesive. Cross-walls take on an H-shape when viewed in optical section. Vegetative reproduction occurs by means of zoospores that are released through a wall pore and by fragmentation achieved by programmed cell death (Cook 2004b). Programmed cell death is an important component of development in multicellular organisms; its occurrence in *Entransia* may be the earliest recorded for streptophytes.

Zygnematales + Desmidiales

Although some authors still use the term Zygnematales to include all desmids and close relatives, Zygnematales actually form a distinct monophyletic group that is sister to the Desmidiales (Turmel et al. 2002; Gontcharov et al. 2003). These two orders together form a clade (see Figure 20.1) known as the class Zygnemophyceae (Kenrick and Crane 1997), class Zygnematophyceae (van den Hoek et al. 1995), or Conjugatophyceae (Guiry 2013). About 3,500 species of conjugating green algae have been described, a number that represents about 10% of all known algal species. About one-third of conjugating green algae are classified in Zygnematales and two-thirds in Desmidiales (Guiry 2013).

Zygnematales are currently defined as unicells or unbranched filaments. The order takes its name from such the filamentous genus *Zygnema* (Figure 20.9), whose outer walls lack pores and whose cells are not constricted (Gerrath 2002). Unicellular zygnemataleans such as *Mesotaenium* are informally known as the **saccoderm desmids** (Figure 20.10a). By contrast, desmidialean unicells such as *Micrasterias* typically have constricted cells with porose walls, and are informally known as the **placoderm desmids** (Figure 20.10b).

No flagellate stages are known for Zygnematales or Desmidiales, and, in both orders, sexual reproduction occurs by means of a conjugation process involving non-flagellate gametes. Zygotes having distinctive shapes and wall structure (Figure 20.11) result from sexual reproduction; these typically confer the ability to survive long periods when conditions are not favorable for growth, germinating when the environment improves. Zygote persistence has been attributed to the presence of acid hydrolysis-resistant, sporopolleninlike polymers in the cell wall (DeVries et al. 1983). Such polymers may explain why zygotes similar to those of modern zygnematalean algae have been recovered from sediments of Carboniferous age (some 250 million years old) and younger (e.g., Worobiec 2014).

Zygnematalean green algae can be environmentally conspicuous. Ponds, ditches, sheltered nearshore regions of lakes, and slow-flowing streams may exhibit blooms of *Spirogyra* or related forms each spring, the growths sometimes assuming nuisance proportions (Graham et al. 1995). *Mougeotia* and some relatives can produce metaphytic (subsurface) clouds in lake waters that have been affected by acidic precipitation (acid rain) or experimental acidification (Schindler et al. 1985; Watras and Frost 1989; Howell et al. 1990; Turner et al. 1991).

Figure 20.8 ***Entransia.*** This filamentous genus has lobed plastids that resemble dripping paint. Filaments are seen with DIC optics in (a), while autofluorescence of photosynthetic pigments—a technique helpful in visualizing the number, size, and shapes of plastids within algal cells—is seen in (b). (a: M. E. Cook; b: L. W. Wilcox)

Figure 20.9 ***Zygnema.*** This unbranched filament lends its name to the order Zygnematales. (Photo: L. W. Wilcox)

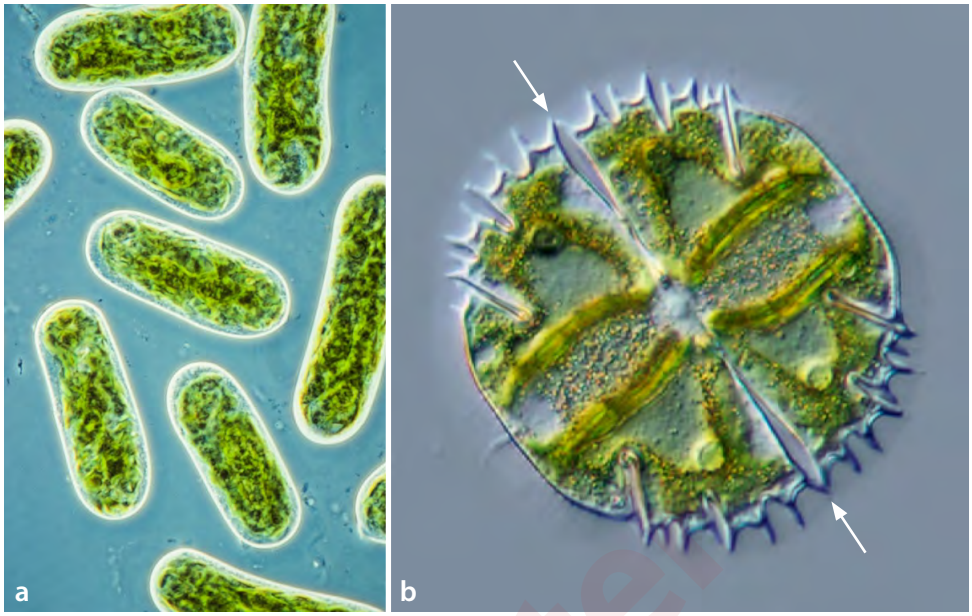

Figure 20.10 Saccoderm versus placoderm desmids. (a) The saccoderm desmid *Mesotaenium* is not constricted. (b) The placoderm desmid *Micrasterias* shows a distinctive constriction (arrows). (a: L. E. Graham; b: L. W. Wilcox)

Cell biology of Zygnematales

Cell walls and mucilage.

The extracellular matrix of zygnematalean cells typically includes several layers. An outer mucilage layer of calcium pectate and hemicelluloses, a thin fibrillar primary wall, and a thicker, fibrillar secondary wall are commonly produced by *Spirogyra*, *Zygnema*, *Mougeotia*, and other filamentous forms, as well as by saccoderm desmids. The production of copious amounts of outer mucilage explains why zygnematalean algae feel slimy to the touch. The mucilage envelope appears to be extruded through the cell wall (Gerrath 1993). Possible functions for zygnematalean mucilage include water retention and resistance to desiccation, nutrient trapping, absorption of harmful ultraviolet radiation, or habitat for symbiotic microbes (Figure 20.12). Iron is sometimes deposited in the outer wall layers, giving cell walls a yellow or brown color (Gerrath 1993). The fibrillar portion of the wall of one *Mougeotia* species was found to consist of both noncellulosic carbohydrates (64%) and cellulose (13%), comparable to the cell walls of *Klebsormidium* (Hotchkiss et al. 1989).

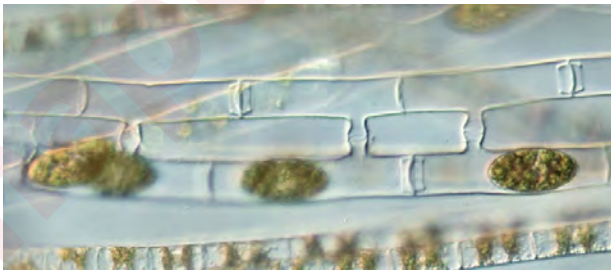

Figure 20.11 Conjugated *Spirogyra* from a field collection with zygotes. (Photo: M. E. Cook)

Mitosis and cytokinesis of zygnemataleans.

The nuclei of zygnemataleans are often large and conspicuous (Figure 20.13), consistent with the fact that large, polyploid nuclear genomes are known to occur (Kapraun 2007). Cell division in zygnemataleans typically occurs during the dark portion of the light–dark cycle. Mitosis and cytokinesis in zygnemataleans resemble those of *Chlorokybus* and *Klebsormidium*, except that centrioles are absent. In *Spirogyra* and *Mougeotia*, the nuclear envelope remains intact until metaphase, when it disintegrates, as in other streptophyte algae. These algae, and *Zygnema*, are also unusual among zygnemataleans in that a small phragmoplast—an array of perpendicular microtubules as well as membranous tubules and vesicles—occurs at the central region of the cell at the plane and, at the same time, as furrow extension from the periphery (Fowke and Pickett-Heaps 1969a,b; Pickett-Heaps and Wetherbee 1987). This small phragmoplast has been suggested to represent an intermediate stage in the evolutionary origin of more highly developed, plantlike phragmoplasts of *Coleochaete* and

Charales (Pickett-Heaps 1975; Graham and Kaneko 1991). A study of the *Spirogyra* phragmoplast via fluorescent tagging of cytoskeletal components and video microscopy revealed several differences between phragmoplast structure and behavior as compared to those of other streptophyte algae and higher plants (Sawitzky and Grolig 1995). Furrowing in *Spirogyra* involves actin microfilaments (Goto and Ueda 1988; Nishino et al. 1996); these presumably help to constrict the cytoplasm in a fashion analogous to the tightening of purse strings. Immunolocalization of microtubules has been followed throughout the cell cycle in *Mougeotia* (Galway and Hardham 1991). In contrast to *Coleochaetales* and Charales, plasmodesmata are not known to occur in the cross-walls of zygnemataleans.

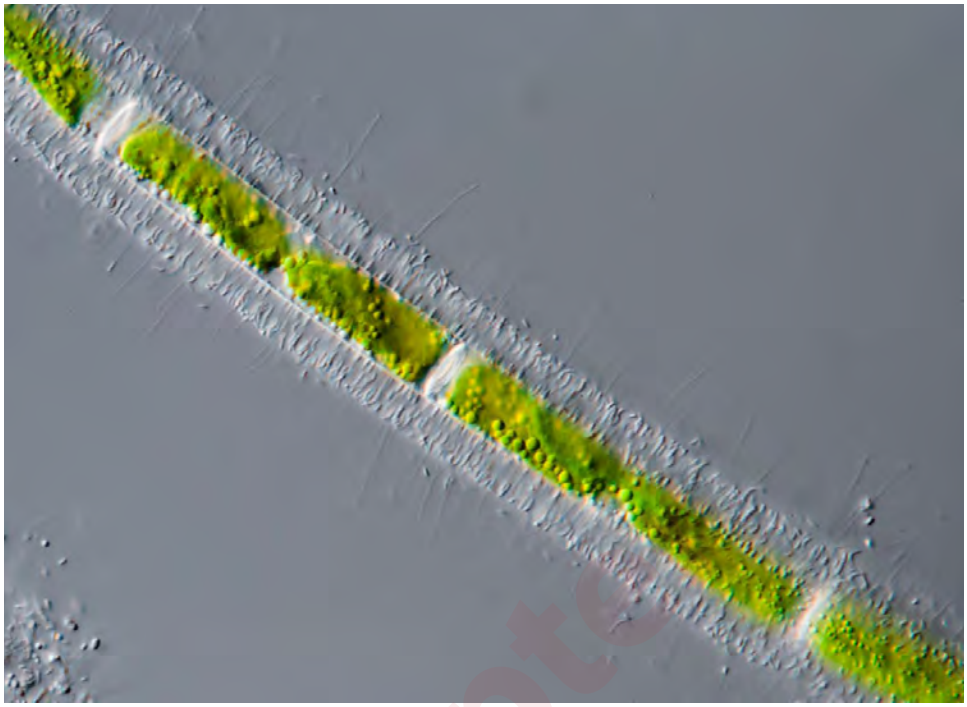

Figure 20.12 ***Mougeotia*** **with numerous microbial associates within mucilage.** (Photo: L. W. Wilcox)

Reproduction in Zygnematales

Asexual reproduction. As noted previously, zygnemataleans do not produce flagellate zoospores, in contrast with *Chlorokybus*, *Klebsormidium*, and Coleochaetales. Such lack of flagella correlates with absence of centrioles, which are necessary for the generation of flagella (Pickett-Heaps 1975). However, filamentous zygnemataleans may reproduce asexually by fragmentation, and populations of single-celled zygnemataleans grow by means of mitosis and cytokinesis. Vegetative cells are dispersible by wind, insects, and water birds (as are desiccation-resistant zygotes) (Hoshaw et al. 1990). Zygnemataleans may sometimes produce thick-walled resting cells known as akinetes, aplanospores, asexual spores, or parthenospores, the latter originating from unpaired cells of sexual populations.

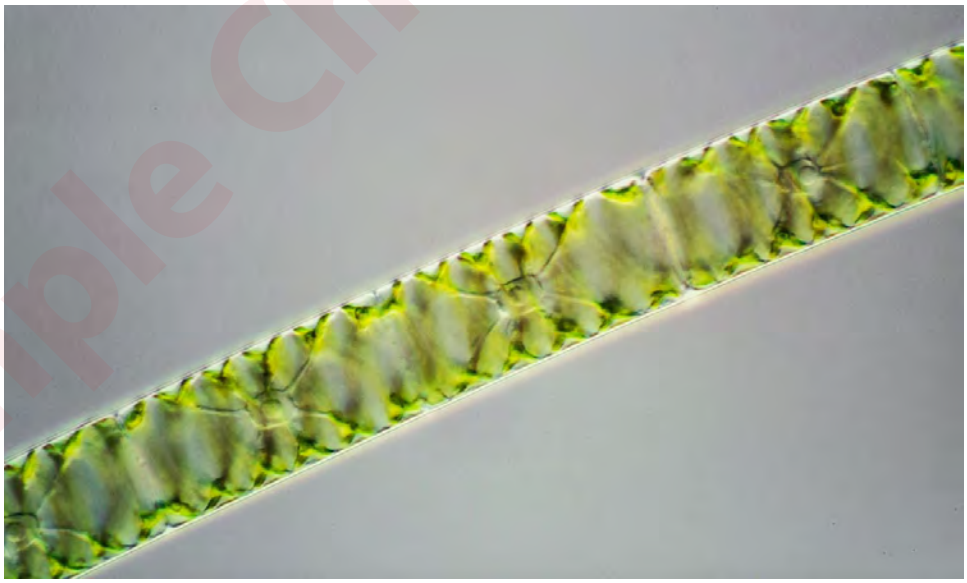

Figure 20.13 **Zygnematalean nuclei.** The nucleus of zygnemataleans is suspended in cytoplasm in the cell center, as illustrated here by *Spirogyra*. (Photo: M. E. Cook)

Sexual reproduction. Zygnematalean mating is accomplished by the physical pairing of filaments or single cells, their enclosure within common mucilage, and subsequent fusion of nonflagellate gametes—a process known as **conjugation**. In the laboratory, cultures of zygnematalean algae have been induced to undergo conjugation by reducing the combined nitrogen concentration in their growth medium (Biebel 1973), by increasing carbon dioxide levels (Starr and Rayburn 1964), or by increasing temperature, light levels, and/or concentrations of Ca^{2+} and Mg^{2+} (Gerrath 1993), but little is

known about the factors responsible for inducing sexual reproduction in nature.

Spirogyra, *Mougeotia*, *Zygnema*, and other filamentous forms may undergo **scalariform conjugation**, where filament pairs align themselves laterally and develop modified branches known as **conjugation tubes**, that link opposing cells (Figure 20.14). The conjugation tube is composed of an outgrowth (papilla) from each opposing cell; when they meet, the wall at their interface is degraded to form an open tube through which gametes can move. Ultrastructural studies have suggested that papilla growth occurs by addition of new cell wall material, under the influence of enlarging vacuoles in the papilla, but the mechanism by which the end walls disintegrate is unclear (Pickett-Heaps 1975). Although gamete motion is often described as "amoeboid," there is no direct evidence that zygnematalean cells can actually move in the same way as true amoeboid cells. Gamete protoplasts can be observed to shrink as they lose water; increased external hydrostatic pressure or mucilage accumulation in the surrounding area may be responsible for their propulsive movement (Pickett-Heaps 1975), but the mechanism is not well understood. In some cases, the cytoplasm of only one of the connected cells moves across the tube, forming a zygote within the confines of the second cell (see Figure 20.15a). Sometimes, both gametes move into the conjugation tube, whereupon a zygote is formed in the center of the tube (see Figure 20.15b). The timing of gamete nuclear fusion and meiosis in filamentous zygnemataleans appears to vary from organism to organism.

An alternate form of conjugation, known as **lateral conjugation**, occurs when gametes develop from adjacent cells within the same filament (Figure 20.15). In this case, filament pairing does not occur. Rather, a short curved tube extends from one cell to the next in the filament. Because lateral conjugation is a form of selfing, this process would be expected to result in reduced levels of genetic variability compared to scalariform conjugation, which may involve filaments that are genetically distinct. Both result in the production of resistant-walled zygotes that serve a perennation function (persistence to the next favorable period for growth). Also, zygote germination involves meiosis, and thus the potential for recombination events that increase population genetic diversity. Among the single-celled saccoderm desmids, sexual reproduction involves cell aggregation, formation of gametes through mitotic division, papilla formation, release of gamete protoplasts from enclosing walls, and zygote formation.

Zygote development involves formation of a thick wall consisting of as many as six distinct layers. Callose and sporopollenin (DeVries et al. 1983) have been demonstrated to occur among the layers of zygote walls of at least some zygnematalean species. Mature zygotes are often highly ornamented and colored orange-brown as a result of wall formation and chlorophyll degradation. In nature, zygotes germinate in spring, or the end of a period of dry dormancy, as when a temporary pool is re-formed in the wet season. Zygnematalean zygotes can withstand burial in mud for long periods (Brook and Williamson 1988), and zygotes have been germinated after dry storage for more than 20 years (Coleman 1983). Zygote germination can be induced in the laboratory by allowing the culture medium

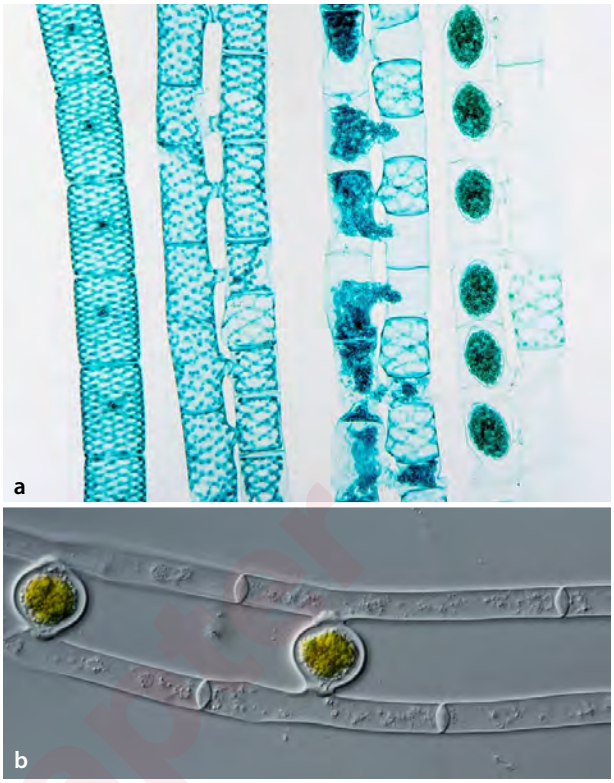

Figure 20.14 Scalariform conjugation in Zygnematales. Flagellate gametes are not produced in any of the modern zygnemataleans. Conjugation tubes allow transfer of gamete cytoplasm from one filament to the other or to the midregion of the tube, where zygote formation may occur. (a) In this prepared slide of *Spirogyra*, moving left-to-right, are a nonreproductive filament; paired filaments in which conjugation tubes have formed; a stage in which gamete cytoplasm is moving from one filament into the other; and zygotes that have formed in the left filament (one cell in the right filament did not pair up with a cell in the left filament). In (b) gametes in a species of *Mougeotia* moved into the conjugation tubes from both filaments such that zygotes formed within the conjugation tubes. (Photos: L. W. Wilcox)

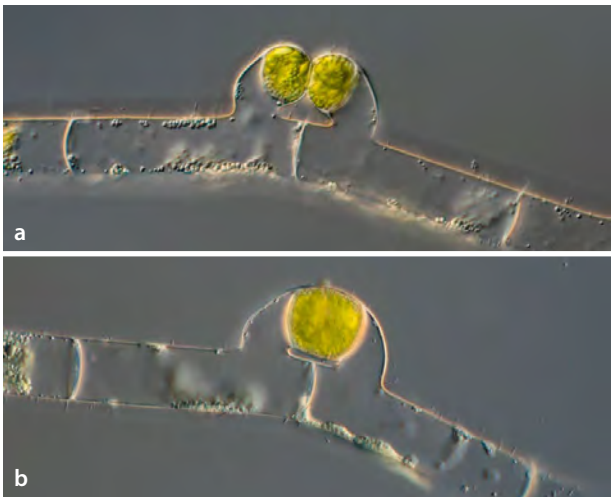

Figure 20.15 Lateral conjugation in *Mougeotia*. This type of conjugation involves only one filament. (a) Gametes from adjacent cells have migrated into tubes prior to fusion. (b) A later stage, in which a zygote has formed. (Photos: L. W. Wilcox)

to slowly evaporate; storing the zygotes in the dark, with or without refrigeration, for 1 to 12 months; and then rewetting zygotes with fresh culture medium. A few hours following rehydration, zygotes become green due to synthesis of chlorophyll, and one to three days later, the wall ruptures, and a germination vesicle containing the meiotic products emerges.

Ecology of Zygnematales

Zygnemataleans are almost exclusively found in freshwater habitats, although a few have been collected from brackish waters. They are ubiquitous in freshwaters, occurring in pools, lakes, streams, rivers, marshes, and especially bogs and mildly acidic, nutrient-poor streams (Figure 20.16). In addition, zygnemataleans may be abundant in reservoirs, cattle tanks, roadside ditches, irrigation canals, and other water bodies of human construction. *Spirogyra*, for example, was found at nearly one-third of the more than 1000 locations sampled by McCourt et al. (1986), and in a North American continent-wide survey, Sheath and Cole (1992) located *Spirogyra* in streams from a wide variety of biomes, including tundra, temperate and rain forests, and desert chaparral. In streams and shallow lakes, *Spirogyra* is typically attached to stable substrates but can also occur as free-floating mats that originate from benthic zygotes or filaments (Lembi et al. 1988). As growth and photosynthesis occur, oxygen bubbles become entrapped in the mats and provide flotation; at the water surface the algal mats are exposed to high temperatures and light levels. Optimal temperature and irradiance conditions for photosynthesis for one species of *Spirogyra* were determined to be 25°C and 1500 µmol photons m^{-2}, respectively. Net photosynthesis was observed to be positive at 5°C under high irradiance conditions, explaining the widespread occurrence of surface growths in the cool waters of early spring. However, the alga could not maintain positive photosynthesis at the low light levels that can result from self-shading when temperatures were high (30°C–35°C), explaining late spring and summer declines in zygnematalean mats (Graham et al. 1995).

Mougeotia, a filamentous zygnematalean, often forms large nuisance growths in subsurface freshwaters affected by acid precipitation or experimental acidification. Such waters are characterized by increases in the concentration of metals such as aluminum and zinc, reduced levels of dissolved inorganic carbon, and food web changes, including reduction in numbers of herbivores (Stokes 1983; Webster et al. 1992; Fairchild and Sherman 1993). The appearance of conspicuous *Mougeotia* growths is widely regarded as an early indicator of environmental change (Turner et al. 1991). The optimal light, temperature, and pH conditions for photosynthesis, as well as effects of the metals zinc and aluminum on photosynthesis, were determined for this alga so that its common and specific association with acidification could be better understood. Net photosynthesis was high (on average over 40 mg O_2 was produced per gram dry weight per hour) over a wide range of irradiances (300–2300 µmol quanta m^{-2} s^{-1}). The optimal temperature was 25°C, and

Figure 20.16 A large growth of *Spirogyra*. This bloom occurred in a slow-moving backwater of a stream in South-Central Wisconsin. (Photo: L. W. Wilcox)

the organism exhibited tolerance of a wide range of pH (3–9) and metal concentrations. These results, together with release from herbivores, help explain the rise to dominance of large subsurface *Mougeotia* growths in acidified lakes (Graham et al. 1996a,b). Conditions that induce desiccation tolerance have been determined for polar isolates of *Zygnema* and related species (Pichrtova et al. 2014).

Diversity of Zygnematales

SPIROGYRA (Gr. *speira*, coil + Gr. *gyros*, twisted) is a filament composed of cells having 1–16 spiral, ribbon-shaped chloroplasts per cell (Figure 20.17). The plastid edges are often beautifully sculpted, and numerous pyrenoids are present (Figure 20.18). Sometimes rhizoidal processes occur at the basal end of the filament; these are involved in attachment to substrates. Actin filaments are known to be involved in rhizoid formation in Spirogyra (Yoshida and Shimmen 2008). Cytoplasmic streaming—based on the action of actin microfibrils—can often be observed in the peripheral cytoplasm. The nucleus is suspended in the center of the cells (see Figure 20.14). Both scalariform and lateral conjugation occur. During conjugation, previously bright-green filamentous masses turn noticeably brownish in color, reflecting the loss of chlorophyll pigments from zygotes and development of brown zygote walls. Zygotes germinate to form a single filament; from this it is inferred that only a single meiotic product survives. Hundreds of species have been described. *Spirogyra* is not monophyletic, nor is its close relative *Sirogonium*, but these two zygnemataleans consistently diverge closest to the base of the Zygnematales + Desmidiales clade (Drummond et al. 2005; Hall et al. 2008).

MESOTAENIUM (Gr. *mesos*, middle + Gr. *tainia*, ribbon) unicells are shaped like cylinders, each with a single platelike plastid having several pyrenoids (see Figure 20.11a). Culture studies have shown that sexual reproduction in *Mesotaenium kramstei* involves the formation of a broad conjugation tube that can grow from any portion of the cell wall. Mucilage is secreted inside the wall as gametes shrink during development. Mature zygotes are mahogany brown (Biebel 1973). *Mesotaenium* is not monophyletic, with some species (*M. kramstei*) more closely related to another saccoderm desmid known as *Cylindrocystis*, and others (*M. caldariorum*) linked with the filamentous *Mougeotia* (Hall et al. 2008).

MOUGEOTIA (named for Jean Baptiste Mougeot, an Alsatian physician and botanist) consists of long, unbranched, free-floating filaments, each cell of which is characterized by a single platelike chloroplast (Figure 20.19). The plastids are suspended in the central area of the cell (i.e., are axial in location). Pyrenoids are either arranged in a single row or scattered throughout the chloroplast. Conjugation in *Mougeotia* is usually scalariform, and zygotes typically form in the conjugation tube. Only a single filament is produced upon zygote germination. *Mougeotia* cells contain numerous small vacuoles filled with phenolic compounds, believed to serve in protecting the cells from herbivores (Wagner and Grolig 1992).

Mougeotia and *Mesotaenium* possess the ability to orient their single, axial, platelike plastids to achieve optimal exposure to light, much as a motor-driven solar panel can be repositioned to follow changes in direction of solar radiation through the day (Figure

Figure 20.17 Several *Spirogyra* species. This genus is an unbranched filament of cells attached end to end. Note the helically twisted ribbon-shaped chloroplasts with multiple round pyrenoids. (Photos: L. W. Wilcox)

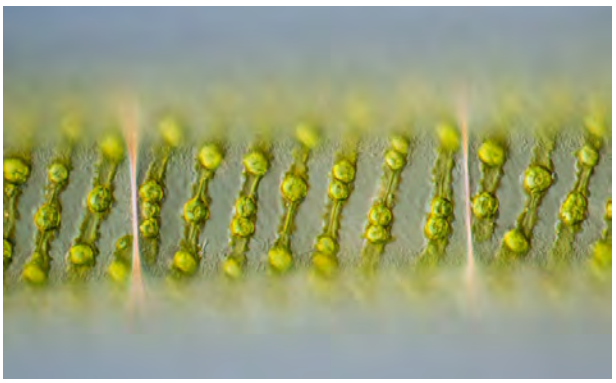

Figure 20.18 Pyrenoids. Higher-magnification view of the spiral, lobed, ribbonlike plastid of *Spirogyra* with numerous globular pyrenoids. Starch is visible around each of the pyrenoids. (Photo: L. W. Wilcox)

20.20). This process requires three components: one or more sensory pigments to perceive the wavelength and direction of the light signal, a transducer to convert the light signal into chemical information, and a mechanical effector to receive the message and move the chloroplast accordingly. In *Mougeotia scalaris*, a blue-light sensor that is likely phototropin and a red-sensing chimeric photoreceptor of phototropin and phytochrome called neochrome are involved in signal perception (Suetsugu and Wada 2007; Kagawa and Suetsugu 2007).

Red-light sensing is thought to effect changes in the binding of actin microfilaments at the cell periphery with myosin molecules that are linked to the chloroplast surface. The actomyosin motor then causes changes in positioning of the chloroplast. The result is that the platelike chloroplast is pulled into a position such that its broad face is directed toward the light when irradiance is low or optimal but so the edge faces the light when irradiance is too high (Wagner and Grolig 1992). Calcium influx, interaction of calcium ions with the calcium-binding protein calmodulin, and the binding of calcium ions with protein kinases are also thought to be involved in the light signal transduction process in these algae. Actomyosin interactions are also involved in organelle movements that occur in interphase cells of other zygnemataleans, such as *Spirogyra*, whose plastids do not rotate (Grolig 1990). *Spirogyra pratensis* produces ethylene and shows a cell elongation response to ethylene, suggesting properties present in earliest plants (Ju et al. 2015).

SPIROTAENIA (Gr. *speira*, coil + Gr. *taenia*, band) (Figure 20.21) has unicells that are straight or somewhat curved, with a single spirally twisted, ribbonlike chloroplast. No flagellate cells are known. Sexual reproduction was studied in *S. condensata* (Hoshaw and Hilton 1966). This occurs by the pairing of cells of differing sizes within a blanket of mucilage. The paired cells then function as gametangia, producing non-flagellate gametes. Zygotes develop an unusual honeycomblike wall, lose pigmentation, and form large cytoplasmic oil droplets. Spontaneous germination of *Spirotaenia* zygotes begins 22 days after their formation; meiosis occurs at the onset, and four germling products are released. These four cells may be observed in the vicinity of the empty zygote wall, surrounded by mucilage. As noted earlier, *Spirotaenia* has been grouped with *Chlorokybus* based on sequence analysis (SSU rDNA and *rbcL* gene sequence data); *Spirotaenia* lacks an intron typically found in zygnematalean SSU rDNA (Gontcharov and Melkonian 2004). The plastid genome of *Spirotaenia* should be examined for evidence of gene content similar to that of *Chlorokybus*. If *Spirotaenia*'s placement within Chlorokybales is upheld by future investigations, *Spirotaenia* may be of evolutionary interest as an early case of sexual reproduction in streptophyte algae.

Figure 20.19 *Mougeotia*. This genus is an unbranched filament having a single platelike plastid per cell. (Photo: L. W. Wilcox)

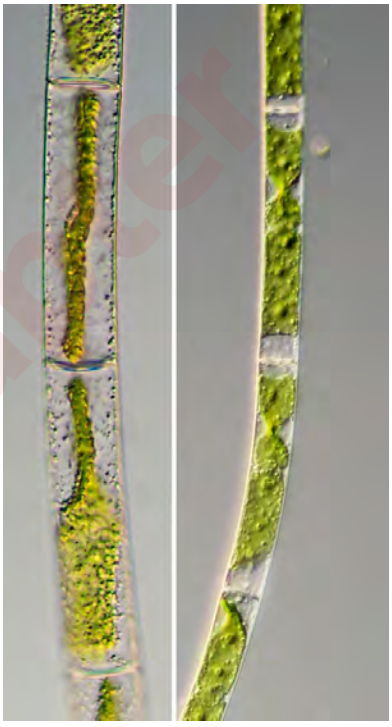

Figure 20.20 Two examples of plastid rotation in *Mougeotia*. The *Mougeotia* plastid can rotate around a lengthwise axis, and portions of the plastid can turn independently, generating a twisted plastid. (Photos: L. W. Wilcox)

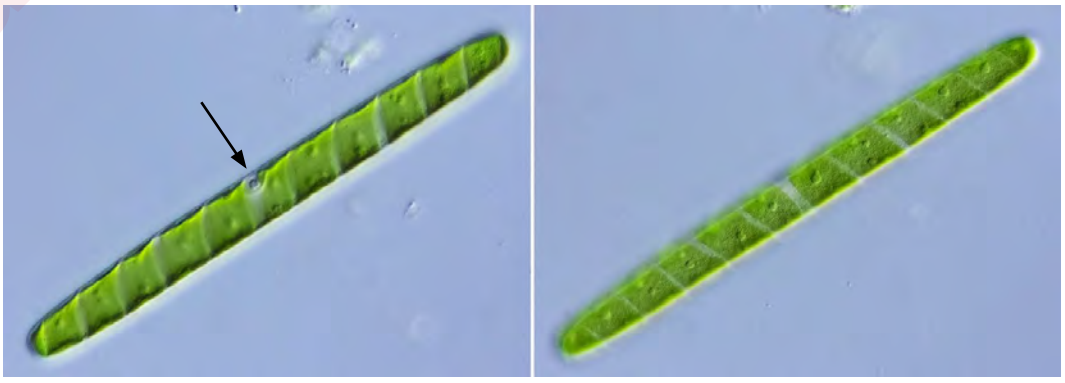

Figure 20.21 *Spirotaenia*. This genus is single celled and has a spirally twisted, ribbonlike chloroplast. Two planes of focus are shown. The nucleus with nucleolus (arrows) is evident in the left image and pyrenoids within the spiral plastid are visible in the right image. (Photos: L. W. Wilcox)

CYLINDROCYSTIS (Gr. *kylindros*, cylinder + Gr. *kystis*, bladder) unicells are cylindrical and contain two axial, stellate chloroplasts similar to those of *Zygnema* (Figure 20.22). Sexual reproduction of a number of species has been studied in culture (e.g., Biebel 1973). Easily grown forms that might prove useful in molecular genetics studies are strains of *C. brebissonii* (UTEX 1922 and 1923) isolated by Biebel (1973). They grow well in defined culture medium (Bold's Basal Medium—Stein 1973). *Cylindrocystis* is not monophyletic (Hall et al. 2008).

ZYGNEMA (Gr. *zygon*, yoke + Gr. *nema*, thread) occurs as relatively short, unbranched filaments of cylindrical cells, enclosed by a mucilage sheath. As is the case for *Spirogyra*, there may be basal rhizoidal outgrowths that serve in attachment. There are two stellate chloroplasts per cell, each with a central pyrenoid (Figure 20.23). The chloroplast genome of *Z. circumcarinatum* is significantly larger than that of some other streptophyte algae and land plants, suggesting extensive expansion by addition of intergenic spacers and introns (Turmel et al. 2005). Conjugation is similar to that of *Spirogyra*; both scalariform and lateral conjugation are observed. Akinetes—asexually produced, thick-walled cells containing abundant oil and starch storage products—can be formed; these are able to survive for over one year before germinating into vegetative filaments. Prescott (1951) observed that in the western Great Lakes region, species growing in high-pH waters more often possessed more zygotes than forms occurring in low-pH habitats. Zygotes germinate to form a single filament, suggesting that only a single meiotic nucleus survives. *Zygnema* is frequently found in nature together with *Spirogyra* and/or *Mougeotia*. At least 80 species have been described.

NETRIUM (Gr. *netrion*, small spindle) unicells are elongated and cylindrical, usually with rounded ends (Figure 20.24). Two large, elaborately lobed and ridged chloroplasts, each with a pyrenoid, occur per cell. Copious mucilage is excreted into the surrounding environment. In *N. digitus*, pectin-containing mucilage may be secreted from one cell tip, thereby causing cells to glide at a velocity of about 1 µm per minute; mucilage may also be secreted from the entire cell surface (Eder and Lütz-Meindl 2010). At the start of sexual reproduction a broad conjugation tube is formed, gametes shrink when they contact each other, the protoplasts then fuse, and the zygote develops in the conjugation tube. The plastid pigmentation disappears, and a golden-brown wall develops on the zygote. After two months in culture medium, the zygotes germinate spontaneously. Typical plastid pigmentation reappears, and the protoplast swells, causing the zygote wall to burst. Usually only two meiotic products survive to form germlings.

Overview of Desmidiales (placoderm desmids)

Desmidiales, also known as placoderm desmids, have distinctive cell walls that explain characteristic features of their ecology. The cells of Desmidiales are often constricted into two parts, known as semi-cells, by a narrow region known as the isthmus. The walls surrounding such semi-cells are formed at different times, for which reason the desmidialean cell wall is said to be composed of two pieces. Desmidialean cell walls are also perforated with pores (Figure 20.25), and often highly ornamented. The presence of wall pores helps explain the common ability of placoderm desmids to move by mucilage secretion, and

Figure 20.22 ***Cylindrocystis.*** This unicellular zygnematalean is a saccoderm desmid (Photo: M. E. Cook)

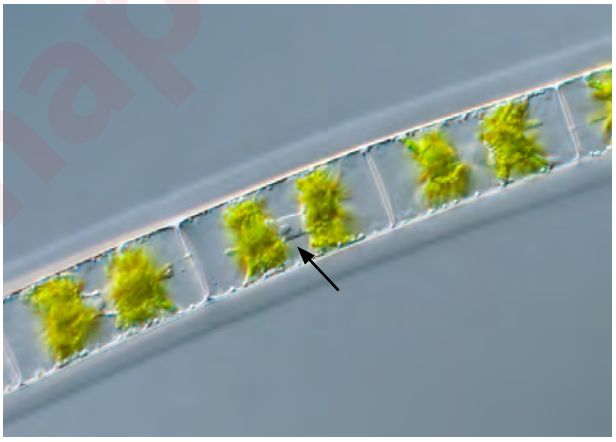

Figure 20.23 ***Zygnema.*** This genus is an unbranched filament having two stellate plastids per cell, each with a large conspicuous pyrenoid. Arrow points to cytoplasmic bridge between plastids, which contains the nucleus. (Photo: L. W. Wilcox)

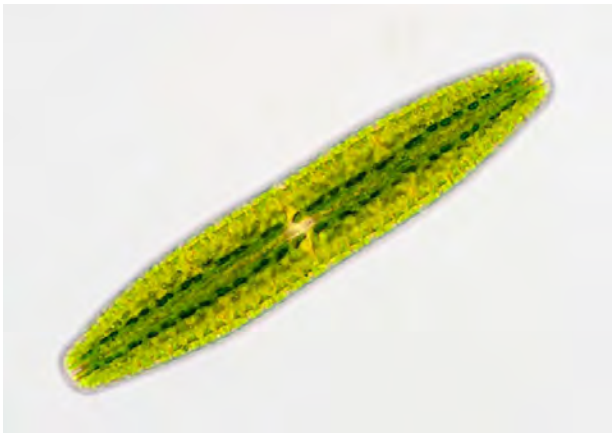

Figure 20.24 ***Netrium.*** This genus is a unicellular saccoderm desmid having two elaborately lobed chloroplasts, one in each cell half. (Photo: L. W. Wilcox)

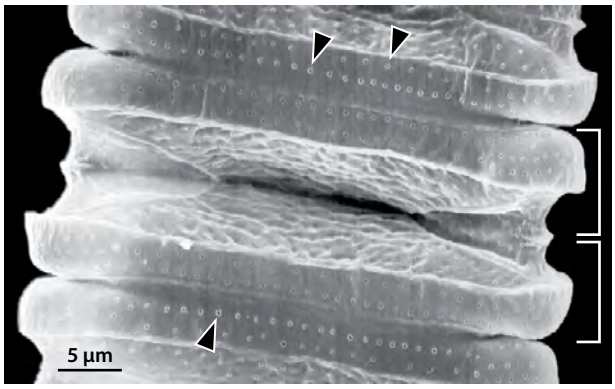

Figure 20.25 Scanning electron microscope (SEM) view of mucilage pores (arrowheads) in the semi-cell walls of *Desmidium*. The brackets indicate the two semi-cells of the cell that is shown in its entirety. (Micrograph: S. Cook)

Figure 20.26 Mucilage. Prisms of mucilage are produced from individual pores (arrows), forming a confluent sheath on *Desmidium grevillii* (shown here in a captured video image of a filament viewed with light microscopy). The small black dots (arrowheads) are end-on views of bacteria that live in the mucilaginous sheath. (Photo: L. W. Wilcox)

mucilage production from pores may serve other functions. Some desmids produce acid and microbe-resistant polyphenolic polymers in vegetative cell walls (Gunnison and Alexander 1975a,b; Kroken et al. 1996; Graham et al. 2004). Such wall compounds, together with favorable conditions for preservation, may explain the oldest- known fossil vegetative cells of desmids, *Paleoclosterium leptum*, from the Middle Devonian (about 380 million years ago) (Baschnagel 1966). Cells similar to modern *Cosmarium* have been reported from amber of Triassic age (220 million years old) (Schmidt et al. 2006a). Resistant cell wall polymers may also explain the survival of placoderm desmids such as *Closterium*, *Micrasterias*, *Pleurotaenium*, and *Euastrum* in drying mud at the edges of lakes. Such desmids have been found to be alive after three months of drying and at depths of 6 cm in sediments (Brook and Williamson 1988).

Cell wall structure, mucilage extrusion, and cell motility of Desmidiales

Placoderm desmids have a primary wall consisting of pectins and cellulosic microfibrils; this wall is often discarded after the development of a secondary wall that is often highly ornamented. However, the cells of filamentous placoderm desmids are held together by retained primary wall material (Krupp and Lang 1985). Pectinaceous mucilage extruded from wall pores can be visualized at the light microscopic (Figure 20.26) and ultrastructural (Figure

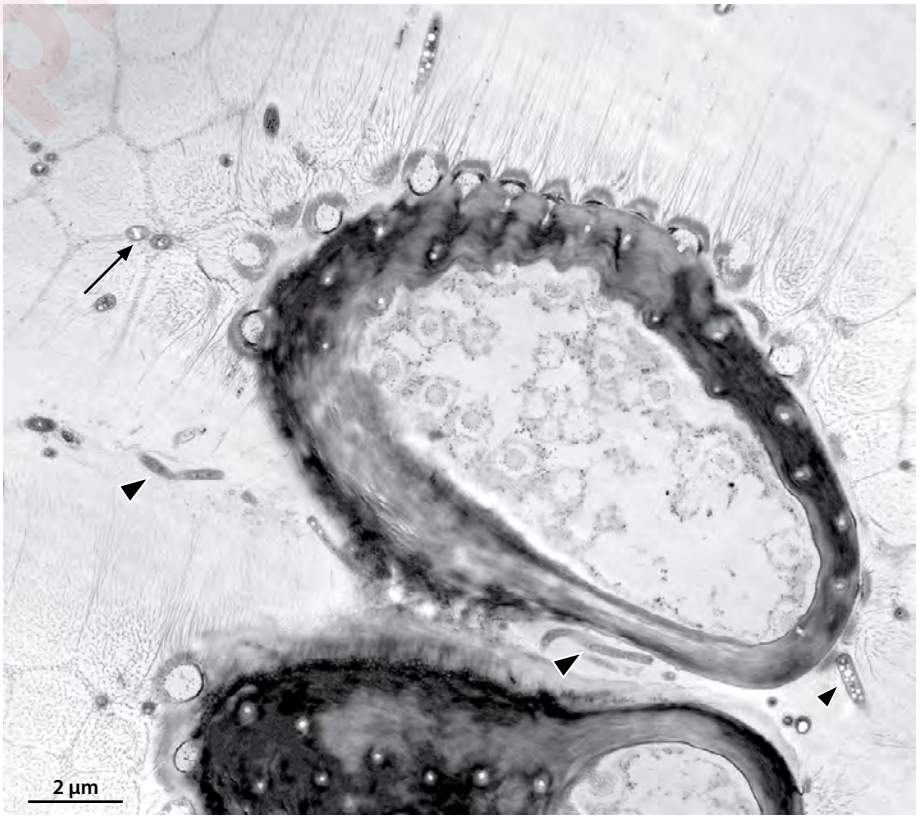

Figure 20.27 Mucilage, pores, and epibacterial communities. Prisms of mucilage and their association with wall pores of *Desmidium grevillii*, as viewed with TEM. Note occurrence of associated bacterial cells (arrowheads) in the pocket formed at the isthmus region and at the interfaces of mucilage prisms (arrow). (From Fisher and Wilcox. 1996. Desmid-bacterial associations in *Sphagnum*-dominated Wisconsin peatlands. *Journal of Phycology* 32:543–549)

20.27) levels as "prisms" of extruded material. Ultrastructural and immunocytochemical analysis of *Closterium* mucilage revealed that mucilage vesicles are produced by Golgi bodies and then released through flask-shaped cell wall pores. Under normal conditions, each cell can produce about 3 µg of mucilage in 30 days, but mucilage production increases three to four times when cells are grown under low-phosphate or low-nitrate conditions (Domozych et al. 1993; Domozych and Domozych 1993). Mucilage extrusion plays several roles, including fostering microbial symbioses, attachment, or motility.

A number of zygnematalean algae harbor bacteria within the confines of their mucilaginous sheaths (see Figures 20.13 and 20.27) (Gerrath 1993; Fisher and Wilcox 1996; Fisher et al. 1998). In other cases, extracellular mucilage may confer a reduced sinking rate to planktonic forms or serve as an attachment mechanism for periphytic species. Many desmids are capable of motility through mucilage extrusion. Movement is typically quite slow, only about 1 µm per second. Gliding movement is accompanied by continuous secretion of a slime trail that can be visualized at the light microscopic level with the addition of india ink to the preparation. *Closterium* and some other desmids secrete mucilage only from opposing cell tips, in alternate fashion, resulting in a somersault-like movement. The placoderm desmid *Cosmarium*, among others, secretes mucilage from the older cell wall half, while the younger wall half is lifted from the substrate at a slight angle. Subsequent swelling of the mucilage by water absorption then propels the cell forward (Häder and Hoiczyk 1992). Phototaxis has been observed in many desmids (Gerrath 1993).

Mitosis and development of new semi-cells in Desmidiales

In *Closterium*, the first sign of impending mitosis is the appearance of indentations in the two chloroplasts at positions about two-fifths of the distance from the centrally located nucleus. These represent the early stages of chloroplast division by constriction, which is mediated by actin microfilaments (Hashimoto 1992). The nuclear envelope breaks down in prophase, and a precocious cleavage furrow begins to appear at the same time. At telophase, as furrowing continues, the spindle abruptly disintegrates, and transverse microtubules develop near the forming cross-wall (Pickett-Heaps and Fowke 1970), but a phragmoplastlike apparatus is absent from *Closterium*. Upon completion of cross-wall development, the chloroplast constrictions become deeper, and a vacuole forms in the resulting groove. The vacuole then increases in size as the chloroplasts continue to constrict. After the daughter cells separate from each other, the chloroplasts complete their division, and the nucleus moves into a central position between them. The cells then elongate on the side closest to the cross-wall so that cell symmetry is reestablished.

In placoderm desmids that are constricted into semi-cells, mitosis is followed by expansion in both daughter cells of a new semi-cell (Figure 20.28). The process by which these new semi-cells come to look almost identical to the older ones is still not completely understood. It has primarily been studied in *Closterium*, *Cosmarium*, *Euastrum*, and *Micrasterias* (Waris 1950; Gerrath 1993; Pickett-Heaps

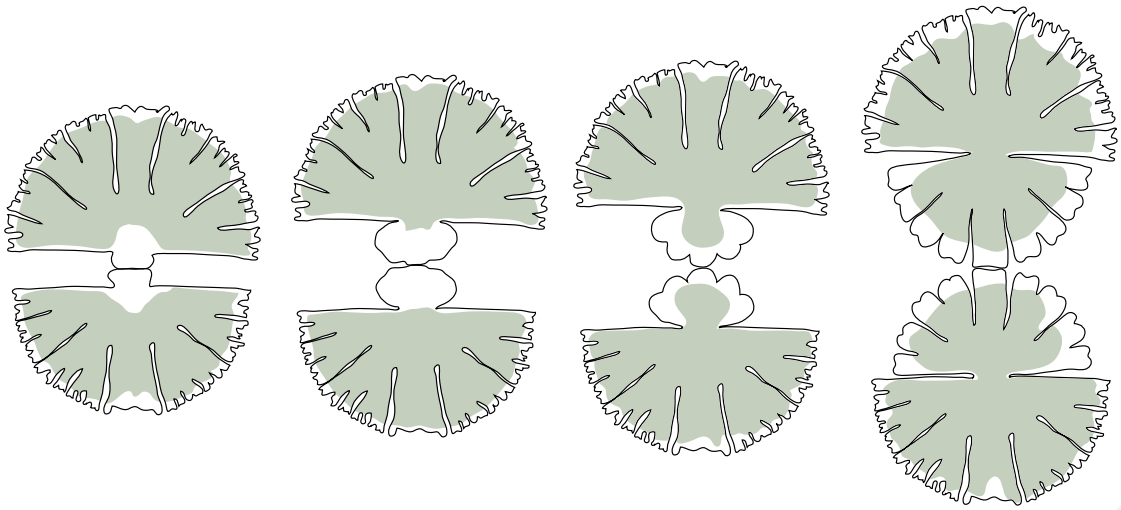

Figure 20.28 Development of new semi-cells in *Micrasterias*. New semi-cells expand from and gradually develop the intricate detail of the older semi-cells.

1975). In highly constricted desmids, the nucleus undergoes what is known as post-telophase migration after the formation of a cross-wall; this is necessary to provide a pathway through the constricted isthmus region for expansion of the chloroplast from the old semi-cell into the newly developing semi-cell. An urn-shaped cage of microtubules radiating from a microtubular organizing center often surrounds the moving nucleus, suggesting a mechanism for organellar transport—possibly microtubule-associated motor proteins. In *Euastrum oblongatum*, nuclear movement begins about 80 minutes after cross-wall formation (Url et al. 1993). Usually the nucleus gets out of the way by moving into the expanding semi-cell, but in some cases the nucleus migrates into the older semi-cell. Expansion of the new semi-cell appears to be driven by turgor pressure because plasmolysis stops the expansion process. As the new semi-cell develops, the nucleus migrates back to its usual position in the isthmus, between old and new semi-cells. In *Euastrum oblongatum*, the nucleus begins to move back to the isthmus about 12 hours after the new semi-cell has been completely formed (Url et al., 1993). A new semi-cell is completely formed within about 16 hours in *Micrasterias* (Kiermayer 1981; Meindl 1983).

Lobing of new semi-cells in genera such as *Micrasterias* seems to occur by greater deposition of primary wall material at the tips of the growing lobes as compared to regions between lobes (Kiermayer and Meindl 1989). The factors influencing this differential distribution of wall material are not well understood. However, it is hypothesized that at the cross-wall development stage, the cell membrane is chemically imprinted with a pattern (Kiermayer 1981) that may later influence localized accumulation of calcium ions and targeted fusion of pectin-bearing Golgi vesicles at the lobes. Cellulose-synthesizing rosettes also appear to be directed to lobe regions, such that cellulose synthesis occurs more rapidly there. Rosettes are thought to arrive at the cell membrane as Golgi-derived vesicles, but the mechanisms by which they are directed to lobe regions are unknown. After primary wall deposition ends, development of the secondary wall of *Micrasterias denticulata* occurs for eight or so hours. Flat Golgi-derived vesicles deliver hexagonal arrays of cellulose-synthesizing rosettes to the cell membrane; these spin out bands of 2–17 adherent cellulose microfibrils (Giddings et al. 1980; Kiermeyer and Meindl 1984). At the beginning of secondary wall deposition, the cell membrane

develops concave, circular invaginations that are about 0.2 μm in diameter. These mark the positions where pores will develop. Pore vesicles containing neutral polysaccharides then deposit these materials at the invaginations. These polysaccharides prevent cellulose microfibril deposition across the sites; subsequent disappearance of the plugs occurs when secondary wall development has been completed, leaving open pores in the walls.

Reproduction in Desmidiales

Some evidence indicates that desmidialeans mediate sexual reproduction by chemical communication. A 20 kDa heat-labile, diffusible protein produced by one of the mating types of *Closterium ehrenbergii* induces mitotic divisions that generate gametes in the other mating type (Fukumoto et al. 1997). In this species, vegetative cells were observed to possess two or four times the nuclear DNA level of gametes. There is no increase in DNA level immediately prior to the divisions that give rise to gametes, and thus gametes possess half the DNA of parental vegetative cells. Desmid zygotes often include unfused gamete nuclei that fuse only shortly before or during germination, which involves meiosis. When zygotes undergo meiosis, DNA levels are reduced, as expected, but frequently two or three of the nuclear products do not survive. In the surviving meiotic nuclei, the DNA level is duplicated twice and then the cell divides, partitioning DNA such that each daughter cell nucleus receives at least two copies of the genome. This process restores the typical vegetative DNA level.

In placoderm desmids, the first cells produced from zygotes are known as **gones**. These cells do not generally resemble normal vegetative cells; they are typically less ornamented. When gones divide by mitosis, new semi-cells are produced that exhibit a normal morphology. Early spring collections from nature or laboratory cultures established from recently germinated zygotes may contain some cells having both a typical semi-cell and a gone semi-cell. Asexually produced resting cells have been reported for several genera of placoderm desmids (Gerrath 1993).

Ecology of Desmidiales

Placoderm desmids are particularly common and diverse in oligotrophic (low-nutrient) and dystrophic (highly colored) lakes and ponds (Woelkerling 1976). In nutrient-poor streams, desmids can make up some 2%–10% of the community, and they are persistent residents, rather than forms that have come to be there via incidental drift. More than 200 desmid species have been observed among stream periphyton (algae attached to substrates), associated with plants such as the moss *Fontinalis*. There, desmids may achieve cell concentrations as high as 10^6 per gram of substrate (Burkholder and Sheath 1984). Some species occur in mesotrophic (higher-nutrient) and eutrophic (high-nutrient) water bodies. *Closterium aciculare* is regarded as an indicator of eutrophic conditions and is sometimes abundant, occasionally growing to bloom proportions. This species is unable to utilize nitrate as a source of combined nitrogen because nitrate reductase is lacking (Coesel 1991), and a requirement for ammonium ion may explain the occurrence of this desmid in highly eutrophic waters. Cells of the very unusual, slow-growing *Oöcardium stratum*

live at the tops of branched calcareous tubes in calcareous streams and waterfalls, in association with deposits of tufa and travertine. A number of placoderm desmids occur in soils and other terrestrial habitats, such as on moist rocks and among bryophytes.

Diversity of Desmidiales

Desmidiales appear to form a monophyletic group (McCourt et al. 2000; Turmel et al. 2002; Hall et al. 2008) of about 3000 described species (Gerrath 1993). Genus and species definitions have classically depended upon characters of the cell wall, including wall pores, ridges, knobs, and spines; chloroplast morphology; and zygote structure. A valuable source of classic taxonomic information is the series by Prescott et al. (1975, 1977, 1981, 1982) and Croasdale et al. (1983) on North American desmid taxa.

In recent years, researchers have used molecular approaches to test the reliability of morphological features in predicting relationships. A phylogenetic analysis of 39 strains of *Staurastrum* and the related genera *Staurodesmus*, *Cosmarium*, *Xanthidium*, and *Euastrum* was performed by Gontcharov and Melkonian (2005), who found that some genera are not monophyletic. Hall et al. (2008) discovered that several of the classic desmid genera are polyphyletic, and certain of the structural features used in taxonomy are questionable. Some species originally placed in *Cosmarium*, *Staurodesmus* and *Triploceras* nest within the monophyletic genus *Micrasterias*, according to multigene phylogenetic analysis (Škaloud et al. 2011). Surprises such as these reveal that form is not always a reliable indicator of desmid relationships. Representative unicellular placoderm genera are described here first, followed by colonial and filamentous forms.

CLOSTERIUM (Gr. *klosterion*, small spindle) (Figure 20.29) cells are not constricted into semi-cells, but pores occur in the walls. Vegetative cells are crescent shaped or elongate and somewhat curved. There is a single axial, ridged plastid with several pyrenoids in each semi-cell and a central nucleus. Conspicuous vacuoles occur at the cell tips; these contain barium sulfate crystals (Brook 1980) that move by Brownian motion. The composition and synthesis of pectin and protein components of the cell wall of *C. acerosum* were studied by Baylson et al. (2001). Almost all natural populations of the *Closterium ehrenbergii* species complex have been observed to have distinct mating types segregated in a 1:1 ratio upon zygote germination. A single gene (*mt*) determines mating type, and the mt^- allele is dominant to mt^+ (Kasai and Ichimura 1990; Tsuchikane et al. 2003). MIKC-type MADS-box genes similar to those important in land plant development were identified in the *C. peracerosum–strigosum–littorale* complex; gene expression increased when vegetative cells started to develop into gametangia and decreased following fertilization (Tanabe et al. 2005). About 140 species of *Closterium* have been described.

COSMARIUM (Gr. *cosmarion*, small ornament) (Figure 20.30) occurs as single cells that are deeply divided at the midregion to form a short isthmus and two semi-cells that are rounded in front view but flattened, oval, or elliptical in side view. Walls may be smooth or ornamented; spines are not present on vegetative cells. One or sometimes

Figure 20.29 *Closterium*. (a) Needlelike species. (b) Autofluorescence of ridged, axial plastid. (c) Crescent-shaped species. (d) Large species with numerous pyrenoids. Note the terminal vacuoles with barium sulfate crystals in (c) and (d). (Photos: L. W. Wilcox)

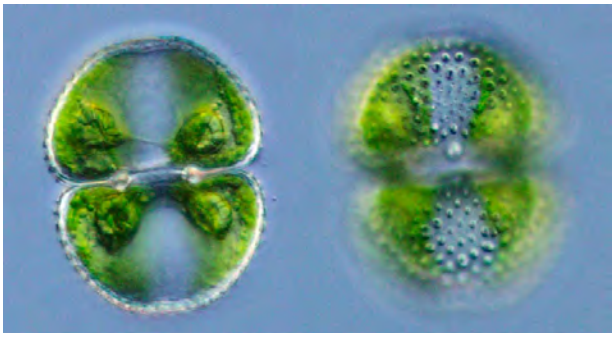

Figure 20.30 *Cosmarium* thru-focus pair. (a) The pyrenoids and associated starch are visible at this level. (b) Nearer the surface, mucilage-extruding pores are visible. (Photos: L. W. Wilcox)

more axial or parietal pyrenoid-bearing chloroplasts occur in each semi-cell. Sexual reproduction in *Cosmarium turpinii* involves separate mating types, with pairs of cells becoming enclosed by mucilage. Mating cells open at the isthmus, and the emerging protoplasts function as gametes. Their fusion results in the production of a thick-walled, spiny zygote, which can often be found together with the empty parental cell walls, enclosed in the same enveloping mucilage. More than 1000 species have been described, but this genus does not form a monophyletic group (Gontcharov and Melkonian 2005; Hall et al. 2008).

TETMEMORUS (L. *tet*, four + L. *morus*, referring to mulberry) (Figure 20.31) occurs as single cells with a distinctive notch at the apex of each semi-cell and a relatively shallow constriction at the isthmus. Hall et al. (2008) found *Tetmemorus* to group with some *Euastrum* species. Both genera have a notch at the tips of the semi-cells; semi-cells of *Euastrum* are typically more compressed than those of *Tetmemorus*.

Figure 20.31 *Tetmemorus*. This desmid has a prominent incision in the apex of each semi-cell. (Photo: L. W. Wilcox)

EUASTRUM (Gr. *eu*, good, true, or primitive + Gr. *astron*, star) has flattened cells (Figure 20.32). There is a characteristic notch in the apices of most species. Semi-cells are typically lobed, and there may be small bumps on the surface that are visible at the light microscopic level in side view. The incision between semi-cells is usually closed, as the semi-cell walls come close together. One or sometimes two pyrenoid-bearing chloroplasts occur in each semi-cell. Chloroplasts are often conspicuously ridged. Zygotes are round and ornamented with spines or other protuberances. There are some 265 described species that do not form a monophyletic group (Hall et al. 2008).

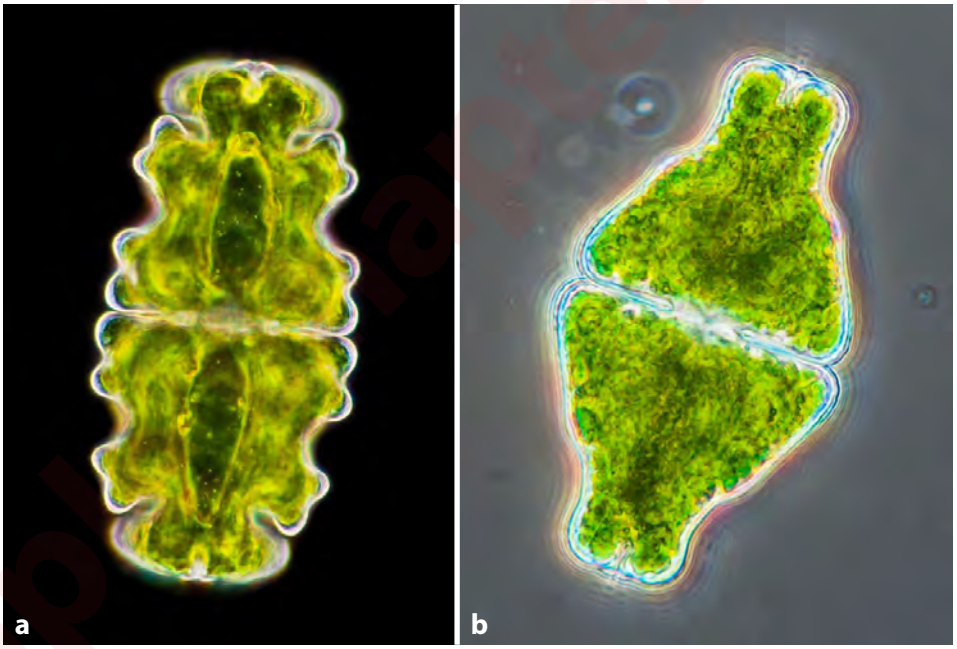

Figure 20.32 *Euastrum*, a lobed, flattened placoderm desmid. Two species are shown here. (Photos: L. W. Wilcox)

XANTHIDIUM (Gr. *xanthos*, yellow) unicells are mostly characterized by wall protuberances rising from semi-cell surfaces perpendicular to the flat plane of the surface (Figure 20.33). The protuberances may be ornamented with pits or granules or may be pigmented, but these features are difficult to see at the light microscopic level. Another distinctive feature is robust, often paired spines. In general, the wall is less ornamented than that of *Cosmarium*, *Euastrum,* or *Staurastrum*. There are usually four axial or parietal plastids, each with a pyrenoid, per semi-cell.

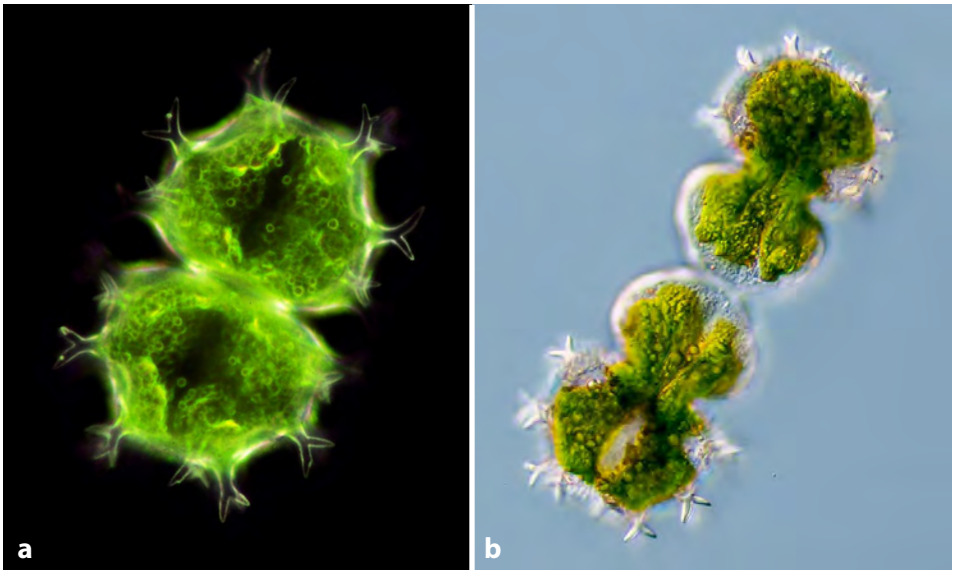

Figure 20.33 *Xanthidium*. This genus is characterized by three-dimensional protuberances on each of the semi-cells (a). In (b), a dividing cell is shown. Note that the characteristic protuberances have yet to form on the new semi-cells. (Photos: L. W. Wilcox)

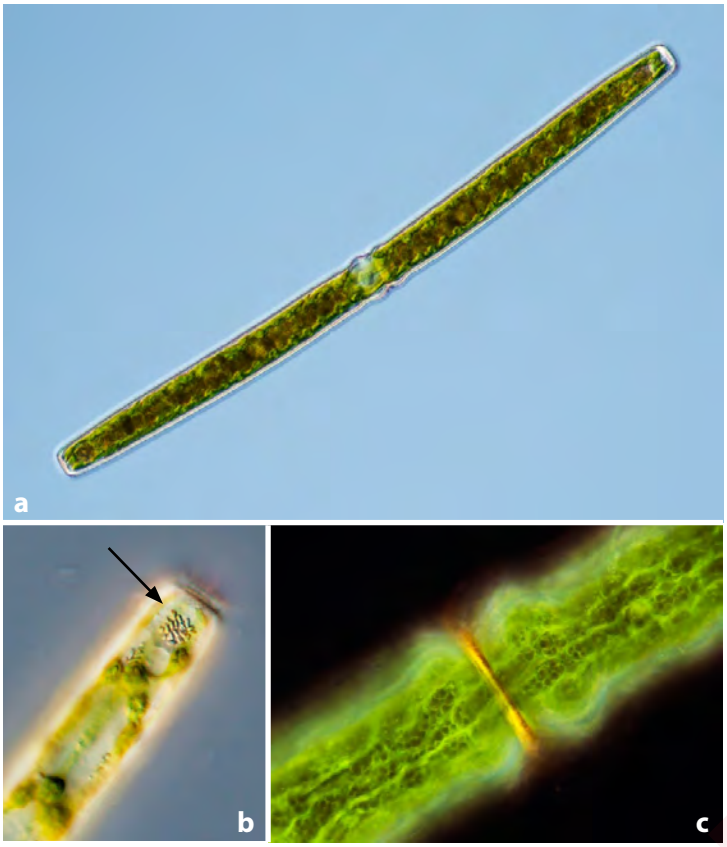

Figure 20.34 ***Pleurotaenium.*** In (a), an entire cell is shown. Chains of several cells that adhere to one another following cell division are sometimes encountered. (b) As in *Closterium*, barium sulfate crystals are seen in terminal vesicles (arrow). (c) Some species have an orange-yellow ring at the semi-cell junction. (Photos: L. W. Wilcox)

Zygotes have only rarely been observed; they are round, and most bear spines. Approximately 115 species have been described.

PLEUROTAENIUM (Gr. *pleuron*, rib + Gr. *tainia*, ribbon) (Figure 20.34) is a cylindrical desmid with long, blunt-ended cells. Cells are 4–35 times longer than they are wide. There may be a noticeable ringlike thickening where semi-cells join. The chloroplast appears as parietal bands or axial with lamellae, and pyrenoids are present. Sometimes apical vacuoles with crystalline inclusions are present.

MICRASTERIAS (Gr. *mikros*, small + Gr. *aster*, star) is flattened and often highly incised and lobed. Some species look like flattened disks, and others are so highly dissected that they look like stars (Figure 20.35). Each semi-cell has a single large lobed plastid that extends into lobes of the cell. Plastids are studded with numerous pyrenoids. The nucleus lies in the isthmus. Conjugation involves the formation of papillae that allow gamete fusion. *Micrasterias foliacea* is exceptional within the genus for its ability to form filamentlike arrays by overlapping polar lobes and interlocking apical teeth (Lorch and Engels 1979). Zygotes are usually spherical, with spines that are sometimes forked. Gones are very simple in construction. *Micrasterias* appears to be a monophyletic lineage (Škaloud et al. 2011).

TRIPLOCERAS (Gr. *triploos*, triple + Gr. *keras*, horn) cells are elongate, 8–20 times longer than they are wide, and not noticeably

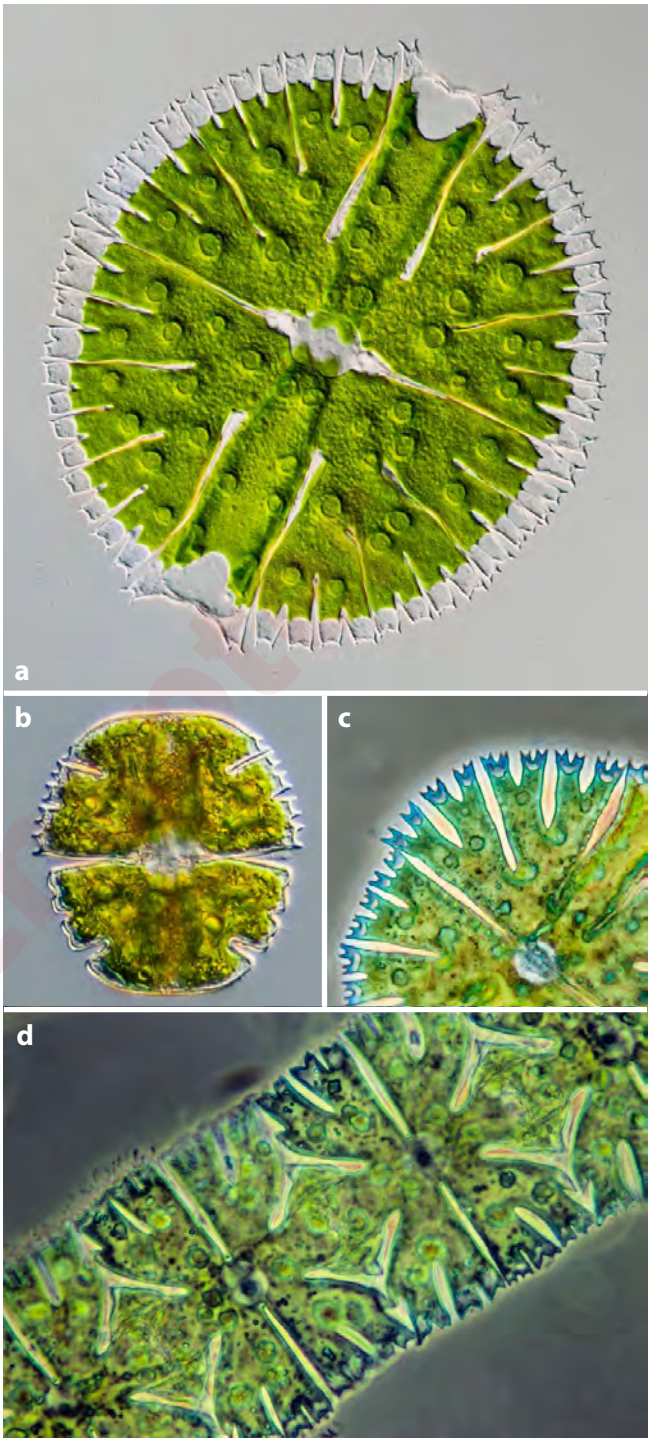

Figure 20.35 **Diversity of *Micrasterias*.** (a)–(c) Three unicellular species. (d) *M. foliacea*, which forms filaments by means of interlocking prongs on the ends of semi-cells. (Photos: L. W. Wilcox)

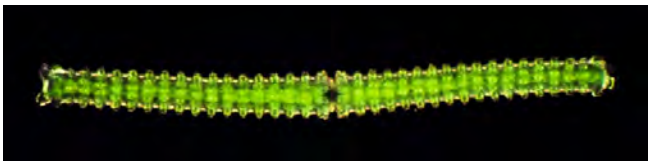

Figure 20.36 ***Triploceras.*** (Photo: L. W. Wilcox)

constricted. The cells are ornamented with various kinds of spines and have undulating margins (Figure 20.36).

STAURASTRUM (Gr. *stauron*, cross + Gr. *astron*, star) are highly constricted unicells that are radially symmetrical in end-on (polar) view. The semi-cells are often triradiate or hexaradiate and may be highly ornamented with spines and other protuberances (Figure 20.37). The walls are impregnated with polyphenolic compounds that confer decay resistance (Gunnison and Alexander 1975a,b). These materials explain the recovery of fossil remains of *Staurastrum* walls from lake sediment cores that are thousands of years old. At conjugation, gamete protoplasts escape as the semi-cell walls separate at the isthmus; their fusion generates spiny zygotes. Zygote germination, presumably by meiosis, produces one to four gones. Some 800 species have been described, primarily on the basis of cell wall characters. Sequencing of the chloroplast genome of *S. punctulatum* revealed substantial differences from those of some other streptophyte algae and bryophytes in terms of size, gene order, and intron content (Turmel et al. 2005).

SPONDYLOSIUM (Gr. *spondylos*, vertebra) is a filamentous form consisting of flattened cells that are deeply constricted (Figure 20.38). Cell walls are not highly ornamented. Plastids are axial. Filaments are often twisted and have an extensive sheath. Zygotes are globose and smooth walled or have short spines. The genus is not monophyletic, according to a phylogenetic analysis by Lane et al. (2008).

ONYCHONEMA (Gr. *onyx*, claw + Gr. *nema*, thread) is a twisted filamentous arrangement of small, deeply constricted cells (Figure 20.39). Semi-cells possess distinctive overlapping apical processes that can be nearly as long as the semi-cells themselves. There is a wide mucilage sheath. Zygotes are globose with short spines.

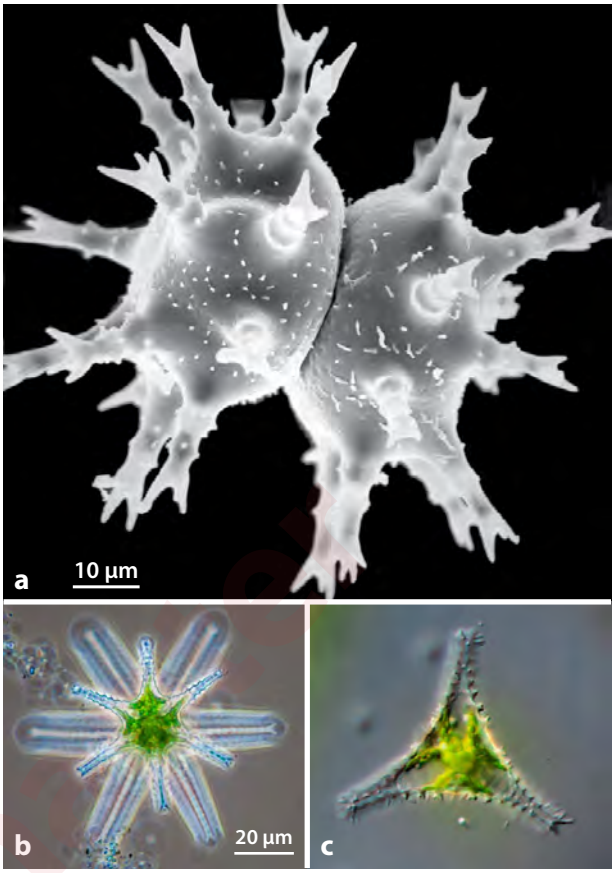

Figure 20.37 *Staurastrum*. (a) A species with multiple arms viewed with SEM. (b) An end-on view of one of the semi-cells of a species having six arms. The longer arms on the other semi-cell are out of focus. (c) A species with three arms. (a: S. Cook; b, c: L. W. Wilcox)

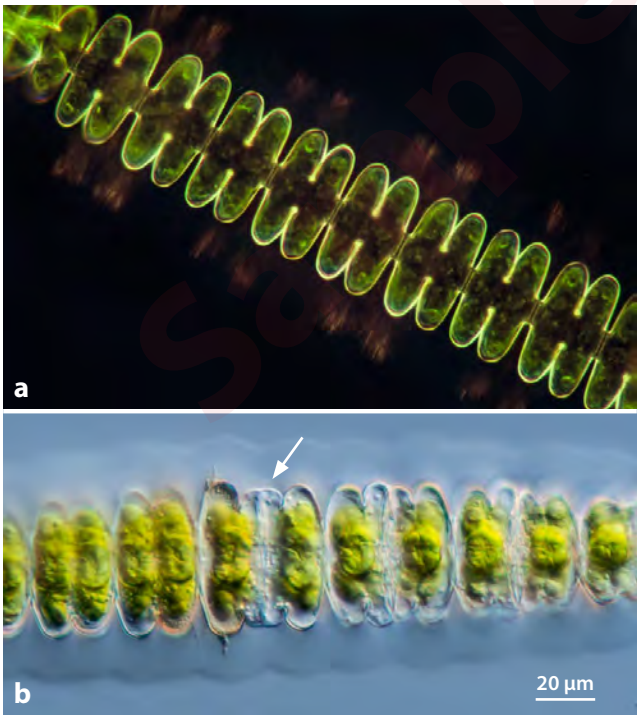

Figure 20.38 *Spondylosium*, a filamentous desmid whose semi-cells are deeply incised. There is a substantial mucilaginous sheath in which bacterial cells often occur. (a) The fine details in the sheath of this specimen represent structure inherent in the sheath. (b) A dividing cell is evident (arrow). (Photos: L. W. Wilcox)

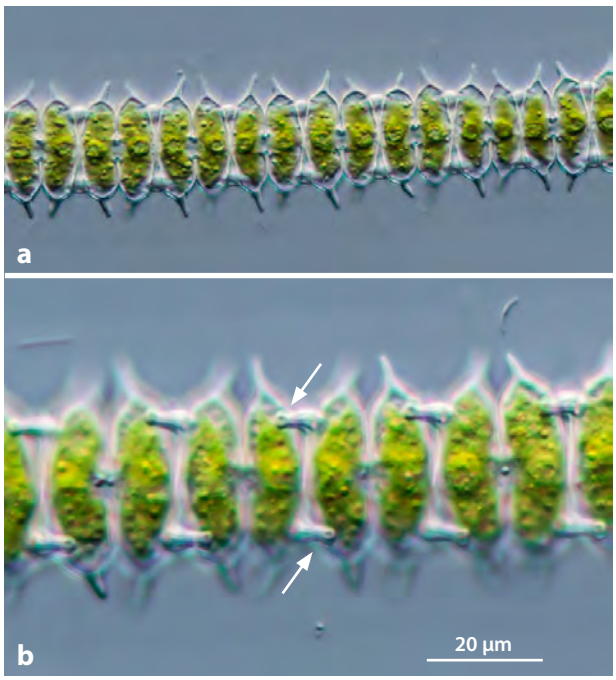

Figure 20.39 *Onychonema*, a filamentous desmid. (a) Arrangement of cells in the filament. (b) Closer view, focused on the apical processes (arrows) that interlock adjacent cells. (Photos: L. W. Wilcox)

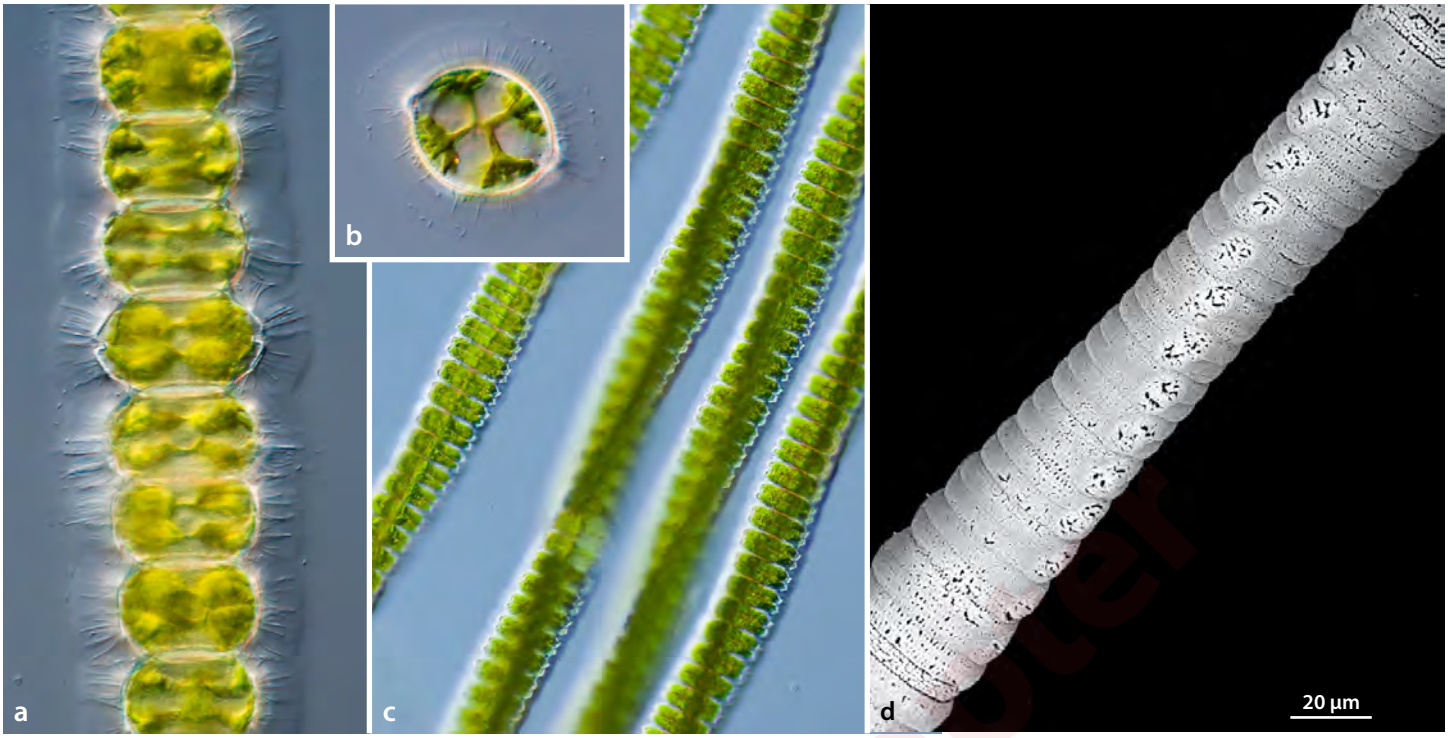

Figure 20.40 *Desmidium*, a filamentous genus. (a) *Desmidium* species with numerous bacteria living within the mucilaginous sheath. Note that the cell profiles change in appearance due to twisting of the filament. (b) View from the end of an isolated semi-cell from a biradiately symmetrical species showing sheath and branched chloroplast. (c) Another species with more obvious twisting of the filaments. (d) SEM view showing the twisted orientation of adjacent cells. The mucilaginous sheath was maintained in this preparation. The periodic indentations reflect underlying constrictions between semi-cells and adjacent cells in the filament. (a–c: L. W. Wilcox; d: From Fisher and Wilcox. 1996. Desmid-bacterial associations in *Sphagnum*-dominated Wisconsin peatlands. *Journal of Phycology* 32:543–549)

DESMIDIUM (Gr. *desmos*, bond) often has a prominent gelatinous sheath. Cells are bi-, tri-, or quadriradiate in end view, depending upon the species. The filaments appear to be spirally twisted because the axes of adjacent cells are offset by a slight angle (Figure 20.40). There are two axial chloroplasts with pyrenoids per cell; there is one plastid per semi-cell. Filaments can fragment. Conjugation tubes are formed between opposing cells of paired filaments, and zygotes form within the tubes or in one of the parental cell walls, depending upon the species. Zygotes are round or ellipsoidal and are smooth walled or have short projections.

BAMBUSINA (named for *Bambusa*, a genus of bamboo [bambu, Indian vernacular name]) cells are cylindrical or barrel shaped and arranged in linear series to form filaments (Figure 20.41). There is only a slight constriction, and the wall region on either side of the isthmus is swollen. Fine striations may be visible at the apices. The axial chloroplasts have radiating lamellae and a single central pyrenoid. Zygotes are globular or elliptical, and their walls are smooth. According to the phylogenetic analysis of Lane et al. (2008), *Bambusina* is sister to *Hyalotheca*.

HYALOTHECA (Gr. *hyalos*, glass + Gr. *theke*, sheath or box) is a filamentous member of the placoderm desmids (Figure 20.42). Each cell is indented at the midpoint, but the indentation may be very slight. Cells contain two axial, radiately ridged chloroplasts with pyrenoids, one per semi-cell. There is an extensive mucilaginous sheath. Filaments can break apart into fragments, a form of asexual reproduction. Conjugation involves formation of conjugation tubes; zygotes may form in the tubes or within one of the parental cell

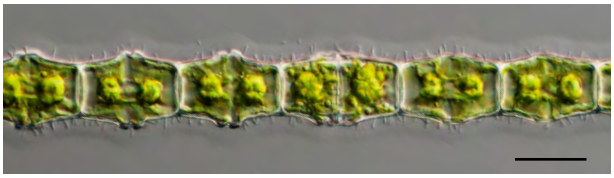

Figure 20.41 *Bambusina*, a filamentous desmid whose semi-cells are not deeply incised. Scale bar = 20 µm. (Photo: L. W. Wilcox)

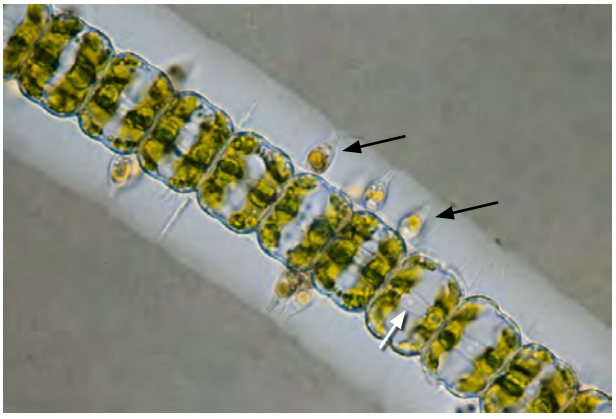

Figure 20.42 *Hyalotheca*. This filamentous desmid often has a wide mucilaginous sheath; it is shown here in an India-ink preparation, which allows the sheath to be better visualized. Sessile chrysophytes (black arrows) are sometimes found associated with *Hyalotheca* and other filamentous desmids having similar sheaths. Note also the central nuclei in the cells (white arrow). (Photo: L. W. Wilcox)

walls. Zygotes are globose and smooth walled. Meiosis occurs during zygote germination and there are two filaments produced. Cells of filaments can round up and dissociate into thick-walled aplanospores, a form of asexual reproduction.

Coleochaetales

CHAETOSPHAERIDIUM (Gr. *chaite*, long hair + Gr. *sphaira*, ball), with only 4 described species (Thompson 1969) (Figure 20.43), and ***COLEOCHAETE*** (Gr. *koleos*, sheath + Gr. *chaite*, long hair), including approximately 16 species (Pringsheim 1860; Jost 1895; Printz 1964; Szymánska 1989; Szymánska 2003) (Figure 20.44), are the only widely accepted members of the order Coleochaetales. Molecular data support the concept that Coleochaetales forms a monophyletic group (Lewandowski and Delwiche 2001; Delwiche et al. 2002) (see Figure 20.1).

Prominent among the characters that *Chaetosphaeridium* and *Coleochaete* share are sheathed hairs, known as seta cells. These extensions of the cell wall enclose a small amount of cytoplasm and are believed to serve as protection against herbivores (Marchant 1977) (Figure 20.45). The hairs may attain a length greater than 100 times the vegetative cell diameter; hairs are typically straight but may be coiled. A rigid sheath of wall material encloses the bases of hairs, which often break off at the sheath rim. Whereas all *Chaetosphaeridium* cells bear one or more sheathed hairs, only 3%–5% of the cells of *Coleochaete scutata* bodies produce hairs, and then only one per cell (McBride 1974). The walls of *Coleochaete* seta cells are composed of several layers whose complex ultrastructure has been compared with that of liverwort rhizoids (Graham et al. 2010). Seta cell bases of *Chaetosphaeridium* are of simpler construction. The chloroplasts of *Coleochaete* seta cells typically have a C-shaped appearance (Figure 20.46) and may rotate.

Figure 20.43 *Chaetosphaeridium*. The spherical cells of *Chaetosphaeridium* are actually linked to form branched filaments, though this is often difficult to discern. (a) The top cell has divided to form the lower cell. Note the sheathed hair (arrow), a hallmark of the order Coleochaetales. (b) SEM view of connected *Chaetosphaeridium* cells. The largest, central cell has given rise to three others and one of these (at left) has recently divided. Sheathed hairs have yet to develop on the cells lacking them. (a: M. E. Cook; b: Kevin M. Kocot)

Structure and development of Coleochaetales

Although *Chaetosphaeridium* appears, at first glance, to be composed of single cells held together by a gelatinous matrix, it is actually a branched filament (Thompson 1969). Thin colorless branches that are difficult to discern with the light microscope interconnect *Chaetosphaeridium* cells. Division is regularly oblique to the plane of the substrate, giving rise to a new cell located somewhat beneath the parental cell. A bulge develops into which the new protoplast moves,

Figure 20.44 Morphological diversity of *Coleochaete*. (a) *Coleochaete orbicularis* has a body that is one cell layer thick. It grows by means of a marginal meristem. (b) *C. soluta* is a pseudoparenchymatous species, composed of laterally adherent branched filaments enclosed within a mucilage layer. (c) *C. pulvinata* is a heterotrichous, branched filament; there is a prostrate system of branches and an erect system of branches, all enclosed by extensive mucilage. (a, b: M. E. Cook; c: L. W. Wilcox)

Figure 20.45 Sheathed hairs of *Coleochaete pulvinata*. (a) Close-up of a single sheathed hair (arrow). (b) *C. pulvinata* thallus at lower magnification showing the numerous hairs as well as the extracellular mucilage. (Photos: L. W. Wilcox)

and then the new cell grows into a position next to the parental one. The portion of the cell lying beneath the parental cell collapses, and the new cell develops a hair. Thompson (1969) did not consider this form of growth to be equivalent to the apical growth exhibited by *Coleochaete*. *Chaetosphaeridium* and *Coleochaete* generate abundant extracellular mucilage that includes prokaryotic and eukaryotic microbes (Figure 20.47).

Many species of *Coleochaete* appear to possess terminal or marginal meristems, meaning that the only cells that undergo vegetative mitotic divisions are those at the tips or edges of bodies. *Coleochaete* species exhibit an unusual degree of body variability (see Figure 20.44). *Coleochaete pulvinata* consists of both radially symmetrical prostrate portions whose branched filaments grow flat against the substrate and radially symmetrical, erect branching systems. Some other species occur only as radially branched prostrate filaments, and yet others are prostrate branched filaments that lack radial symmetry. *Coleochaete orbicularis* and *C. scutata* are radially symmetrical species that grow as a single discoid, tissuelike layer of cells; such forms have been used as model systems for understanding principles of plant morphogenesis (Dupuy et al. 2010). Surfaces of the tissuelike forms are coated with a ridged material (Marchant and Pickett-Heaps 1973) (Figure 20.48) that exhibits some similarities to cuticles of land plants, especially those of various bryophytes (Cook and Graham 1998).

When grown under aeroterrestrial conditions, *Coleochaete* species that are disk-shaped in aquatic media instead occur as degradation-resistant, hairless, multilayered hemispheres (Figure 20.49) or irregular clusters of cells that resemble certain Cambrian microfossils (Graham et al. 2011). Air-dried *Coleochaete* is also resistant to desiccation, retaining integrity and green coloration for several months, and *Coleochaete* has the ability to generate zoospores when moistened after being exposed to air for a week. These features suggest that ancient,

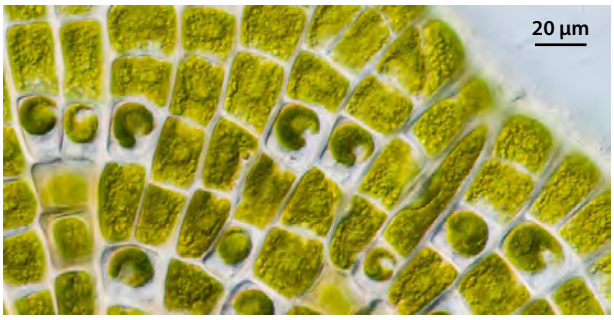

Figure 20.46 C-shaped plastids in hair cells of *Coleochaete orbicularis*. (Photo: L. W. Wilcox)

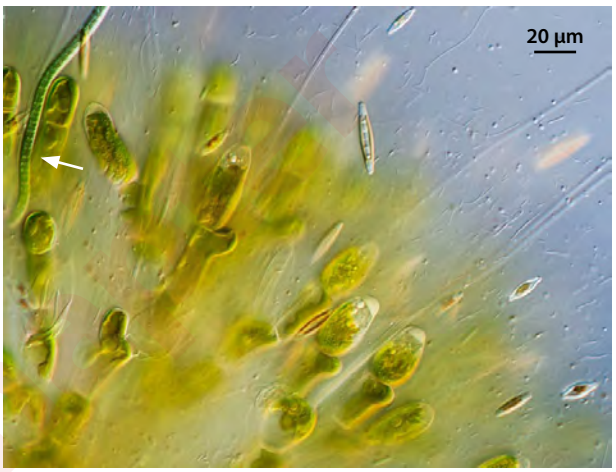

Figure 20.47 Microbes within mucilage of *Coleochaete pulvinata*. Various types of bacteria (including a cyanobacterium—arrow) are present in this image as well as diatoms. *Coleochaete* hairs are also evident. (Photo: L. W. Wilcox)

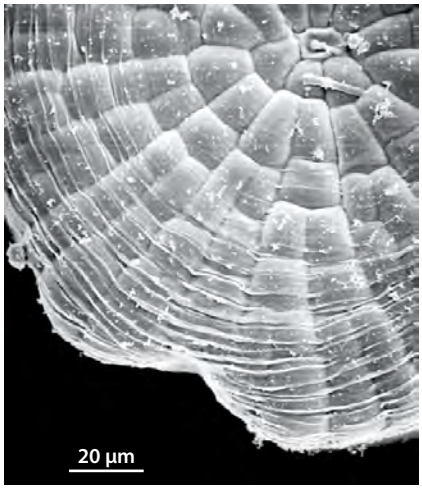

Figure 20.48 Surface cuticle-like material on *Coleochaete*. (SEM: S. Cook)

complex streptophyte algae might have been able to colonize land and leave behind distinctive microfossil remains.

Cell biology of Coleochaetales

Chloroplasts occur singly in most cells of *Chaetosphaeridium* and *Coleochaete*, and these plastids contain one or more pyrenoids that are similar to those of other streptophyte algae and hornworts (Graham and Kaneko 1991). The thylakoids of *Coleochaete* and *Chaetosphaeridium* are organized into grana, as are those of land plants.

In addition to plantlike cellulose (see Figure 20.2) and xyloglucans (Fry et al. 2008), *Coleochaete* is known to produce hydrolysis-resistant ligninlike cell wall phenolic polymers (Delwiche et al. 1989; Sørenson et al. 2011) (Figure 20.50). Such tough polymers have been proposed to allow the formation of microbial associations with reduced risk of hydrolytic damage to host tissues, a property that would have fostered survival of early terrestrial streptophytes and provided a metabolic foundation for the evolutionary diversification of key plant phenolic pathways (Graham et al. 2014).

Vegetative cell division has been studied at the ultrastructural level in *Coleochaete scutata* (Marchant and Pickett-Heaps 1973) and *C. orbicularis* (Graham 1993; Brown et al. 1994; Cook 2004a; Doty et al. 2014), as well as in *C. soluta*, *C. irregularis*, and *Chaetosphaeridium globosum* (Doty et al. 2014). Pairs of centrioles appear at the poles of developing spindles, and the nuclear envelope begins to disintegrate at prometaphase, such that mitosis is open. In *Coleochaete*, the plantlike peroxisome becomes closely associated with the plastid and divides (by invagination) at the same time, thus achieving regular partitioning to daughter cells. In contrast, as noted earlier, other streptophyte algae partition their peroxisomes to daughter cells in different ways.

Despite earlier reports, *Coleochaete* does not display two types of cytokinesis. Rather, cytokinesis consistently involves a phragmoplast similar to that present in land plants. Polarized cytokinesis, in which the phragmoplast and cell plate contact one cell wall and then progress toward the opposite wall, occurs in *Coleochaete* (Cook 2004a; Doty et al. 2014) and *Chaetosphaeridium* (Doty et al. 2014) and in highly vacuolated cells of *Arabidopsis*. As in the cases of land plants and charalean algae, the cross-walls of *Coleochaete* are penetrated by numerous plasmodesmata, whereas these are absent from the cross-walls of other streptophyte algae. The evolutionary origin of land plant plasmodesmata is significant because these structures are developmentally very important in land plants (Cook et al. 1997). Plasmodesmata may have been a necessary prerequisite to the origin of histogenetic (tissue-producing) meristems (Cook and Graham 1999).

Asexual reproduction in Coleochaetales

Both *Coleochaete* and *Chaetosphaeridium* produce biflagellate zoospores (lacking eyespots) that serve as asexual reproductive cells. Zoospores generate new bodies of the same type from which they were produced and are capable of rapidly generating a clonal population from a single parental body. In *Chaetosphaeridium*, zoospores can be produced from any cell. Zoospore production in

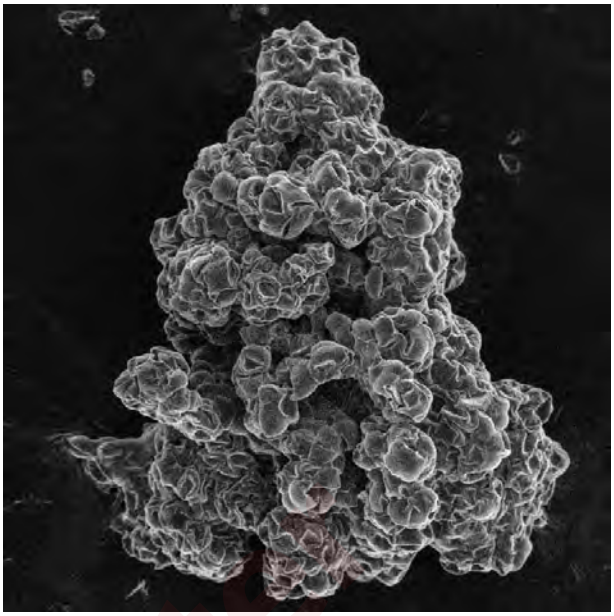

Figure 20.49 Aeroterrestrial *Coleochaete*. When grown exposed to air, *C. orbicularis* differs in form compared to thalli grown in aquatic habitats (as in Figure 20.44). (SEM: M. E. Cook)

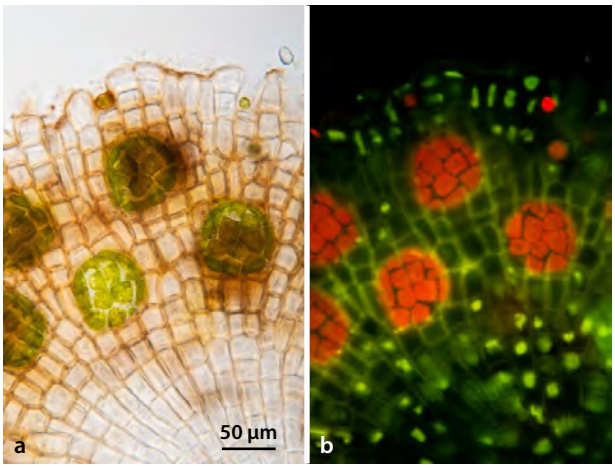

Figure 20.50 Resistant compounds in *Coleochaete*. A portion of a *C. orbicularis* thallus with developing meiospores is seen with brightfield (a) and fluorescence microscopy (b). Brownish walls in brightfield, and yellow-green autofluorescence under blue light localizes ligninlike resistant wall compounds to cell walls covering or near the zygotes. (The bright, yellow-green dots represent the condensed, dead cytoplasm of vegetative cells.) (Photos: L. W. Wilcox)

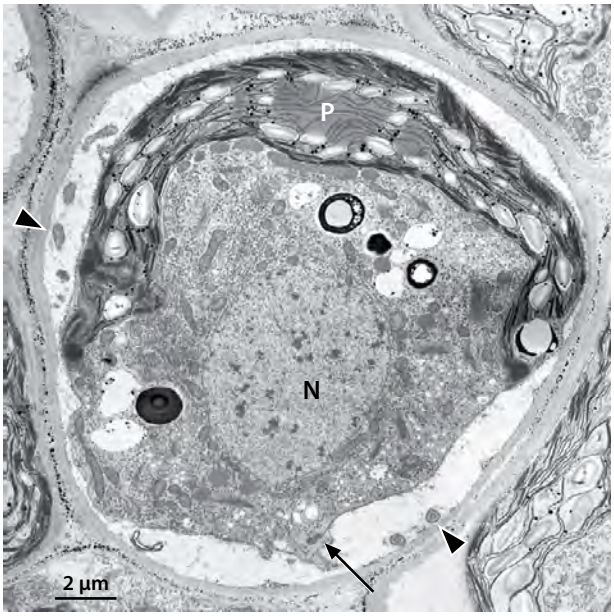

Figure 20.51 *Coleochaete* zoospore prior to release from parental body cell viewed using TEM. Note the single parietal plastid (P) with numerous starch grains, and multilayered structure (arrow). Profiles of the two flagella, which are coiled around the cell, are visible just inside the parental cell wall (arrowheads). (From Graham and McBride. 1979. The occurrence and phylogenetic significance of a multilayered structure in *Coleochaete scutata* spermatozoids. *American Journal of Botany* 66:887–894)

Chaetosphaeridium involves a precursor cell division, and typically the lower cell differentiates into the zoospore, which escapes by dissolution of the cell wall. Usually only a single zoospore is produced per parental cell of *Chaetosphaeridium*, but sometimes two are generated.

Zoospores of *Coleochaete* are always produced singly (Figure 20.51), a precursor division is not involved, and zoospores escape through a specialized discharge pore (Figure 20.52) that is presumed to arise by the localized action of hydrolytic enzymes on the cell wall. Temperature is more influential than irradiance or day length in inducing *Coleochaete* zoospore production (Graham et al. 1986). Zoospores of *Coleochaete* settle, attach to surfaces, and develop a cell wall beneath the scale layer. A transverse cell division then occurs (reminiscent of the growth pattern in *Chaetosphaeridium*), and the upper cell terminally differentiates into a seta cell. The lower cell continues to divide, and its derivatives serve as the marginal meristem, dividing either radially or circumferentially, depending upon spatial constraints.

In both *Chaetosphaeridium* (Moestrup 1974) and *Coleochaete* (Graham 1993), zoospore surfaces and flagella are coated with scales (Figure 20.53) that resemble the lower layer of body scales of *Mesostigma*. The body and flagella of *Coleochaete* gametes are covered with similar scales. The scale covering gives the surface of motile cells a frosty or granular appearance (Thompson 1969), but its function is not completely understood. Flagellate reproductive cells of *Coleochaete* and *Chaetosphaeridium* also exhibit a single MLS-containing flagellar root (see Figure 20.53). In addition, *Coleochaete* contains another flagellar root composed of just a few microtubules (Sluiman 1983). A fibrous connective structure links the two flagellar bases, and the flagella emerge laterally, as is generally characteristic of charophycean motile cells.

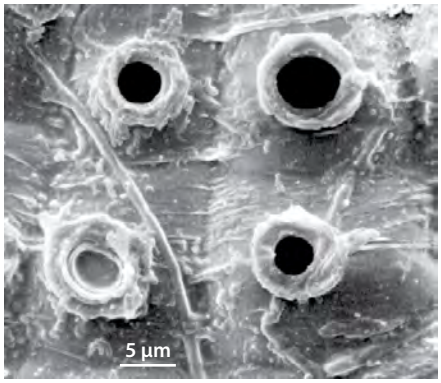

Figure 20.52 *Coleochaete scutata* wall pores through which zoospores were released. (SEM: S. Cook)

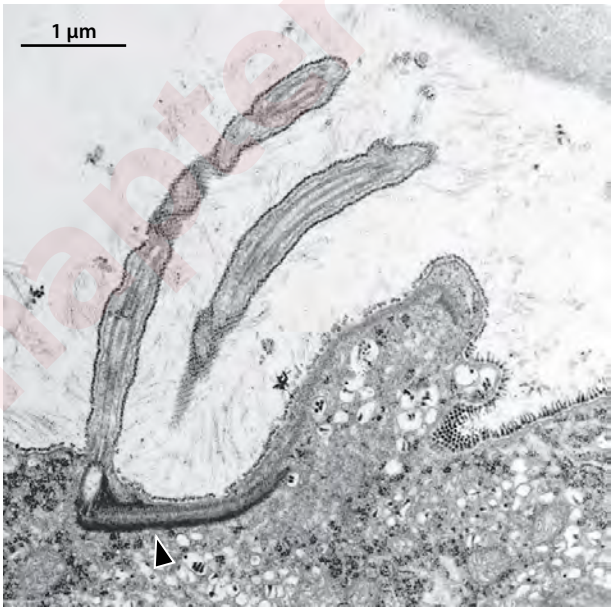

Figure 20.53 *Coleochaete* zoospore. A multilayered structure (arrowhead) is associated with the flagellar bases. Note the presence of numerous scales on the cell and flagellar surface, as well as the numerous long flagellar hairs. These are produced within cytoplasmic vesicles and then transported to the surface. (From Graham and McBride. 1979. The occurrence and phylogenetic significance of a multilayered structure in *Coleochaete scutata* spermatozoids. *American Journal of Botany* 66:887–894)

Sexual reproduction in Coleochaetales

Observations on sexual reproduction made on cultured *Chaetosphaeridium globosum* (Thompson 1969) indicate that egg cells are naked, nonmotile cells that are larger than vegetative cells, and that biflagellate sperm cells are released in pairs from colorless precursor cells. The eggs are expelled before fertilization and held within the body mucilage. Fertilized eggs develop into oval smooth-walled zygotes that are about the same size as egg cells. There is a delay between plasmogamy (cytoplasmic fusion) and karyogamy (nuclear fusion). The zygote wall is several layers thick and internal layers may be yellow or deep brown in color.

In contrast to *Chaetosphaeridium*, egg cells of *Coleochaete* are not released from the body. Rather, they develop a cell wall protuberance that is relatively short in *Coleochaete orbicularis* but may be quite long in *Coleochaete pulvinata* (Figure 20.54). The tip of the protuberance (sometimes called a **trichogyne**) disintegrates when the egg is ready for fertilization (Oltmanns 1898) and exudes cytoplasmic contents that appear to attract flagellate sperm. In *C. pulvinata,* the sperm are produced in small colorless branches that occur in groups on precursor cells near egg cells (see Figure 20.54) (Graham and Wedemayer 1984), while in *C. orbicularis* and *C. scutata,* sperm are formed in small cells occurring in packets derived from asymmetric cell divisions (Figure 20.55). Production of sperm is an exception to the general rule that non-peripheral cells do not divide. Sperm release is by localized wall dissolution.

In *Coleochaete*, sexual reproduction occurs in mid- to late summer, with zygote maturation occurring in the fall. When several species co-occur, it appears that sexual reproduction may be temporally separated, perhaps reflecting a species-isolation mechanism. Zygotes are not released from parental bodies, similar to retention of zygotes within the female gametangia of early-divergent land plants. Immediately after fertilization, zygote cytoplasm at first shrinks and then begins massive enlargement. During enlargement, surrounding vegetative cells are induced to divide, forming a layer of cortical cells that cover zygotes partially or completely, depending on the species (Figure 20.56). In *C. orbicularis* (Figure 20.57), cortical cells exhibit cellular

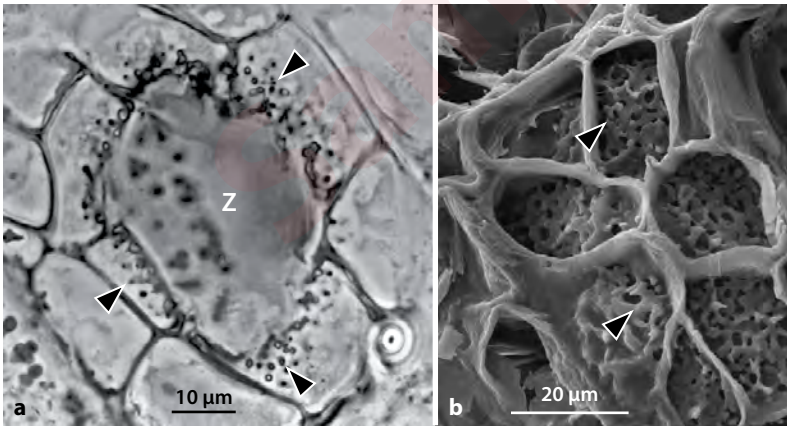

Figure 20.57 *Coleochaete orbicularis* transfer cells. (a) Cells that surround zygotes (Z) have numerous, localized wall projections (arrowheads). Similar wall ingrowths occur at the interface of parent and progeny tissues in land plants. (b) SEM showing transfer cell wall ingrowths adjacent to a zygote on a thallus that had overwintered. The outer walls of the transfer cells had decomposed by the time the image was made. (a: from Graham and Wilcox. 1983. The occurrence and phylogenetic significance of putative placental transfer cells in the green alga *Coleochaete*. *American Journal of Botany* 70:113–120; b: M. E. Cook and L. W. Wilcox)

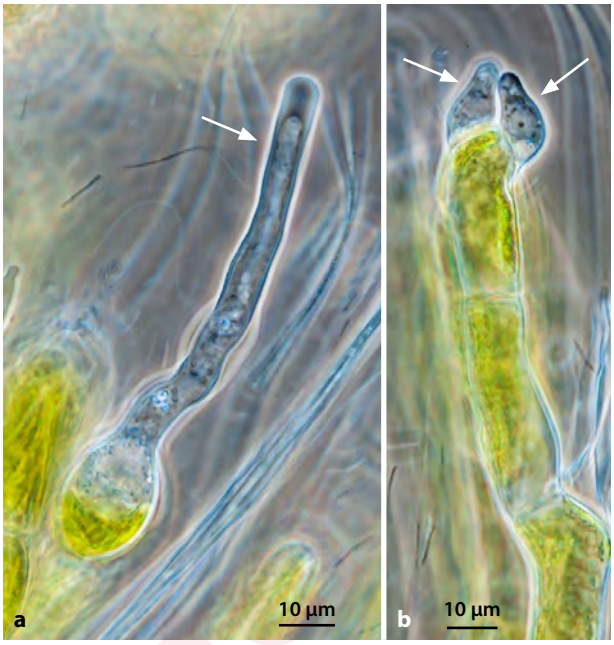

Figure 20.54 *Coleochaete pulvinata* oogonium and antheridia. (a) The oogonium displays an elongate trichogyne (arrow). (b) Small colorless antheridia often occur nearby (arrows). (Photos: L. W. Wilcox)

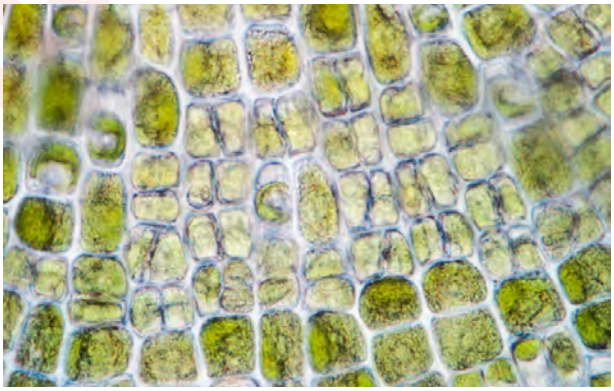

Figure 20.55 *Coleochaete scutata* antheridia. These structures occur as packets of cells formed in concentric rings between the body periphery and its center. (Photo: L. E. Graham)

Figure 20.56 *Coleochaete pulvinata* zygotes. A putative chemical influence emanating from zygotes induces neighboring cells to grow toward and cover them, forming a protective cortical layer. (Photo: L. W. Wilcox)

features similar to those of placental transfer cells at the gametophyte–embryo junction of land plants. In land plants, transfer cells function in the transport of nutrients such as amino acids and sugars across the maternal–embryo interface, a process that has been described as **matrotrophy** (Graham 1996; Graham and Wilcox 2000).

In both placental transfer cells of land plants and the cortical cells surrounding *Coleochaete orbicularis* zygotes, elaborate wall ingrowths develop on a localized basis (see Figure 20.57) (Graham and Wilcox 1983). In the case of *Coleochaete*, the wall ingrowths occur only on the vegetative cell walls that are closest to zygotes, suggesting that zygotes have an inductive influence, perhaps by exuding chemical signals. The wall ingrowths vastly increase the surface area of the cell membrane, across which nutrients moving from vegetative cells to zygotes must pass. The ability of vegetative bodies of *Coleochaete* to take up and utilize sugars (Graham et al. 1994) suggests that these algae may possess the requisite cell membrane carbohydrate transporter molecules. Concurrently, zygotes begin to enlarge through accumulation of massive storages of starch and lipid (Figure 20.58). These storage materials are thought to arise in part from maternal contributions, though zygotes maintain green chloroplasts at this stage and therefore are presumably capable of generating photosynthates. The storage materials are needed to fuel production of an unusually large number of meiotic products when zygotes germinate in early spring. At maturity, *Coleochaete* zygote walls become lined with a thin layer of material that is similar to sporopollenin in higher plant spores and pollen walls (Delwiche et al. 1989; Graham 1990). Further, the walls of vegetative cells that surround zygotes, and, indeed, any cells within the apparent sphere of influence of zygotes, accumulate highly resistant phenolic compounds (Kroken et al. 1996) (Figure 20.59). Such compounds, together with the sporopollenin layer, are presumed to protect zygotes against microbial attack during the dormant period.

In northern temperate latitudes, *Coleochaete* zygotes are induced to germinate in spring, perhaps by warmer temperatures and longer day lengths. At least some of the zygotes remain in shallow nearshore waters—the same location favored by vegetative bodies—because zygotes are attached to senescent parental bodies, which often remain attached to stable substrates. Though dead, cell walls of parental bodies do not completely decay during this period because they are impregnated with phenolic materials. In at least one species of *Coleochaete*, meiosis occurs during the first divisions of the polyploid zygotes, followed by several mitotic divisions (without further DNA replication) (Hopkins and McBride 1976), yielding 8–32 haploid products (Figure 20.60). These cells, known as meiospores, develop two flagella, an MLS flagellar root, and a layer of surface scales, and then they escape from zygotes as the wall cracks open (Graham and Taylor 1986a,b). Upon settling in nearby, well-illuminated nearshore habitats, the meiospores seed the development of vegetative populations of *Coleochaete* during the subsequent growing season, completing the life cycle. Mathematical models have been used to identify factors that may have influenced the evolution of sexual reproduction in *Coleochaete* and early land plants (Haig et al. 2015).

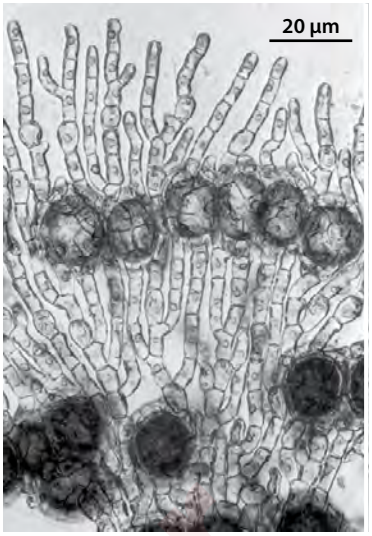

Figure 20.58 ***Coleochaete pulvinata* zygotes.** Those toward the bottom of the image are at a later stage of development than those at the top (located in a younger portion of the thallus). (Photo: L. E. Graham)

Figure 20.59 **Mature *Coleochaete orbicularis* zygotes.** Note that the walls of cells close to the zygotes have taken on a brownish coloration due to the accumulation of resistant phenolic compounds. (Photo: L. W. Wilcox)

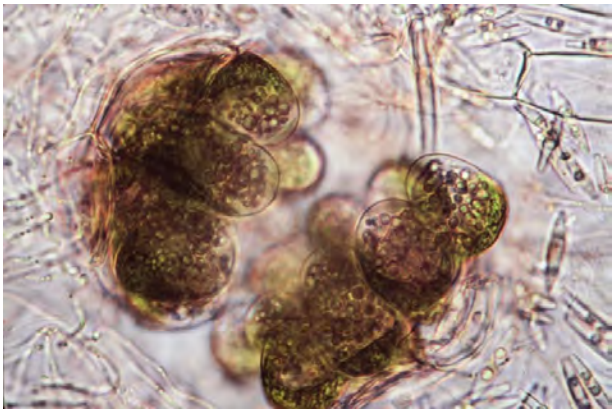

Figure 20.60 ***Coleochaete orbicularis* meiospores.** (Photo: L. E. Graham)

Ecology of Coleochaetaleans

Coleochaete and *Chaetosphaeridium* are periphytic, attached to submerged portions of higher plants such as the great bulrush *Schoenoplectus* (*Scirpus*), *Potamogeton*, and the undersides of water lily leaves. They also grow on nonliving substrates such as pebbles near the edges of oligotrophic (low-nutrient) freshwater lakes and ponds, where they are frequently but unpredictably exposed to desiccation as a result of variable wave action and changes in water levels. *Coleochaete nitellarum* can occur on the surfaces of deep-water charalean algae, particularly *Nitella*. Coleochaetaleans are sensitive to the effects of eutrophication; they disappear from water bodies that suffer excessive input of nutrients. They are not known to occur in brackish waters or extreme habitats. In laboratory cultures, one *Coleochaete* species has been demonstrated to utilize exogenous dissolved organic carbon in the form of hexose sugars and sucrose (Graham et al. 1994). This capability could be useful in low-pH or other environments in which dissolved inorganic carbon levels might be too low to saturate photosynthetic requirements.

Charales

Charalean algae are important both ecologically and evolutionarily. Charaleans are ecologically important for forming massive growths in both deep and shallow lake and pond waters (Figure 20.61), sometimes to the point of being regarded as nuisance weeds. However, charaleans are also an important food for waterfowl and provide a nursery area for fish (Dugdale et al. 2006; Schmieder et al. 2006). A few species, including *Chara evoluta*, occur in brackish waters having salt content of 20–40 ppt. Beds of *C. aspera* dominate shallow, sheltered, soft-bottomed areas of the Baltic Sea where they are exposed to natural and anthropogenic mechanical stresses (Torn et al. 2010). Some form extensive meadows in fairly deep freshwaters (Stross 1979); *Chara contraria*, for example, has been collected from 150 m in Lake Tahoe. Charaleans are generally considered to be adapted in various ways to low-irradiance, benthic habitats (Andrews et al. 1984).

Because many forms accumulate surface layers of calcium carbonate in the form of calcite, the group is known colloquially as stoneworts, muskgrasses, bassweeds, or brittleworts. Calcification gives some forms a white or pale-green appearance (Grant 1990). Charalean algae are now, and for the past few hundred million years have been, major carbonate sediment producers in freshwater lakes because they may be more heavily encrusted by calcium carbonate than aquatic higher plants. Most of the charalean body readily disintegrates in the benthos, forming marl deposits at rates that can reach several hundred g m^{-2} $year^{-1}$ (Tucker and Wright 1990). Charalean zygotes, protected by an inner layer of sporopollenin, thick cell walls, an outer enclosure of vegetative cells, and encrusting carbonates, are resistant to degradation and thus able to survive in benthic sediments.

Charaleans have a long fossil history, based primarily upon degradation-resistant reproductive structures, which provide useful information about the evolutionary process and patterns of extinction. The ancestors of modern genera are thought to have arisen in the late Triassic, but the earliest remains attributable to charaleans

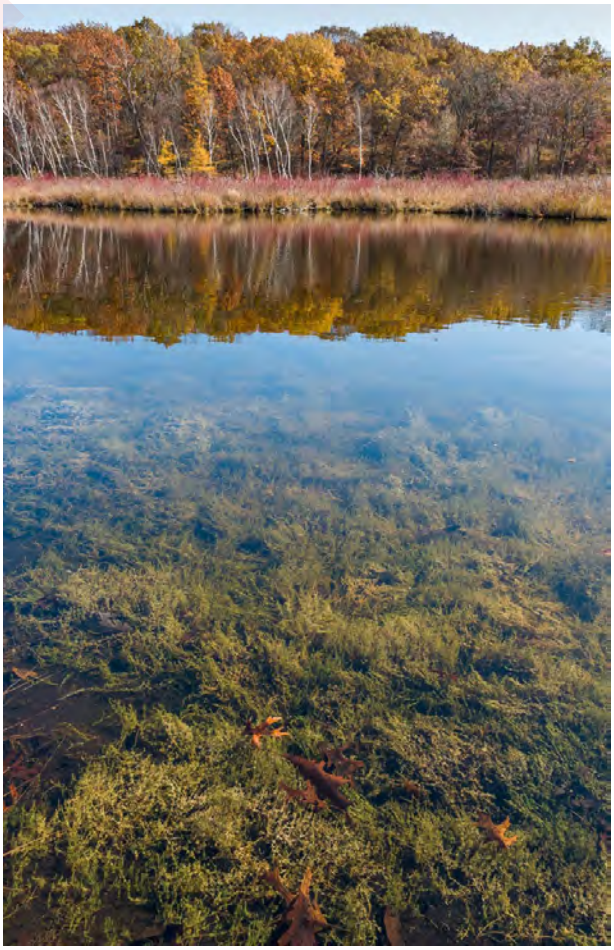

Figure 20.61 Dense growth of *Chara* in a small pond. (Photo: L. W. Wilcox)

are of Silurian age (Feist et al. 2005), and thus much younger than the earliest fossil evidence for land plants (reviewed by Clarke et al. 2011). Modern charaleans are the largest and most morphologically, developmentally, and reproductively complex group of charophycean green algae. Reaching lengths of 1 m or more, with whorls of branches at nodes, some are regularly confused with similar-appearing aquatic flowering plants such as *Ceratophyllum*.

Charalean vegetative structure

Charaleans fundamentally are branched filaments, though the main axis is differentiated at the apex, nodes, and basal region (Figure 20.62). The erect shoot possesses a single specialized apical meristematic cell; this cell cuts off derivatives from its lower surface only, thereby extending the filament in length (Figure 20.63). By comparison, the apical meristematic cells of bryophytes have three or four faces from which new cells appear and thus generate tissues. No centrioles are present at spindle poles in these or other cell divisions in charalean algae, which is consistent with the absence of flagellate zoospores. The immediate derivative of transverse apical cell mitotic division (known as a segment cell) divides again transversely. The uppermost of the resulting two cells will continue to divide to produce a complex node with lateral branches, whereas the lower cell develops into a very long internodal cell without further division. Therefore, the main axis consists of regularly alternating discoidal nodal cells and long cylindrical internodal cells.

Internodal cells can reach lengths of 15 cm. Internodal cells are so large that microelectrodes can easily be inserted for electrophysiological studies. An excellent review of electrophysiological investigations in charalean algae can be found in Wayne (1994). Internodal cells contain well over 1000 nuclei, which are produced by the replication of a single original nucleus through a process that does not involve the typical mitotic apparatus. The interphase nuclei of vegetative cells in young shoots of *Chara* and *Nitella* can undergo endoreduplication—an increase in the amount of DNA beyond the haploid level (Michaux-Ferrière and Soulié-Märsche 1987). An increased number of nuclei and high levels of nuclear DNA presumably balance the large increase in cell volume, which is mediated by development of a large internal vacuole. The cytoplasm nearest the central vacuole of internodal cells is an ideal site for visualizing cytoplasmic streaming, resulting from actin microfibril activity (Allen 1974; Palevitz and Hepler 1975; Williamson 1979, 1992). Presumably such streaming is necessary to achieve mixing and long-distance transport of cell constituents in long cells having large cytoplasmic volumes. It has been hypothesized that large internodal cells represent adaptation to shady benthic habitats (Raven et al. 1979).

Within the internodal cells, a layer of nonmobile peripheral cytoplasm contains numerous discoid chloroplasts arranged in rows (Figure 20.64) having granalike thylakoid stacks and starch grains but lacking pyrenoids. The plastids are generated by repeated fission. Also present are mitochondria, and peroxisomes containing conspicuous catalase crystals (Figure 20.65a). Elaborate invaginations of the cell membrane, known as **charasomes** (Figure 20.65b), may occur along the cell periphery; their function is uncertain. The presence of charasomes is apparently not correlated with the ability to use bicarbonate

Figure 20.62 Complex, branched filamentous body of charalean algae. A species of *Chara* is shown here. The orange structures are antheridia. (Photo: L. W. Wilcox)

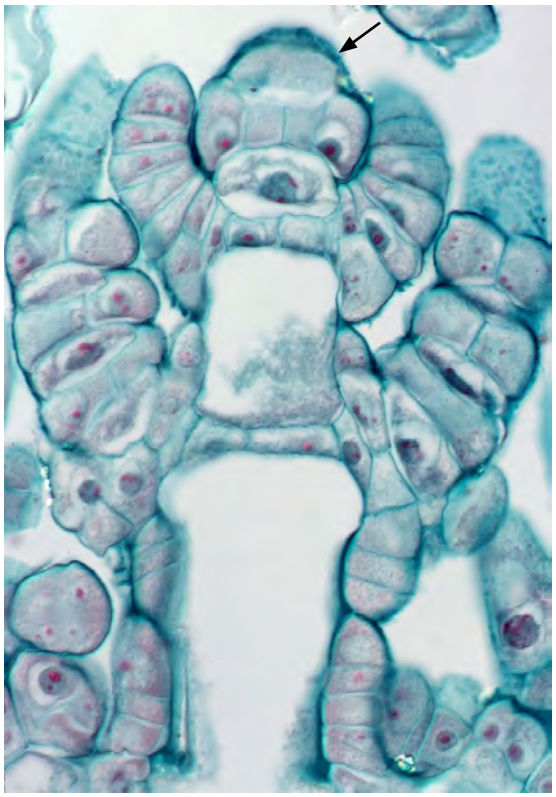

Figure 20.63 Apical meristematic cell of *Chara* (arrow). (Photo: M. E. Cook)

ion in photosynthesis (Lucas et al. 1989) (see Chapter 2). The cell wall of charaleans is composed of distinct layers, including cellulose, and can be relatively thick.

The cell plate that separates nodal from internodal cells is characterized by very large pores. These appear to have originated by the coalescence of several plasmodesmata, themselves reported (in at least one species) to have been derived secondarily by the action of wall hydrolytic enzymes (Franceschi et al. 1994; Lucas 1995). These large pores are thought to facilitate passage of materials throughout the vertical axis of the body (Cook et al. 1997).

Nodal initial cells first divide vertically (commonly into two halves), and then each of the resulting cells undergoes a very highly controlled series of asymmetric divisions that produce the branch initials (Cook et al. 1998). Divisions are synchronized in the two cells making up the node; divisions leading to the formation of branch initials occur in a sequential, radial manner. Charalean branch initials serve as the apical cells for development of branches having the same kind of alternating nodal and internodal cells as the main axis. The branches in turn produce smaller branchlets. Unlike the apical cell of the main axis, which can continue to divide on an indeterminate basis, the branch apices cease division after a determined number of cells have been produced. Branches of two *Chara* species (but not all species examined) have been observed to grow toward light by cell elongation, particularly in high- irradiance conditions. This behavior is suggested to protect gametangia or aid penetration of light into the canopy (Schneider et al. 2006). Cells of the nodal region may generate other branches that mirror the growth habit of the main axis (i.e., have indeterminate growth). In addition, in most species of *Chara*, basal nodal cells of the branches can generate multiple rows of filaments that grow up or down over the internodal cell surfaces, forming what is known as a corticating layer. Corticating filaments originating from adjacent nodes meet in the middle of the internodal cell (Figure 20.66).

A land plantlike phragmoplast is present during cytokinesis in charalean algae. In addition to the characteristic longitudinal microtubule array, charalean algae possess actin microfilaments, membrane tubules, coated vesicles, and fenestrated sheets such as are associated with cell plate development in higher plants (Pickett-Heaps 1975; Cook et al. 1997; Braun and Wasteneys 1998). However, charalean algae that have been investigated differ in a number of ways from plants in regulation of the cytokinetic process. These include later dissolution of the phragmoplast, absence of an organellar and ribosome exclusion zone from developing cell plates, cell plate development that is patchy and not regularly centrifugal, co-occurrence of different cell plate developmental stages, and an earlier development of plasmodesmata (Cook et al. 1997). Although some of the literature (Grant 1990) implies that charalean algae produce pre-prophase microtubule bands homologous to those of land plants, to date, such bands have not been demonstrated to occur in any streptophyte alga.

Ultrastructural studies of the charalean node suggest that it can be considered to be a tissuelike, or parenchymatous, structure (Pickett-Heaps 1975; Cook et al. 1998). Comparative immunolocalization studies of actin and microtubules in internodal and nodal cells suggest

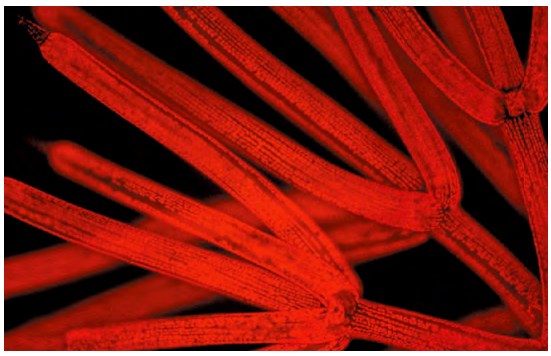

Figure 20.64 *Nitella* viewed using fluorescence microscopy. Note the large internodal cells, branches emerging in whorls from much smaller nodal cells, and the numerous autofluorescent, discoid plastids arranged in vertical files. (Photo: L. E. Graham)

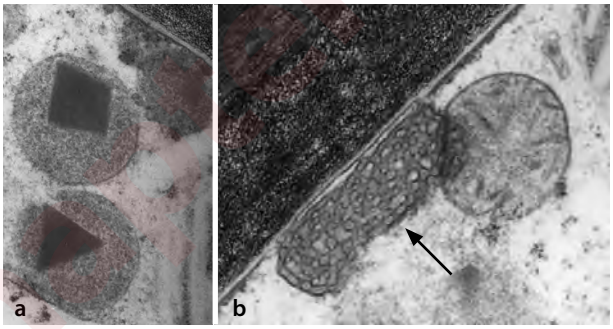

Figure 20.65 Peroxisomes and charasome in *Chara*. (a) Peroxisomes with catalase crystals, and (b) charasome (arrow) with overlapping mitochondrion. (TEMs: M. E. Cook)

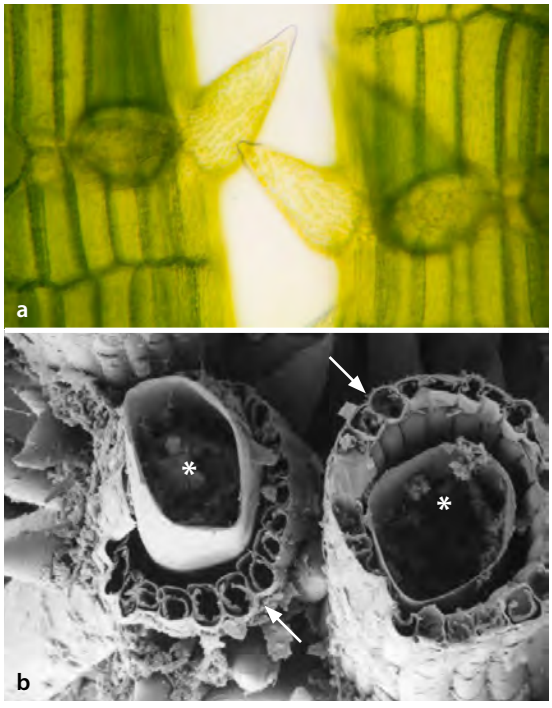

Figure 20.66 *Chara zeylanica* corticating filaments. (a) Corticating filaments grow from both nodes over the surface of internodal cells. (b) Cross-sectional view of two *Chara* thalli viewed with SEM (internodal cells are indicated by asterisks and arrows point to corticating filaments). (a: L. W. Wilcox; b: M. E. Cook)

that the latter more closely resemble higher plant meristem cells (Braun and Wasteneys 1998). Moreover, cells in the nodal region of at least one charalean species possess primary plasmodesmata, i.e., those produced during formation of the cell plate at cytokinesis, much like plasmodesmata of land plants (Cook et al. 1997).

The basal portion of charalean algae in nature is typically attached to muddy or silty substrates by numerous colorless rhizoids. These are very long cells containing unpigmented plastids and 30–60 barium- and sulfur-containing crystals, the latter having geotropic function. Rhizoids grow at the tip and are not differentiated into nodes and internodes, but they do exhibit a definite polarity, with cells showing at least seven distinct zones (Kiss and Staehelin 1993). Evidence was found in several charalean species for the production and function of strigolactone hormones in stimulating rhizoid elongation (Delaux et al. 2012).

Reproduction of charaleans

Asexual reproduction in charaleans can occur by means of adventitious (from the shoot) development of new shoots from rhizoids and nodal complexes. Additional asexual structures include bulbils, which are white spherical or star-shaped structures that form on rhizoids of some species and function in dispersal and perennation, persistence through stressful periods.

Charaleans have probably the most conspicuous sexual structures of any green algae (Figures 20.67 and 20.68). These specialized structures (gametangia) are of two types—(male) antheridia, where thousands of biflagellate spermatozoids develop, and (female) oogonia, each containing a single egg cell. The antheridia of charalean algae are bright orange at maturity and are visible without the use of a microscope. In monoecious species, antheridia and oogonia are usually borne together at the node of a branchlet. Microscopic examination of antheridia reveals that the orange pigmentation is generated by carotenoid droplets within an outer layer of cells arranged in groups of eight, forming a flowerlike pattern (see Figure 20.68). These are known as the **shield cells**, and their form is used in classification and identification of some species. The shield cells are attached to a columnar-shaped cell known as a **manubrium**, which is also associated, at its other end, with a group of eight cells known as the **primary capitulum**. Cells derived from division of the primary capitulum generate long, unbranched filaments of small cells, each producing a single thin, helically twisted spermatozoid (Figure 20.69). Antheridial development is illustrated in Figure 20.70. Changes that occur in the configuration of the flagellar apparatus during sperm development have been studied in *Chara contraria* var. *nitelloides* (Vouilloud et al. 2005). Additional details of sperm structural development can be found in Moestrup (1970) and Pickett-Heaps (1975). When sperm are ready for release, the shield cells separate, allowing the sperm to swim away.

Oogonia arise from branchlet nodal cells as well. A primordial cell divides twice transversely, and the uppermost of the resulting cell stack becomes the egg. The cell just below the egg repeatedly divides, generating a ring of five peripheral cells surrounding a central cell (Figure 20.71). The five peripheral cells elongate to form **tube cells** (also known as sheath cells) that grow upward along the surface of the egg, extending to keep pace with enlargement of the egg. As each tube cell elongates, it takes a counterclockwise helical

Figure 20.67 *Chara* oogonium and antheridium. The egg cell (with starch grains) and the surrounding tube cells and coronal cells are evident, as is an antheridium to the lower right. (Photo: M. E. Cook)

Figure 20.68 *Chara* antheridia. Note the petal-like arrays of surface shield cells. (SEM: M. E. Cook)

Figure 20.69 *Chara zeylanica* spermatangial filaments. Each of the equal-sized colorless cells will produce a single, elongate, spirally twisted biflagellate sperm. (Photo: L. E. Graham)

path, and at its tip, one or more transverse divisions give rise to one or two **coronal (crown) cells** (the number depending on the genus) (Figure 20.72). This process resembles that of zygote cortication in *Coleochaete*, and it has been proposed that both are based on similar cell–to-cell signal–response events (Graham 1993). As the egg enlarges, it becomes filled with storage products—usually many white starch grains, but lipid droplets are also abundant. Such storage buildup resembles that occurring in *Coleochaete* zygotes, and its role is similar—supporting later zygote germination. At maturity, openings form between the tube cells that allow sperm to reach the egg.

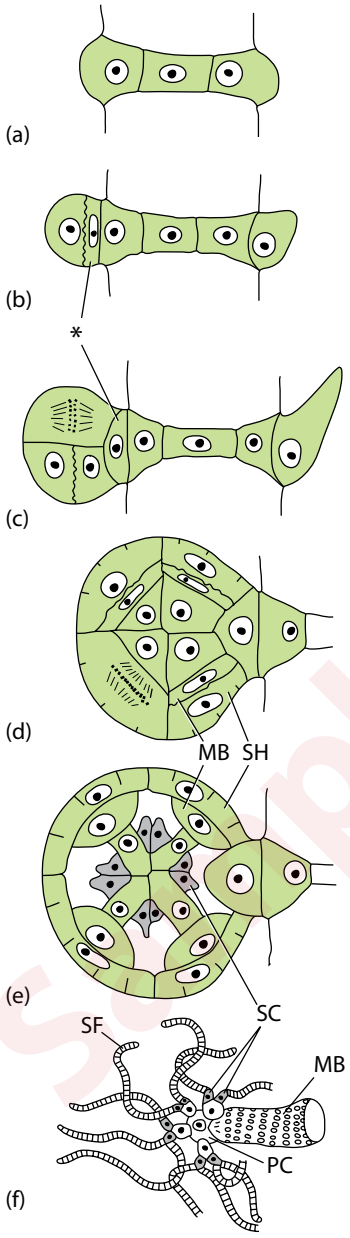

Figure 20.70 Antheridial development. Antheridia develop on short branches generated at the nodes (a). (b) Antheridial induction results in an unequal division, with the smaller cell (*) serving as a stalk. (c) The larger cell undergoes further division. (d) Diagonal divisions are integral to the formation of specialized regions within the developing antheridia. (e) An innermost set of columnlike manubria (MB) are attached to radiating shield cells (SH) at the surface and also generate sperm filaments (SF) in the intervening space. (f) Detail of the manubrium and attachment of sperm filaments. PC = primary capitula, SC = secondary capitula. (Re-drawn from Pickett-Heaps 1975. *Green Algae: Structure, Reproduction and Evolution of Selected Genera*, Sinauer Associates, Sunderland, MA)

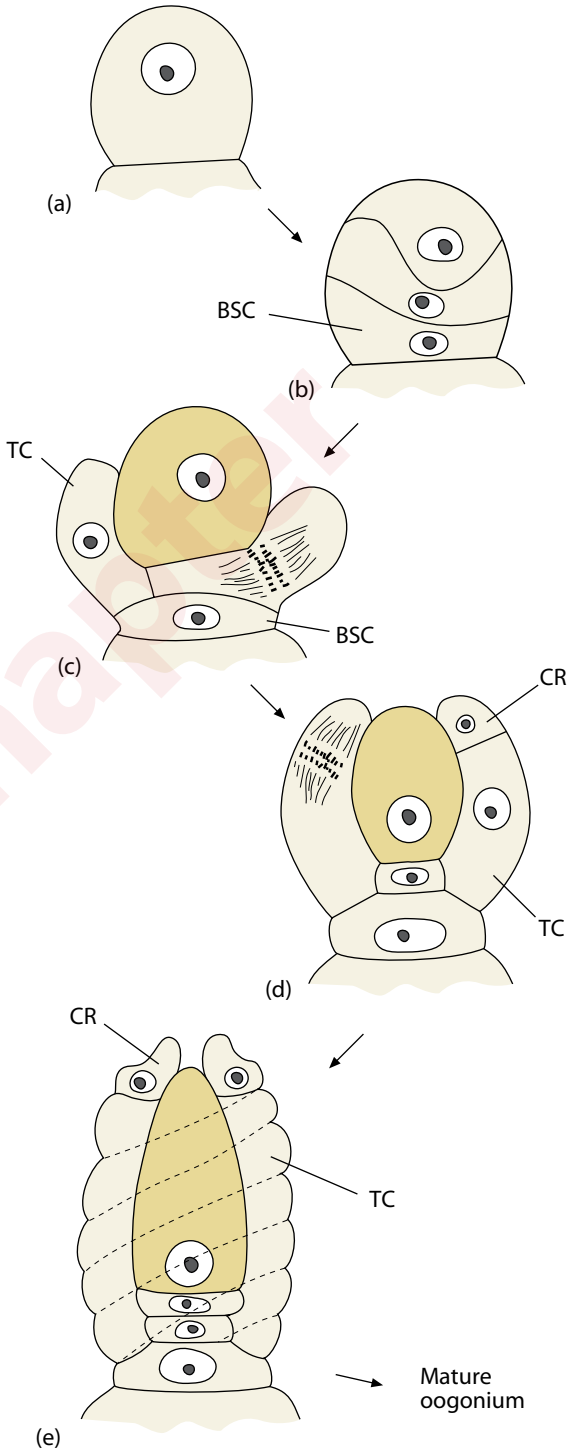

Figure 20.71 Oogonial development. (a) A cell derived by division of a nodal cell undergoes further division (b) into a basal stalk cell (BSC), a middle cell that generates the five tube cells (TC) and the terminal egg (darker shaded cell). (c–e) Elongation of the tube cells forms a twisted cortical layer that covers the egg cell entirely except for a pore at the top. A final division at the tip of each of the five tube cells gives rise to the five coronal cells (CR) of *Chara*. *Nitella* undergoes two such divisions at the ends of each of the 5 tube cells, giving rise to a total of 10 coronal cells. (Re-drawn from Pickett-Heaps 1975. *Green Algae: Structure, Reproduction and Evolution of Selected Genera*, Sinauer Associates, Sunderland, MA)

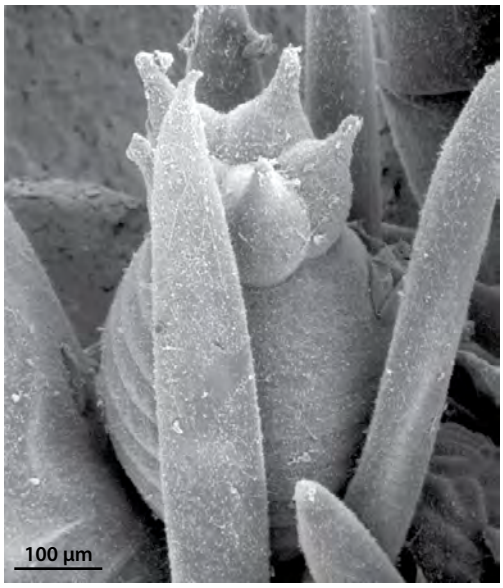

Figure 20.72 Mature oogonium of *Chara*. This SEM view shows the five coronal cells. (Micrograph: M. E. Cook)

After fertilization a thick, darkly pigmented zygote wall develops that contains an inner sporopollenin layer. Calcification of the concave inner walls of the spiral tube cells of *Chara* also typically occurs after fertilization. In *Tolypella*, calcium carbonate is deposited on the outside of the tube cells; the tube cells of *Nitella* zygotes are not calcified. As vegetative bodies are degraded at the end of the growing season, enveloped zygotes (also known as oospores or zygospores), together with their protective tube cells and perhaps a few other vegetative remnants, fall to the sediments. The thick, resistant wall and calcified tube-cell layer (if present) contribute to zygote survival during a period of dormancy. The calcified impressions of the tube cells and enclosed structures may persist in the fossil record; these are known as **gyrogonites** (Figure 20.73). Fossil gyrogonites reveal that the ancient relatives of modern charaleans often had more than five tube cells; the number has apparently been reduced over time. Another interesting (and unexplained) change has occurred in charalean tube cell orientation over time. Tube cells of older taxa, such as the lower Devonian *Trochiliscus*, were twisted to the right, whereas tube cells of taxa appearing near the end of the Devonian were twisted toward the left, as are those of modern forms and intervening ages. A few examples of calcified or silicified remains of antheridia and vegetative parts are also known (Figure 20.74). *Paleonitella* had noncalcified vegetative bodies with nodal organization and was preserved in the geological deposit known as the Rhynie Chert as petrifactions (mineral-impregnated remains) (Kelman et al. 2003).

Zygotes of charaleans are believed to germinate by meiosis, with only one meiotic product surviving. However, this assumption is based entirely upon circumstantial evidence, such as the observation of four nuclei within germinating zygotes (van den Hoek et al. 1995) and the fact that sperm and vegetative nuclei contain the same level of DNA, suggesting that meiosis is not gametic (Shen 1967). Analysis of DNA-level changes or chromosome counts during zygote germination have been difficult to accomplish because the massive

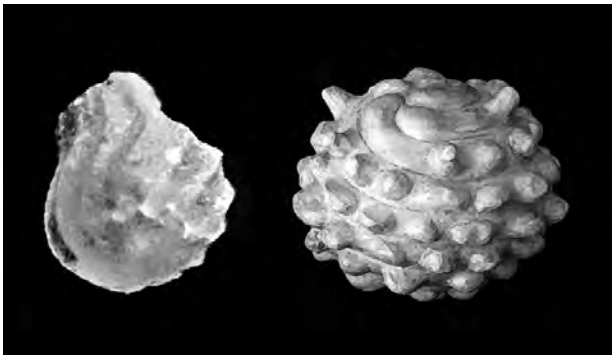

Figure 20.73 Fossil gyrogonites. On the left is an image of a fossil known as *Ampullichara*. On the right is a model made of a more complex gyrogonite fossil. (Photos: L. W. Wilcox)

Figure 20.74 Fossilized *Chara* from Argentina. Note the dark zygotes and remnants of cortical and tube cells. (Photo: M. E. Cook)

amounts of storage photosynthate and thick, dense zygote wall preclude easy observation of nuclear phenomena. Zygote germination occurs when a colorless filamentous protonema (meaning "first thread") emerges from a break in the zygote wall. The protonema possesses a colorless primary rhizoid and, under the influence of blue or white light, undergoes transverse divisions to form a short filament with green chloroplasts appearing in the uppermost cells. Germination of charalean zygotes is notoriously difficult to achieve at high levels of efficiency in the laboratory, further adding to difficulties in clarifying the life history of these algae. Cold-temperature and red-light treatments (Takatori and Imahori 1971) are said to increase the rate of zygote germination. Resistance to germination and germination only after extended storage in wet conditions or treatment with fluctuating water levels, irradiance, or temperature are regarded as adaptations that increase the chances that wetland charaleans will survive one or more unfavorable seasons. Variation among species in zygote germination behavior helps explain seasonal and geographical distribution patterns. For example, *Nitella cristata* var. *ambigua* zygotes germinated well in response to cues for the onset of winter, explaining the occurrence of this winter form (Casanova and Brock 1996).

Charalean diversity

According to Wood and Imahori (1965), there are six genera of living charaleans: *Chara*, *Lamprothamnion*, *Lychnothamnus*, *Nitellopsis*, *Nitella*, and *Tolypella*. Most of the 81–400 species (the number depending upon the expert) belong to *Chara* or *Nitella* (Grant 1990). Species determinations are based on such characters as the arrangement of the gametangia, the presence or absence of cortical layers, and the number and arrangement of cortical cells. Molecular (*rbcL*) data suggest that modern charaleans form a monophyletic group, and there is some support for a monophyletic tribe Chareae (*Chara*, *Lamprothamnion*, *Nitellopsis*, and *Lychnothamnus*), but monophyly of the tribe Nitelleae (*Nitella* and *Tolypella*) was not supported (McCourt et al. 1996). Molecular sequence data suggest that the genera *Nitella* and *Tolypella* are basal to the more derived Chareae.

CHARA (pre-Linnaean name of unknown origin) is characterized by structures known as **stipules** (or stipulodes), which are single-celled, often sharply tipped structures occurring below the branchlets. The main axes of most species are corticated, but some species, such as *Chara braunii* (Figure 20.75), lack this corticating layer and can be mistaken for *Nitella*. There is a single layer of 5 oogonial coronal cells. (In contrast, *Nitella* possesses two tiers of coronal cells, totalling 10.) *Chara* species are often calcified and thus may have a stony, gray-green appearance. Calcification, together with cortication, gives *Chara* a generally more robust appearance than *Nitella*. Species such as *Chara vulgaris* are regarded as marl formers because they deposit large amounts of calcium carbonate at the bottoms of water bodies. They primarily occur in relatively high-alkalinity waters. Some forms, including *C. vulgaris*, produce an odor that is variously described as "skunky" or "like spoiled garlic." Light conditions affecting sexual reproduction in *C. braunii* are noted by Sato et al. (2014).

Figure 20.75 *Chara braunii*, an uncorticated *Chara* species. (Photo: L. W. Wilcox)

NITELLA (L. *nitella*, brightness or splendor) is characterized by very regular, symmetrical branching. The branches that bear the gametangia are repeatedly forked. Bodies are uncorticated and not typically calcified. The oogonia are either solitary or occur below the antheridia. Oogonia have 10 coronal cells in two tiers of 5 each. The species range greatly in size from meter-long *N. flexilis*, which has very long internodal cells and occupies waters 10–12 m deep (Figure 20.76a), to minute and delicate forms such as *N. tenuissima* (Figure 20.76b), which occurs in shallow waters. In contrast to most *Chara* species, *Nitella* most commonly occurs in soft, slightly acidic waters.

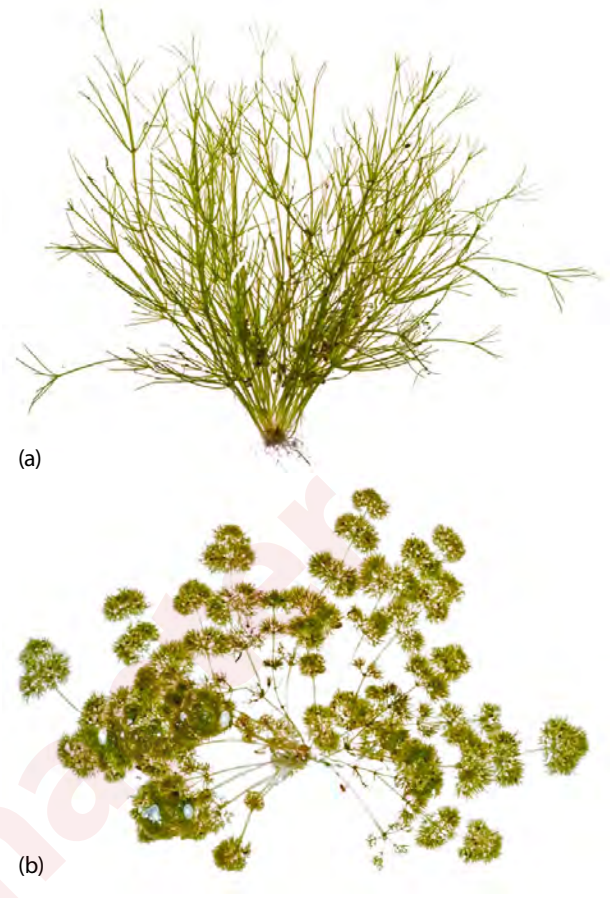

Figure 20.76 ***Nitella*.** (a) *Nitella flexilis*. (b) *Nitella tenuissima*. Note characteristic dense tufts of branches from nodes that are well separated by internodal cells. (Photos: M. Cook and C. Lipke)