Green algae and the origin of land plants[1]

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Over the past two decades, molecular phylogenetic data have allowed evaluations of hypotheses on the evolution of green algae based on vegetative morphological and ultrastructural characters. Higher taxa are now generally recognized on the basis of ultrastructural characters. Molecular analyses have mostly employed primarily nuclear small subunit rDNA (18S) and plastid *rbcL* data, as well as data on intron gain, complete genome sequencing, and mitochondrial sequences. Molecular-based revisions of classification at nearly all levels have occurred, from dismemberment of long-established genera and families into multiple classes, to the circumscription of two major lineages within the green algae. One lineage, the chlorophyte algae or Chlorophyta sensu stricto, comprises most of what are commonly called green algae and includes most members of the grade of putatively ancestral scaly flagellates in Prasinophyceae plus members of Ulvophyceae, Trebouxiophyceae, and Chlorophyceae. The other lineage (charophyte algae and embryophyte land plants), comprises at least five monophyletic groups of green algae, plus embryophytes. A recent multigene analysis corroborates a close relationship between *Mesostigma* (formerly in the Prasinophyceae) and the charophyte algae, although sequence data of the *Mesostigma* mitochondrial genome analysis places the genus as sister to charophyte and chlorophyte algae. These studies also support Charales as sister to land plants. The reorganization of taxa stimulated by molecular analyses is expected to continue as more data accumulate and new taxa and habitats are sampled.

Key words: Chlorophyta; Charophyta; DNA; *Mesostigma*; Streptophytina; ultrastructure.

Twenty years ago, a relatively slim volume with chapters by leading chlorophycologists celebrated the systematics of green algae (Irvine and John, 1984), a field that was undergoing rapid and fascinating changes, both in content and theory. "The present period may be termed the 'Age of Ultrastructure' in green algal systematics," wrote Frank Round (1984, p. 7) in the introductory chapter, which summarized the history and state of the art. Round (1984) argued that light microscopy had laid the foundation in the preceding two centuries, but that the foundation was largely descriptive—alpha taxonomy in the most restricted sense. Ultrastructure, he asserted, had enlarged and presumably would continue to expand our horizons to unify systematics of green algae and overcome the fragmented alpha taxonomy that had dominated the field. Little did Round know that this golden age of green algal systematics was about to go platinum. Molecular systematics, in concert with a rigorous theoretical approach to data analysis and hypothesis testing (Theriot, 1992; Swofford et al., 1996), would at first complement and then transform the age of ultrastructure and usher in the "Age of Molecules."

In this article, we review the major advances in green algal systematics in the past 20 years, with a focus on well-supported, monophyletic taxa and the larger picture of phylogeny and evolution of green algae. We will review the types of data that have fueled these advances. As will become obvious, this perspective entails discussion of some embryophytes as well as their closest green algal relatives. In addition, we will point out major uncertainties in green algal systematics, which pose some of the most provocative areas for further research.

Deconstructing hypotheses of relationships of the green algae and land plants—A link between green algae and land plants has been clear to biologists for centuries, since before Darwin and the advent of evolutionary thinking and phylogenetics (Smith, 1950; Prescott, 1951). Recent new data on morphology, genes, and genomes, as well as new ways of analyzing and synthesizing information, are only the most recent in a long history of change in our understanding of these so-called "primitive" plants. This review focuses primarily on research that has led to both some radical restructuring of the classification of algae and some satisfying confirmations of the careful observations of earlier workers.

First and foremost, green algae, the division Chlorophyta of Smith (1950), are undoubtedly monophyletic with embryophyte green plants, although the Chlorophyta in this sense is paraphyletic (Mattox and Stewart, 1984; Mishler and Churchill, 1985; McCourt, 1995). Embryophytes (land plants; bryophytes and vascular plants) are clearly descended from green algal-like ancestors, but the sister of the embryophytes includes only a few green algae. The remainder of Chlorophyta constitutes a monophyletic group. This major bifurcation in green plant evolution implies a single common ancestor to the two lineages, but, given the diversity of unicellular green algae and our growing understanding of them, there may be additional lineages outside this major bifurcation.

What are green algae?—The term *algae* is not phylogenetically meaningful without qualifiers. Algae in general and green algae in particular are difficult to define to the exclusion of other phylogenetically related organisms that are not algae. This difficulty is a reflection of recent data on algae as well as the way phylogenetic thinking has permeated classification. Green algae are photosynthetic eukaryotes bearing double membrane-bound plastids containing chlorophyll *a* and *b*, accessory pigments found in embryophytes (beta carotene and

xanthophylls), and a unique stellate structure linking nine pairs of microtubules in the flagellar base (Mattox and Stewart, 1984; Sluiman, 1985; Bremer et al., 1987; Kenrick and Crane, 1997). Starch is stored inside the plastid and cell walls when present are usually composed of cellulose (Graham and Wilcox, 2000a).

The plastids of green algae are descended from a common prokaryotic ancestor (Delwiche, 1999; Delwiche et al., 2004), for which descendants are endosymbiotic in the host cells of a number of other eukaryotic lineages. These plastids are termed primary, i.e., derived directly from a free-living prokaryotic ancestor (Delwiche and Palmer, 1997; Delwiche, 1999), although a secondary origin has been proposed (Stiller and Hall, 1997; see Keeling, 2004, in this issue for an overview of this process and variations on the theme of endosymbiosis). Plastid-bearing lineages permeate all of the other major clades of algae (see also in this issue Andersen, 2004; Hackett et al., 2004; Saunders and Hommersand, 2004).

Green algal diversity—Mostly microscopic and rarely more than a meter in greatest dimension, the green algae make up for their lack in size with diversity of growth habit (Figs. 1–17) and fine details of their cellular architecture. Body (thallus) size and habit ranges from microscopic swimming or nonmotile forms (e.g., nanoplankton, benthos, or lichen phycobionts) to macroscopic (benthic attached forms). Thallus structure runs the gamut of complexity, from swimming and nonmotile unicells, to filaments, colonies, and various levels of tissue organization (pseudoparenchymatous, parenchymatous, or thalloid) and branching morphologies. Unicells are spherical to elongate, with or without flagella, scales, and wall layers or other coverings (e.g., loricas). Filaments generally exhibit cylindrical cells arranged end-to-end, although chains of irregularly shaped cells are known. Unbranched (*Oedogonium*) and branching (*Draparnaldia*) forms are known, and many branching forms have attenuated terminal filament tips (*Chaetophora*). Colonies of various sizes occur, from pairs of cells (*Euastropsis*) to thousands (*Hydrodiction*). Cells in colonies may be joined by gelatinous strands or share a common parental wall. Colonies range in form from small sarcinoid packets (nonlinear clusters of cells; *Chlorokybus*) to aggregates of thousands of swimming cells (*Volvox*). Branching forms may be simple bifurcating or reticulating networks of filaments, but a few achieve a complexity that can be called tissuelike (*Nitella*). Cells may be uninucleate or coenocytic, in which many nuclei are dispersed throughout the cytoplasm of so-called giant cells (*Caulerpa*).

THE DATA REVOLUTION(S)

The systematics of green algae, and algae in general, has been driven by observational tools due to the apparent simplicity of the organisms to the naked eye and due to the small size and cryptic features of the organisms. Linnaeus recognized only five artificially inclusive genera (*Tremella*, *Fucus*, *Ulva*, *Conferva*, *Corallina*), plus *Chara* and *Volvox*, the last as an animal-like plant (Prescott, 1951). Light microscopy opened the windows on the microstructures of algae and provided the bulk of observational data for a long time. As described earlier, electron microscopy and molecular data have revolutionized our understanding of green algal phylogeny, which is reflected in modern classification. Table 1 lists the

features and sources of morphological and genetic data, along with representative publications and reviews.

Previous green algal taxonomy had grouped organisms based on growth habit, and several lineages were inferred by arranging taxa according to evolutionary "tendencies" (Smith, 1950). Motile ancestral green algal unicells were hypothesized to have given rise to distinct lines of increasing size and complexity, with each exhibiting a variation on a theme. One line resulted in motile colonies, another in nonmotile branching thalli or colonies (with motile reproductive cells retained in the life cycle), and a third in large nonmotile coenocytic forms (retaining motile uninucleate gametes; Smith, 1950; Bold and Wynne, 1985; McCourt, 1995).

The view that the cellular features involved in the vital processes of cell division and swimming (of gametes or asexual zoospores) would be highly conserved evolutionarily led to numerous comparative studies targeting the mitotic, cytokinetic, and swimming apparatus of the cell (e.g., Stewart and Mattox, 1975). The flagellar apparatus, with its flagellar basal bodies and axonemes and rootlets of microtubules, has been painstakingly compared across a large number of green algae. To a lesser extent, plastid structure has been important for diagnosis of some groups. This focus came about through research by early workers (Pickett-Heaps and Marchant, 1972, and others; Table 1) that revealed evolutionarily conservative characters that cut across misleading convergence in vegetative morphology. In the age of molecular systematics, we are evaluating hypotheses formulated from comparative ultrastructure studies over the last 30 years, and adding new hypotheses as well.

Ultrastructural work showed that filamentousness, coloniality, coccoid habit (nonmotile, lacking flagella), and many other vegetative features evolved numerous times and were generally unreliable as characters marking monophyletic groups. The simple sequencing of forms, flagellate → coccoid → unbranched filament → branched filament → tissuelike (with a cul de sac towards siphonous thalli from coccoids), may have occurred repeatedly in many lineages (Mattox and Stewart, 1984; Round, 1984).

Close on the heels of this first wave of new data and new methods of analysis employing a phylogenetic approach (Theriot, 1992) came a molecular revolution. By and large, the refutation of the classical tendencies as organizing principals in algal evolution and classification was confirmed, and a new dogma arose, which has been refined and enlarged but retains the major scaffolding erected by ultrastructure and biochemistry.

Molecular studies in green algae have been largely driven by slightly earlier studies in embryophytes and, to a lesser extent, cyanobacteria (Palmer, 1985). This is due largely to the development of primers for many genes that were first studied in embryophytes (Table 1). The green alga lineage (eukaryotes containing primary green plastids) originated as much as 1500 million years ago (mya; Yoon et al., 2004), and the divergence of land plants occurred perhaps 700 mya (Heckman et al., 2001) or more likely 425–490 mya (Sanderson, 2003). Despite the antiquity of a shared common ancestor of land plants and their nearest relatives (Karol et al., 2001), ribosomal DNA and many plastid genes are recognizable as homologs in green plants and algae. As a result, molecular phylogenetics of green algae expanded rapidly as methodologies and approaches were transferred to algal taxa.

The complete nuclear genome for *Chlamydomonas rein-*

hardtii (Grossman et al., 2003) was released in early 2004, and annotation is an ongoing process (E. Harris, Duke University, personal communication). Organellar genomes to date include five plastid (Wakasugi et al., 1997; Turmel et al., 1999b; Lemieux et al., 2000; Maul et al., 2002; Turmel et al., 2002b) and 10 mitochondrial genomes (Gray, 1993, direct submission to NCBI; Wolff et al., 1993; Denovan-Wright et al., 1998; Kroyman and Zetsche, 1998; Turmel et al., 1999a; Nedelcu et al., 2000; Turmel et al., 2002b, 2002c; Turmel et al., 2003), and several other green algal plastid or mitochondrial genomes should soon be published (C. Lemieux, Lavall University, personal communication). Several complete genome sequencing projects are also underway (A. Grossman, Stanford University; B. Palenik, Scripps Institution of Oceanography, personal communications).

With the advent of molecular data has come the possibility of finding characters that transcend rampant morphological homoplasy revealed by ultrastructure. However, a recurrent theme of many molecular studies has been a pattern of relatively well-resolved distal branches of a phylogenetic tree with weak or poor support for the internal or deeper divergences. Chapman et al. (1998) blamed this result on the limitations of sequence data and the antiquity of green algae: a stochastically changing molecule cannot be expected to retain enough signal to resolve events that happened nearly simultaneously in the ancient past (Lanyon, 1988). We suspect that a major reason is that most studies have employed only one or two genes and that more data can resolve these major ambiguities.

MAJOR CLADES OF GREEN ALGAE

The green algae have evolved in two major lineages (Fig. 18). One, which we refer to as the chlorophyte clade, includes the majority of what have been traditionally called green algae—flagellate green unicells and colonies (e.g., *Chlamydomonas*, *Volvox*), filamentous branched (e.g., *Chaetomorpha*, *Cladophora*) and unbranched forms (e.g., *Oedogonium*), green seaweeds (e.g., *Ulva*, *Codium*), many soil algae (e.g., *Chlorella*), terrestrial epiphytes (e.g., *Trentopohlia*), and many phycobionts (e.g., *Trebouxia*). The other lineage, the charophyte clade, contains a smaller number of green algal taxa, although some (e.g., *Spirogyra*, *Chara*) are widespread and as familiar as any alga. Charophyte algal thalli include swimming unicells, filaments (branched and unbranched), ornate unicells, and fairly complex forms that have been called parenchymatous. Charophyte algae are found in fresh water, with a few ranging into brackish habitats, and several groups live in soils, crusts, and other aerial settings. A third group of taxa, called prasinophytes, consists of "primitive"-appearing unicells of uncertain affinity—that is these taxa are probably members of one of the two main clades or more likely are representatives of other early-diverging clades (Fawley et al., 2000).

Within the chlorophyte clade are three well-supported groups: chlorophytes, trebouxiophytes, and ulvophytes. The charophyte clade comprises at least five small but distinct groups of green algae leading to a highly diverse clade of land plants. The discussion next focuses on well-supported monophyletic groups in the two major clades, as well as taxa for which a phylogenetic position is unclear.

A working classification of green algae and plants—For the purposes of this review, we will use the classification shown in Table 2, which gives division, class, and order names

of major groups with informal names in parentheses. This is not intended to be a definitive taxonomic revision of green algal classification, but we anticipate that such a revision will incorporate the basic scheme used here (C. F. Delwiche, University of Maryland, personal communication). The use of some terms or prefixes (e.g., charo-) is inevitably confusing because of the historical claim that such terms have on us. In other cases, paraphyly of traditional classes, orders, families, genera, or even species, makes classification difficult.

Chlorophyte clade—This clade contains three major groups and the majority of described species of green algae: chlorophytes, trebouxiophytes, and ulvophytes. Mattox and Stewart (1984) assigned the groups class-level rank, i.e., Chlorophyceae, Pleurastrophyceae (now at least in part Trebouxiophyceae), and Ulvophyceae. All members of this clade have swimming cells with two or four anterior flagella. Within the cell, the flagellar basal bodies are associated with four microtubular rootlets. All three groups share the character of cruciately arranged rootlets that alternate between two and higher numbers of microtubules (X-2-X-2). From group to group, differences in the offset from this cruciate pattern are seen, and arrangements are categorized according to relative positioning (O'Kelly and Floyd, 1984). When viewed from above the cell, the basal bodies and rootlets can have a perfect cruciate pattern (i.e., with basal bodies directly opposed, DO) or they are offset in a counterclockwise (CCW) or clockwise (CW) position. The ancestral condition has been inferred to be a CCW offset (Trebouxiophyceae and Ulvophyceae; Mattox and Stewart, 1984), with CW and DO the derived states (chlorophytes).

Molecular work, primarily on the small subunit of ribosomal DNA (18S rDNA) has strongly supported the monophyly of this triad of green algal groups and shows the ulvophytes as sister to a clade containing chlorophytes and trebouxiophytes. A combined analysis of morphology and 18S rDNA data strongly supported the monophyly of the three groups (Mishler et al., 1994). Later studies provided more evidence for this topology, often with high support, while greatly increasing taxon sampling (Friedl and Zeltner, 1994; Friedl, 1995; Bhattacharya et al., 1996; Krienitz et al., 2001).

Charophyte clade—This group contains a number of green algae plus a large number of what are considered to be the mostly highly derived green autotrophs, the land plants (Graham, 1993). Nomenclaturally, the group has led a confused life. Mattox and Stewart (1984) placed the algae in this group in Charophyceae, although the exclusion of the land plants made this taxonomic arrangement paraphyletic. Graham and Wilcox (2000a) acknowledged the paraphyly of the group and used the term "charophyceans" to refer to them. Bremer et al. (1987) assigned the division name Streptophyta to the green algae plus land plants, although Jeffrey (1982) had used this name more restrictedly, including only stoneworts (Charales) and embryophytes (archegoniate land plants). We will refer to the green algal groups of the charophyte clade as charophyte algae. The clade (charophyte + embryophytes) is characterized by biflagellate cells (when motile cells are present), with asymmetrically inserted flagella and two dissimilar flagellar roots (including a multilayered structure, or MLS, and a smaller root), persistent mitotic spindles, open mitosis, and several enzyme systems not found in other green algae (Mattox and Stewart, 1984; Graham and Wilcox, 2000a).
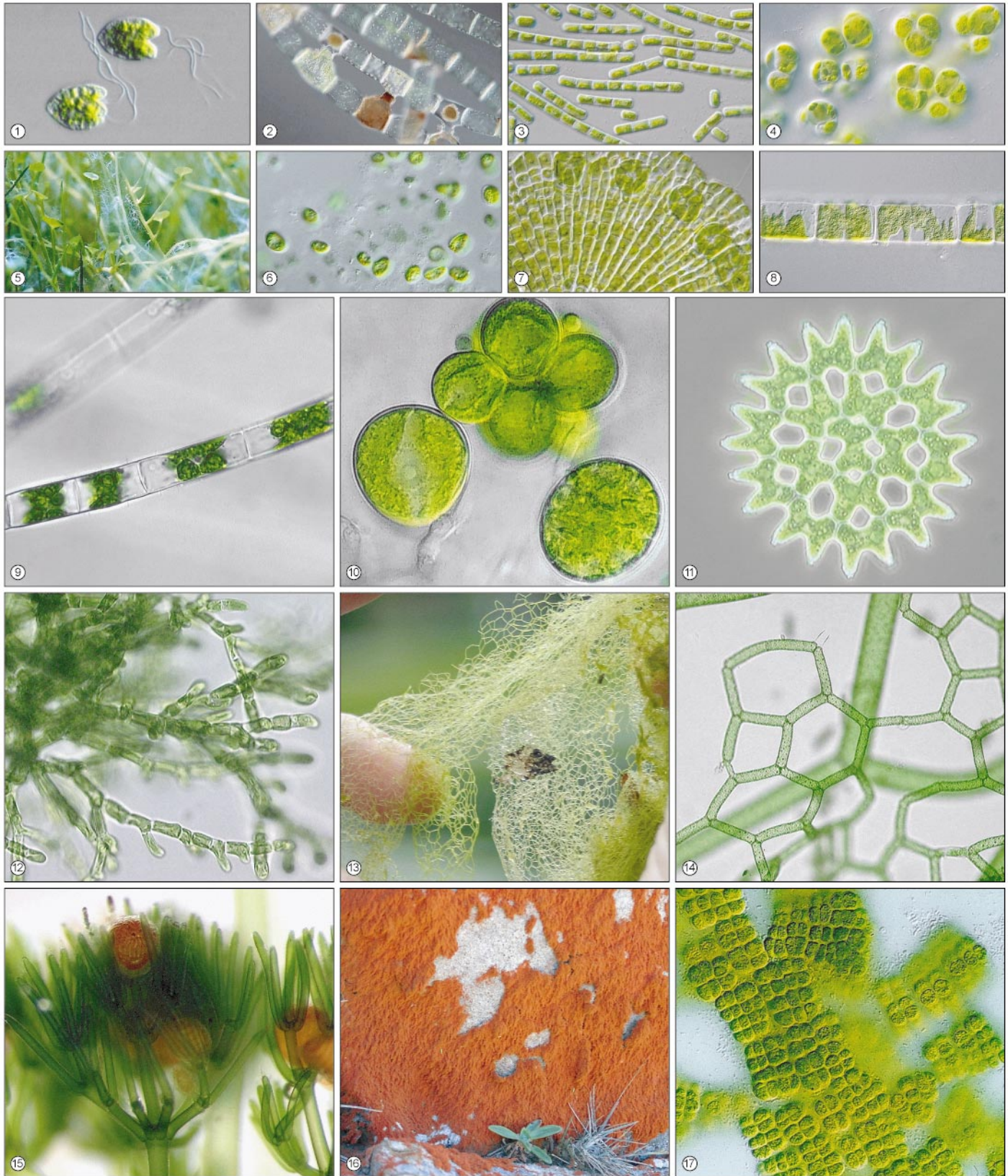
Figs. 1–17. Representative green algae. 1. *Halosphaera* cf. *minor*; prasinophyte (photo by C. O'Kelly). 2. Two conjugating filaments of *Spirogyra maxima*; charophyte (photo by C. Drummond). 3. *Klebsormidium flaccidum*; charophyte (photos 3–9 by C. F. Delwiche). 4. *Chlorokybus* sp.; charophyte. 5. Marine macro-alga, *Caulerpa*; an ulvophyte. 6. *Mesostigma*; flagellate charophyte. 7. View of part of a *Coleochaete orbicularis* thallus, with eggs, charophyte. 8. *Entransia fimbriata*; charophyte. 9. *Ulothrix* sp.; ulvophyte. 10. *Myrmecia* sp.; trebouxiophyte (photo by V. Flechtner). 11. Colonial planktonic alga, *Pediastrum duplex*; chlorophyte. 12. *Microthamnion* sp.; trebouxiophyte. Figs. 13 and 14. Macroscopic and microscopic view of the water net, *Hydrodictyon reticulatum*

TABLE 1.   Examples of comparative ultrastructural, biochemical, and molecular characters that have been used to distinguish major groups of green algae. Representative and particularly comprehensive references are provided. See Appendix (in Supplemental Data accompanying the online version of this article) for figure of cellular locations.

Characters	References
I. Cell ultrastructure (internal and surface features)	
Absolute orientation of flagellar apparatus	O'Kelly and Floyd, 1984; Watanabe and Floyd, 1996
Multilayered structure (MLS) presence/absence	Melkonian, 1984
System I (SMAC) and System II (rhizoplast) fibers, presence/absence	Sluiman, 1989; Watanabe and Floyd, 1996
Pyrenoid presence/absence, morphology	Watanabe and Floyd, 1996
Scale presence/absence, morphology	Becker et al., 1994
Flagellar hairs, morphology	Marin and Melkonian, 1994
Cytokinesis via infurrowing, phycoplast, phragmoplast	Mattox and Stewart, 1984
Biochemistry	
Photorespiratory enzymes	Floyd and Salisbury, 1977; Suzuki et al., 1991; Iwamoto and Ikawa, 2000
Accessory pigments	Zignone et al., 2002
Single or combined genes (nuclear, plastid, mitochondrial)	
18S rRNA (nuclear)	Huss and Sogin, 1990; Krienitz et al., 2003
26S rRNA (nuclear)	Buchheim et al., 2001; Shoup and Lewis, 2003
actins (nuclear)	An et al., 1999
rbcL (plastid)	Daugbjerg et al., 1994, 1995; Manhart, 1994; McCourt et al., 2000; Nozaki et al., 2003; Zechman, 2003
atpB (plastid)	Karol et al., 2001
*nad*5 (mitochondrial)	Karol et al., 2001
Genome-level Characters	
Intron presence	Manhart and Palmer, 1990; Dombrovska and Qiu, 2004
Plastid genome arrangement	Lemieux et al., 2000; Turmel et al., 2002c
Mitochondrial genome arrangement	Nedelcu et al., 2000; Laflamme and Lee, 2003

RELATIONSHIPS OF MAJOR GROUPS OF GREEN ALGAE

In the following sections, we present an overview of each of the major groups listed in Table 2, along with a brief summary of recent phylogenetic work within the group.

Prasinophytes—Prasinophyte algae have received attention recently because they are some of the important bloom-forming marine planktonic algae (O'Kelly et al., 2003) that are known mixotrophs and can account for a significant component of biomass in marine planktonic systems (Diez et al., 2001). Prasinophyceae also occupy a critical position at the base of the green algal tree of life and are viewed as the form of cell most closely representing the first green alga, or "ancestral green flagellate" (AGF). One of the first green algae to be examined using transmission electron microscopy was a member of class Prasinophyceae (Manton and Parke, 1960). Prasinophyceae (Micromonadophyceae of Mattox and Stewart, 1984) was proposed to segregate flagellate unicellular green algae with organic body scales from the other green flagellates (Christiansen, 1962; Moestrup and Throndsen, 1988; Melkonian, 1990c).

Members of Prasinophyceae have diverse morphologies, ranging from bean- to star-shaped cells with one to eight flagella often inserted in a flagellar pit and up to seven distinct types of organic scales. Recently, coccoid forms have been added to this group (Fawley et al., 2000). They occur in marine and brackish water, although some members live in freshwater habitats (e.g., *Pedinomonas*, *Mesostigma*). Prasinophyte algae are among the smallest of the eukaryotic planktonic marine flagellates (Zignone et al., 2002). Sexual reproduction has only been demonstrated in *Nephroselmis olivacea* (Suda et al., 1989).

Scale morphology has been used to differentiate the major groups of prasinophytes (Norris, 1980; Melkonian, 1984; Moestrup, 1984). In all but a few genera, organic scales are produced in the Golgi apparatus and coat the body and flagella. Some taxa possess up to seven distinct scale types, but others have a single type of scale. Flagellar behavior and morphology also reflect a tremendous diversity in this group. Some prasinophyte taxa have cruciately arranged rootlets, but others have asymmetrical rootlets. Some cells push with undulating flagella, and others swim with flagella forward. The number of rootlets varies between two and four and can include associated system I (SMAC) and system II (rhizoplast) fibers. *Pyramimonas octopus* has eight flagella and at least 60 flagellar structures connecting the basal bodies (Moestrup and Hori, 1989). Multilayered structures (MLSs) are found in flagellate sperm cells of embryophytes and occur in *Mesostigma*, a putative charophyte alga, and Pyramimonadales but are absent from all other groups of prasinophytes and all other taxa in the chlorophyte clade (assuming that the MLS-like struc-

Figs. 1–17.   Continued. from a pond in Connecticut; chlorophyte. 15. *Nitella hyalina* with orange sex organs; charophyte (photo by K. Karol). 16. *Trentepohlia* sp., with abundant orange secondary pigments forming a shaggy coat on rocks at Point Reyes, California; ulvophyte. 17. *Chlorosarcinopsis* sp.; chlorophyte (photo by V. Flechtner).
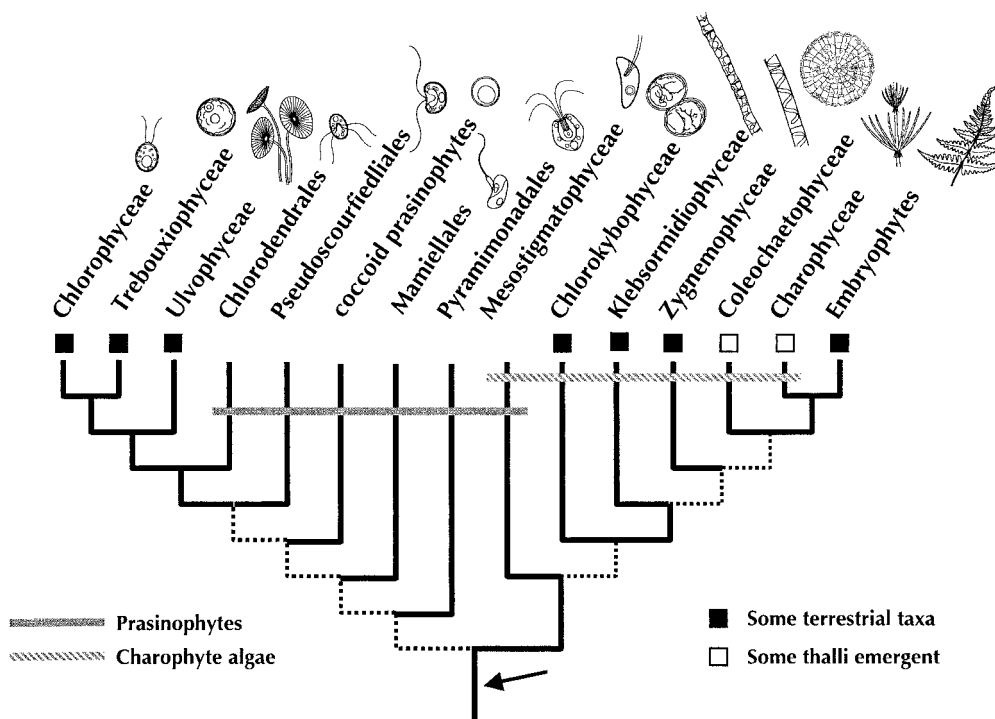
Fig. 18. Summary of the phylogenetic relationships among the major lineages of green algae determined by analysis of DNA sequence data. Branches of the tree depicted by dotted lines indicate relationships that are weakly supported with molecular data. Dotted lines within the ''charophyte algae'' indicate poorly resolved regions based on Karol et al. (2001). The arrow at the base of the tree indicates the possible placement of *Mesostigma* supported by Lemieux et al. (2000) and Turmel et al. (2002c). Boxes at the tips of the branches indicate the lineages containing at least some terrestrial taxa (solid boxes) or taxa that are emergent (open boxes). No box indicates all taxa in group are aquatic. The drawings are thumbnail composites meant to show representative taxa.

tures of Trentopohliales are not homologous; Chapman, 1984). MLSs have also been described in dinoflagellates and jakobids (Wilcox, 1989; O'Kelly, 1992), and if these are homologous to those in green plants, then the presence of an MLS can be interpreted as the ancestral condition in the green algae. The structure of the flagellar transition region has been used to distinguish among some of the orders and provides evidence for the relationship between *Mesostigma* and charophytes (Melkonian, 1984). Characteristic flagellar hairs can also be used to distinguish among the main groups (Marin and Melkonian, 1994). Lateral flagellar hairs occur in all studied members, but tip hairs do not occur on flagella of Chlorodendrales and *Nephroselmis*. Ultrastructural differences in the manner of mitosis and cytokinesis are also apparent. Most members have persistent telophase spindles, the exception being members of Chlorodendrales.

The diversity in cell shape, number of flagella, flagellar apparatus organization, organic scales covering the cell surface and flagella, and differences in cell division led to the conclusion that Prasinophyceae as proposed are not a monophyletic group (Mattox and Stewart, 1984; Mishler and Churchill, 1984, 1985; but see Melkonian, 1984). Various taxonomic treatments based on morphological and ultrastructural data have been proposed. Moestrup (1991) segregated the uniflagellate, nonscaled taxa *Pedinomonas*, *Marsupiomonas*, *Resultor*, and *Scourfieldia* sp. M561 into the class Pedinophyceae. Moestrup and Throndsen (1988) reclassified Prasinophyceae (excluding *Micromonas*).

Such dramatic morphological variation in unicells led to striking differences in opinion regarding the AGF. Some authors hypothesized that smaller taxa such as *Pedinomonas*,

with their simpler flagellar apparatus and scales, were the best candidates (Moestrup, 1991). Other authors proposed that the AGF was a larger, more complex, and multiflagellate taxon. O'Kelly (1992) suggested that the complex flagellar apparatuses found in the larger, multiflagellate taxa were adaptations for prey capture in these algae or in their recent ancestors. Food particle ingestion and digestion has been shown in members of the Pyramimonadales (Bell and Laybourn-Parry, 2003) and has also been suggested (Delwiche, 1999) as a means of obtaining a cyanobacterial symbiont that ultimately became permanently incorporated as the plastid.

The advent of molecular systematics permitted evaluation of many morphologically and ultrastructurally based hypotheses regarding diversity and evolution of scaly, green flagellates, most of which are detailed in three comprehensive reviews (Melkonian, 1984; O'Kelly, 1992; Sym and Pienaar, 1993). Molecular analyses of 18S rDNA data have echoed the morphological diversity seen in prasinophytes, identifying at minimum seven separate lineages that form a grade at the base of the green tree of life (Kantz et al., 1990; Marin and Melkonian, 1994; Steinkötter et al., 1994; Nakayama et al., 1998; Fawley et al., 2000; Zignone et al., 2002). The major lineages that have been recovered by molecular data are discussed next (Fig. 18). It is evident that most (perhaps all) of the remaining orders require further attention and should be reclassified.

Pyramimonadales—This monophyletic order is usually considered as sister to the rest of Prasinophytes and includes the quadriflagellate taxa *Halosphaera*, *Cymbomonas*, *Pyramimonas*, and *Pterosperma*. Some taxa have complicated scales. Moestrup et al. (2003) detailed the ultrastructural evidence for

TABLE 2.   A working classification of green algae and land plants.

Kingdom Chlorobionta
  Division Chlorophyta (green algae sensu stricto)
  Subdivision Chlorophytina
    Class Chlorophyceae (chlorophytes)
      Order Chlamydomonadales[a] (+ some Chlorococcales + some Tetrasporales + some Chlorosarcinales)
      Order Sphaeropleales[b] (sensu Deason, plus *Bracteacoccus, Schroederia*, Scenedesmaceae, Selanastraceae)
      Order Oedogoniales
      Order Chaetopeltidales
      Order Chaetophorales
      Incertae Sedis (*Cylindrocapsa* clade, *Mychonastes* clade)
    Class Ulvophyceae (ulvophytes)
      Order Ulotrichales
      Order Ulvales
      Order Siphoncladales/Cladophorales
      Order Caulerpales
      Order Dasycladales
    Class Trebouxiophyceae (trebouxiophytes)
      Order Trebouxiales
      Order Microthamniales
      Order Prasiolales
      Order Chlorellales[b]
    Class Prasinophyceae[a] (prasinophytes)
      Order Pyramimonadales
      Order Mamiellales
      Order Pseudoscourfieldiales
      Order Chlorodendrales
      Incertae sedis (Unnamed clade of coccoid taxa)
  Division Charophyta[c] (charophyte algae and embryophytes)
    Class Mesostigmatophyceae[d] (mesostigmatophytes)
    Class Chlorokybophyceae (chlorokybophytes)
    Class Klebsormidiophyceae (klebsormidiophytes)
    Class Zygnemophyceae (conjugates)
      Order Zygnematales (filamentous conjugates and saccoderm desmids)
      Order Desmidiales (placoderm desmids)
    Class Coleochaetophyceae (coleochaetophytes)
      Order Coleochaetales
  Subdivision Streptophytina
    Class Charophyceae (reverts to use of GM Smith)
      Order Charales (charophytes sensu stricto)
    Class Embryophyceae (embryophytes)

[a] May comprise several distinct lineages that warrant class-level ranking.
[b] Formerly in Chlorophyceae.
[c] This clade of green algae and embryophytes has been termed Streptophyta, although the latter was defined differently by Jeffrey (1982). Charophyta has also been frequently used for the Charales and their extinct relatives, here referred to as Charophyceae.
[d] If Turmel et al. (2002c) and Lemieux et al. (2000) are right, this group is sister to all other chlorobionts, and warrants a new division, Mesostigmatophyta (see text).

a close relationship of *Cymbomonas tetramitiformis* with *Halosphaera*. However, these authors also concluded that *Cymbomonas* possesses scales similar to *Mamiella*. It may be that the presence/absence of scales or a theca is a phylogenetically informative character, whereas scale morphology is not phylogenetically useful. Some members of this order, such as *Halosphaera*, *Pterosperma*, and *Cymbomonas* (Moestrup et al., 2003), are known to produce a cyst or phycoma stage, which at least in *Cymbomonas* contains two chloroplasts and thus is likely the result of sexual reproduction. The resistant walls of phycomata appear in the fossil record in the Early Cambrian and perhaps earlier (Tappan, 1980).

Mamiellales—This order includes some of the smallest eukaryotes known, e.g., *Crustomastix* (3–5 μm long). Cells have one or two laterally inserted flagella and a total of two flagellar roots per cell. Some members lack scales, but most have a single form of scale over the body and flagella (Nakayama et al., 2000). Molecular data indicate that this group also includes coccoid taxa. Presumably all taxa contain the pigment prasinoxanthin, but Zignone et al. (2002) identified siphonoxanthin in *Crustomastix*. Mamiellales are usually reconstructed as sister to the rest after Pyramimonadales.

Mesostigmatophyceae—*Mesostigma viride* is the only member of this class. This asymmetrical cell with two laterally inserted flagella was recently placed in a separate class with the charophyte *Chaetosphaeridium* based on molecular and cellular evidence (e.g., presence of similar maple-leaf-shaped scales on flagella). The flagellar rootlet system of *Mesostigma* has an MLS, unlike most other prasinophytes (except for Pyramimonadales). Although a separate class is warranted, the inclusion of *Chaetosphaeridium* has been refuted. The placement of *Mesostigma* is further discussed in the section on relationships of the charophyte algae and embryophytes.

Pedinophyceae—This class includes *Pedinomonas* and *Resultor*, tiny (<3 μm) uniflagellate cells without scales. The flagellar apparatus is unusual among prasinophytes, consisting of one emergent flagellum and an additional basal body. The rootlets have an X-2-X-2 pattern, and the two basal bodies are offset in a counterclockwise orientation (Moestrup, 1991). Dividing cells have a persistent telophase spindle. Given this combination of characteristics, the phylogenetic position of Pedinophyceae still remains a puzzle. Contrasting hypotheses are that *Pedinomonas* represents either a reduced member of Mamiellales or a reduced ulvophycean taxon (Melkonian, 1990b). The only molecular study to include *Pedinomonas* placed it as sister of the green algae (Kantz et al., 1990), but because this analysis lacked an outgroup, there is no way to interpret its phylogenetic position. To date, no phylogenetic studies have included *Resultor*, although one *rbcL* (Rubisco large subunit) sequence from this taxon is accessioned in GenBank (www.ncbi.nlm.nih.gov).

Pseudoscourfieldiales—Members of this order (*Pseudoscourfieldia marina*, *Nephroselmis pyriformis*, and *N. olivacea*) have two flagella of unequal length, two body scale layers, and two flagellar scale layers. The flagellar apparatus is complex, having three flagellar roots. Molecular data resolve this group into either one or two clades of flagellate and coccoid taxa, both of which are usually placed as sister to Mamiellales.

Chlorodendrales—There is strong support for the monophyly of this order of flagellates and evidence that they share characters with the UTC clade, rather than with the other Prasinophyceae. The members of this group are distinct from other prasinophytes in many morphological and ultrastructural features: they possess a metacentric spindle that collapses at telophase; paired flagella that beat in a breast stroke pattern; and an X-2-X-2 configuration of the flagellar rootlets. These algae are also distinct in possessing a theca, which is formed by fusion of the outer layer of scales. The striking similarity between some members of the UTC clade and Chlorodendrales led Mattox and Stewart (1984) to reclassify *Tetraselmis* into a new class Pleurastrophyceae, along with *Pleurastrum*, *Trebouxia*, and *Pseudotrebouxia* (the latter genera are now placed in Trebouxiophyceae, Friedl, 1995, 1996). Analyses of molecular data always place *Tetraselmis* as sister to the morphologically more complex UTC clade.

Coccoids—In addition to the aforementioned lineages, Fawley et al. (2000) identified at least two well-supported, yet unnamed, clades of coccoid taxa from existing culture collection isolates that had not previously been sampled. These taxa were suspected members of the Prasinophyceae because they possess prasinophyte-specific pigments. It is evident that molecular data have corroborated and even enhanced our understanding of the diversity and nonmonophyly of prasinophyte algae evident from ultrastructural data. Molecular data also have been particularly important in demonstrating that the earliest prasinophyte lineages (closest relatives of the AGF) were large, complex, scaly, and multiflagellate rather than small and naked. Molecular analyses have also confirmed a close phylogenetic relationship of *Tetraselmis* and the UTC clade. As more taxa and genes are sampled, we are gaining greater detail about the evolution of this grade of green algae; however, important questions remain about the influence of data analysis, data set composition, and taxon sampling on the phylogenetic placement of these individual lineages and taxa. Taxonomic revisions to classify the phylogenetic lineages now treated as orders within the Prasinophyceae should be forthcoming as greater resolution is achieved and analyses based on data from more than a single gene are published.

Ulvophytes—This group was named the class Ulvophyceae by Mattox and Stewart (1984), but because of the uncertain status of the class as a clade, we will refer to the group collectively as ulvophytes. Ulvophytes are diverse morphologically and ecologically and comprise some of the more strikingly beautiful green algae, extant or otherwise (Berger and Kaever, 1992). The group is predominantly marine and includes some of the best-known green seaweeds, such as the sea lettuce *Ulva* (Hayden and Waaland, 2002), the weedy *Codium* (Goff et al., 1992), *Caulerpa* (Meinesz, 1999), and the model organism *Acetabularia* (Mandoli, 1998). Other filamentous genera dominate localized freshwater habitats (*Cladophora*, *Rhizoclonium*, and *Pithophora*), sometimes to the detriment of human use (Lembi and Waaland, 1988).

Thallus forms include nonmotile unicells, branched and unbranched filaments, filmy membranes (mono- or distromatic), and cushiony forms of compacted tubes. Many ulvophytes have multinucleate thalli and a siphonous construction, i.e., with few or no cross walls, which makes the thallus one giant, multinucleate cell. The surfaces of thalli of many marine ulvophytes are lightly to heavily calcified, and some species are important contributors to coral reef structure and common in the fossil record (Butterfield et al., 1988; Berger and Kaever, 1992). At the ultrastructural level, bi- and quadriflagellate motile cells have a cruciate (X-2-X-2) flagellar root system with CCW offset and overlapped basal bodies, with scales and rhizoplasts. Cytokinesis occurs by furrowing, with a closed persistent spindle (Mattox and Stewart, 1984; O'Kelly and Floyd, 1984; Sluiman, 1989).

The diplobiontic life cycle (free-living gametophyte and sporophyte phases, which may be iso- or heteromorphic; Bold and Wynne, 1985) is found in four of the five groups of ulvophytes. This type of life cycle, though neither uniform nor universal in the group, contrasts to that of other green algae, which generally have a predominant haploid vegetative phase and a single-celled, often dormant, zygote as the diploid stage (haplobiontic, Bold and Wynne, 1985). The diplobiontic life cycle is thus largely absent from green algae in freshwater habitats.

Because of the lack of clear synapomorphies for the ulvophytes, monophyly has been an open question since the group was established (O'Kelly and Floyd, 1984; Bremer, 1985; Mishler and Churchill, 1985). Analyses using molecular data are lacking. Preliminary studies of 18S and 26S rRNA (Zechman et al., 1990) indicated that Ulvophyceae of Mattox and Stewart (1984) are not monophyletic, but a verdict awaits addition of further taxa and genes. Antiquity of the group and possible large numbers of extinctions may make recovery of the relationships among the major clades within the ulvophytes difficult with a single gene such as 18S rDNA.

Within the ulvophyte lineage, five well-demarcated groups are recognized (O'Kelly and Floyd, 1984), which are usually ranked as orders, although their elevation to class is favored by some authors (e.g., van den Hoek et al., 1995). Figure 19 shows a phylogenetic tree of the ulvophytes based on Hayden and Waaland (2002), O'Kelly et al. (2004), and a preliminary analysis of 18S rDNA sequences (F. Zechman, California State University at Fresno, personal communication).
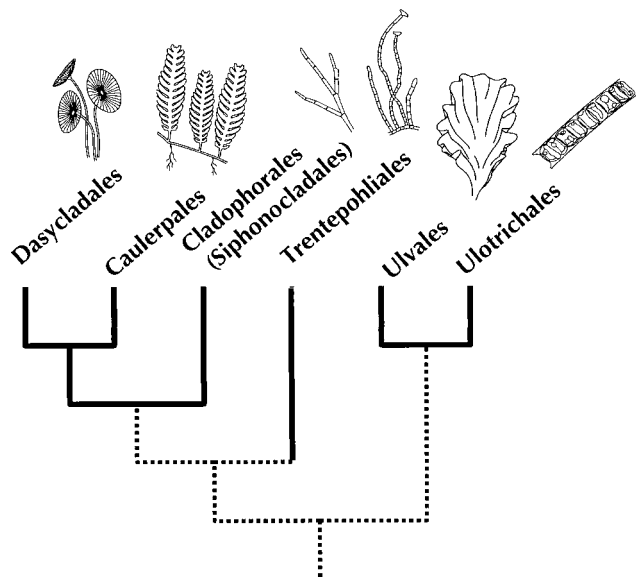
Fig. 19. Summary of the phylogenetic relationships of major lineages within Ulvophyceae based on DNA sequence data (Hayden and Waaland, 2002; O'Kelly et al., 2004) and a preliminary analyses of 18S rDNA sequences (F. Zechman, California State University at Fresno, personal communication). The drawings are thumbnail composites meant to show representative taxa.
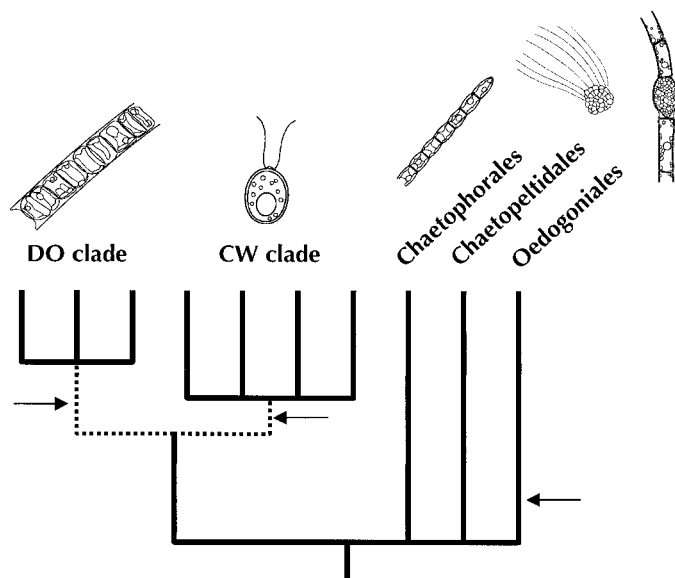
Fig. 20. Summary of the phylogenetic relationships of major lineages within Chlorophyceae, based on DNA sequence data. DO clade includes members of Sphaeropleales and Chlorococcales; CW clade includes Chlamydomonadales, Chlorococcales, and Chlorosarcinales. Dotted lines indicate regions of the tree that are poorly resolved with molecular data. Arrows indicate possible placement of incertae cedis clades. The drawings are thumbnail composites meant to show representative taxa.

Ulotrichales—This group includes nonmotile unicells (*Codiolum*), branched (*Acrosiphonia*) and unbranched filaments (*Ulothrix*), and bladelike forms (*Monostroma*). The order is fairly common in fresh and salt water and on solid substrates or other algae. The simple unbranched filamentous thallus of *Hormidium* led to its being classified in Ulotrichales, whereas cytokinesis and zoospore morphology resulted in its renaming (*Klebsormidium*) and removal to an entirely different major group (charophytes; Silva et al., 1972; Mattox and Stewart, 1984). Assignment to *Klebsormidium* was confirmed by molecular data (Karol et al., 2001).

Ulvales—This order contains several seaweed genera familiar worldwide, including *Ulva* and *Enteromorpha*, with membranous or tubular thalli, respectively. Recent work indicates that these genera are paraphyletic and *Enteromorpha* has been synonymized with *Ulva* (Hayden and Waaland, 2002; Hayden et al., 2003). Morphogenetic switching between the tubular and membranous form may have evolved multiple times in the group (Tan et al., 1999).

Cladophorales (including Siphonocladales)—Cladophorales contain branched and unbranched siphonous (multinucleate) green algae. This group includes one of the most commonly encountered freshwater and marine genera, the branching filamentous *Cladophora*. The genus has been monographed by van den Hoek and colleagues (1963, 1982, 1984; van den Hoek and Chihara, 2000), who placed it in a separate order, Cladophorales. Several recent studies of 18S rDNA sequences (Bakker et al., 1994; Hanyuda et al., 2002) indicate that *Cladophora* is paraphyletic or polyphyletic to other genera in the group (*Chaetomorpha*, *Rhizoclonium*, *Microdictyon*, and *Pithophora*), although additional data are needed to resolve relationships of this group. Hanyuda et al. (2002) provided clear

evidence that marine *Cladophora* has given rise to freshwater forms several times.

Caulerpales and Dasycladales—Caulerpales and Dasycladales are two marine groups of distinctive and often beautiful, siphonous seaweeds. The highly invasive *Caulerpa taxifolia* is a member of Caulerpales. Molecular studies of *Caulerpa* using the *tufA* gene have indicated that many morphological species are not monophyletic and that later diverging lineages in the genus are diversifying faster than ancient ones (Fama et al., 2002).

Dasycladales comprise two families, Dasycladaceae and Polyphysaceae (formerly Acetabulariaceae, Silva et al., 1996), that are estimated to have diverged some 400 mya based on fossil evidence (Berger and Kaever, 1992); molecular data indicate a more recent split approximately 265 mya (Olsen et al., 1994). The group includes the model organism, mermaid's wine glass (*Acetabularia* spp.). Zechman et al. (1990) studied rDNA sequences derived from RNA transcripts, and later Olsen et al. (1994) studied 18S rDNA sequences in Dasycladaceae and Polyphysaceae. Both analyses found support for monophyly of the former family. All dasyclads possess a stem-loop deletion unique among green algae (Olsen et al., 1994). Olsen et al. (1994) used distance and parsimony methods and supported monophyly of the two families, but only when the phylogenetic tree was unrooted; the authors suggested that rooting the tree with ulvophycean outgroups removed signal and resolution because the outgroups were so distantly related to the ingroup families. Olsen et al. (1994) also found that several genera, including *Acetabularia*, were paraphyletic.

Berger et al. (2003) sampled 17 of 19 species from Polyphysaceae (Acetabulariaceae) in a study of 18S rDNA sequences and corroborated the hypothesis of monophyly of Polyphysaceae and paraphyly of Dasycladaceae. Zechman (2003)

sampled a different gene, the plastid *rbcL*, in taxa from the two families and performed parsimony, likelihood, and Bayesian analyses. In contrast to the earlier 18S rDNA studies, Zechman (2003) concluded that both families were paraphyletic, not just Dasycladaceae. He also found a similar problem of paraphyly of genera. Berger et al. (2003) mapped features of cap formation onto the 18S rDNA tree and proposed a series of name changes in genera that would reconcile monophyletic groups on the tree and names of genera. Clearly, there is much to be done in this group, and additional multigene studies to resolve the phylogeny of the two families are underway (F. Zechman, California State University at Fresno, personal communication).

Trentopohliales—With an interesting mixture of ultrastructural characters, this group is sometimes omitted from the ulvophytes or included with several disparate green algal lineages (Chapman, 1984). Although entirely terrestrial, the group has marine relatives. A sea-to-land transition or vice versa would be unique among green, red, and brown algae (Graham and Wilcox, 2000). Trentopohlians have phragmoplast-like cytokinesis (Chapman and Henk, 1986; Chapman et al., 2001), an MLS-like flagellar structure and unusual zoospores (Graham, 1984), and "primitive"-type plasmodesmata, three characters reminiscent of charophyte algae (Chapman, 1984; Chapman et al., 1998). However, 18S rDNA sequence data place these algae firmly in the chlorophyte clade, most likely in the ulvophytes. Clearly, this is one group that bears further investigation of both morphology and DNA sequences.

Chlorophyceae—Chlorophyceae are a monophyletic group that includes some of the most familiar of the microscopic green algae, including many model organisms. The unicellular flagellate *Chlamydomonas* has been used to study flagellar motion and swimming (Mitchell, 2000), photosynthesis mutations (Niyogi, 1999), and plastid genome modifications in secondarily nonphotosynthetic taxa (Vernon et al., 2001). Colonial green algae such as *Volvox* have been models for the evolution of multicellularity, cell differentiation, and colony motility (Hoops, 1997; Kirk, 2003). Chlorophycean algae *Scenedesmus* and *Pediastrum* are also important paleoecological or limnological indicators (Nielsen and Sorensen, 1992; Komarek and Jankovska, 2001).

Green algae in this class have a great range of vegetative morphology, from coccoid to swimming unicells, colonies, and simple flattened thalli to unbranched and branched filaments. All have a haplobiontic life cycle (zygotic meiosis). Orders previously recognized were defined on their vegetative morphology, form of sexual reproduction (either isogamy, anisogamy, or oogamy), and mode of asexual reproduction (zoosporic or autosporic). During cell division, mitosis is closed and cytokinesis involves a phycoplast system of microtubules, sometimes combined with furrowing. Swimming cells are vegetative cells, zoospores (asexual), or gametes, with two, four, or hundreds of flagella. Cells with two or four flagella have cruciate (X-2-X-2) rootlets and flagella that are displaced in a "clockwise" (CW, 1–7 o'clock) direction or are "directly opposed" (DO, 12–6 o'clock). In some swimming colonial forms such as *Volvox*, the flagellar apparatus undergoes developmental modification and the flagella are reoriented for colony swimming (Hoops, 1997). Other variants include taxa with two unequal flagella (*Heterochlamydomonas*) or flagella emerging from a pit (*Hafniomonas*).
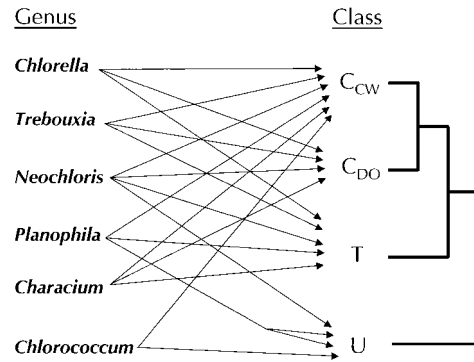

Fig. 21.   Examples of the extreme polyphyly of genera formerly classified in orders Chlorellales, Chloroccocales, Chlorosarcinales. With molecular data and/or ultrastructural data, the named genera were shown to have species belonging to different phylogenetic clades. U = Ulvophyceae, T = Trebouxiophyceae, C_{CW} = chlorophycean CW clade, C_{DO} = chlorophycean DO clade.

A flood of primarily 18S rDNA data collected from chlorophycean green algae in the last two decades has given rise to dramatic modifications at every level of classification (Fig. 20). A number of the traditional orders (Chlorellales, Chlorococcales, Chlorosarcinales), originally circumscribed using vegetative morphology, are now known to contain phylogenetically unrelated taxa. This is especially true for the groups that are morphologically depauperate and exhibit convergent evolution toward reduced morphology or cases in which *absence* of a trait was used as a main distinguishing feature of an order. Numerous evolutionary losses of motile cells are found across the chlorophycean green algae. Members of the Chlorellales (now mostly in the Trebouxiophyceae) completely lack motile cells, and thus the phylogenetically useful features that come from flagellar ultrastructure cannot be scored for these algae (Huss and Sogin, 1990).

Not only are these traditional orders polyphyletic, many of the species within genera are also being reclassified into different taxonomic groups (even classes), largely supported by molecular data (Fig. 21). In nearly all cases that have been (or can be) examined, ultrastructural and other cellular features are congruent with the molecular reclassifications, even though the ultrastructural data alone are often insufficient to support the reclassifications. Based on molecular data and corroborated by a great deal of biochemical evidence, *Chlorella* was split into numerous groups spread across Chlorophyceae and Trebouxiophyceae (Huss and Sogin, 1990; Huss et al., 1999). Buchheim et al. (1990) showed that *Chlamydomonas* is not monophyletic. Since then, many additional genera have been shown to be highly polyphyletic. Congeners of *Neochloris* and *Characium* are now in three classes (Watanabe and Floyd, 1989; Lewis et al., 1992; Kouwets, 1995; Watanabe et al., 2000). Friedl and O'Kelly (2002) used ultrastructural and molecular data to distribute four species of the chlorosarcinoid genus *Planophila* into Ulvophyceae (two species into two distinct clades), one into Trebouxiophyceae, and a fourth species into Chaetopeltidales (Chlorophyceae). Species of *Chlorococcum* were separated into Chlamydmonadales and Ulvophyceae (Watanabe et al., 2001; Krienitz et al., 2003). Thus, molecular data have provided evidence of convergent morphological evolution in many of the characters used to distinguish unicellular genera of chlorophycean green algae. The results of these studies only emphasize the need for additional molecular investigations, especially those that explore morphological
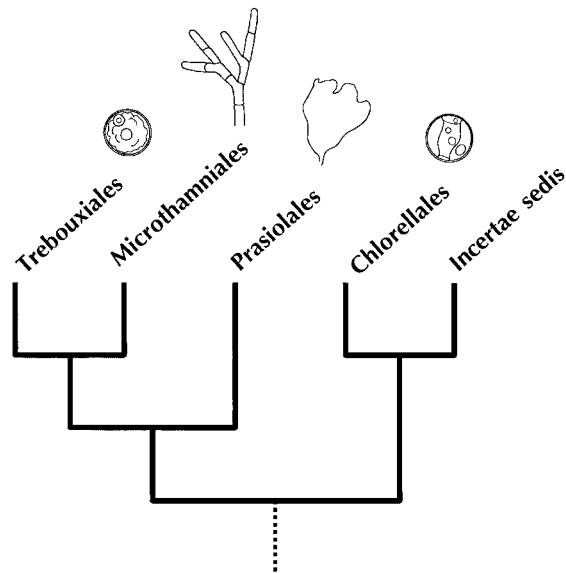
Fig. 22. Summary of phylogenetic relationships of major lineages within Trebouxiophyceae based on 18S rDNA data. Dotted lines indicate regions of the tree that are poorly resolved in some analyses of molecular data. The drawings are thumbnail composites meant to show representative taxa.

character evolution in Chlorophyceae. Two overarching themes are evident in Chlorophyceae. First, superficial similarities in body plans can be misleading, especially for simpler forms, and second, a range of morphology is represented within a single clade, with the coccoid form being nearly ubiquitous. To paraphrase J. B. S. Haldane (cited in Evans et al., 2000), nature must have had an inordinate fondness for coccoids.

CW clade (Chlamydomonadales)—This clade includes a large number of taxa in Chlamydomonadales, plus taxa formerly placed in Volvocales, Dunaliellales, Chlorococcales, Tetrasporales, Chlorosarcinales, and Chaetophorales (Lewis et al., 1992; Wilcox et al., 1992; Buchheim et al., 1994; Nakayama et al., 1996a, b; Watanabe et al., 1996; Booton et al., 1998a, b; Krienitz et al., 2003). Vegetative morphology includes biflagellate unicells and colonies, quadraflagellate unicells, coccoid unicells, weakly branching filaments, or packets that are sometimes in a gelatinous matrix. All of the taxa possess the CW orientation of flagella and rootlets.

Buchheim et al. (1996, 1997), among others, demonstrated that the large and well-recognized genus *Chlamydomonas* represents at least five lineages within the CW clade. Some of the lineages share organismal characters, such as the pigment loroxanthin (Fawley and Buchheim, 1995) and autolysin groups (Buchheim et al., 1990). Wall-less unicellular taxa in the related order Dunaliellales (sensu Ettl) were also shown to be nonmonophyletic, but instead interspersed in the CW clade. The unusual *Hafniomonas*, previously interpreted as having a modified CCW flagellar apparatus orientation or as a relative of Chaetopeltidales, is also a member of the CW clade (Nakayama et al., 1996a).

The colonial flagellates previously placed in Volvocales are not monophyletic based on molecular data (Buchheim and Chapman, 1991; Buchheim et al., 1994) and do not represent a tidy progression of evolution by building ever larger colonies from *Chlamydomonas*-like ancestors with developmentally

modified flagellar apparatus ultrastructure. Some colonial genera (*Gonium*) have been shown to be monophyletic, although most colonial forms with larger cell numbers are not (Nozaki et al., 2000).

Sphaeropleales (DO clade)—As emended by Deason et al. (1991), this order includes vegetatively nonmotile unicellular or colonial taxa that have biflagellate zoospores with the DO flagellar apparatus arrangement: *Sphaeroplea*, *Atractomorpha*, *Neochloris*, *Hydrodictyon*, and *Pediastrum*. All of these taxa possess basal body core connections (Wilcox and Floyd, 1988). With an increase in the number of taxa for which sequence data are available, there is evidence of an expanded DO clade that includes additional zoosporic (*Bracteacoccus*, *Schroederia*; Buchheim et al., 2001; Lewis, 1997) and some strictly autosporic genera such as *Ankistrodesmus*, *Scenedesmus*, *Selanastrum*, *Monoraphidium*, and *Pectodictyon* (Krienitz et al., 2001, 2003). Monophyly of the DO clade is generally weakly supported by phylogenetic analysis of molecular data, even those that have used data from two genes (Buchheim et al., 2001; Wolf et al., 2002; Shoup and Lewis, 2003).

Oedogoniales—This order includes three genera, *Oedogonium*, *Oedocladium*, and *Bulbochaete*, and approximately 600 species, all of which grow attached to submerged surfaces in freshwater habitats. All members of this order form simple or branched filaments. The familiar *Oedogonium* is often used as an example of oogamous sexual reproduction in green algae. All genera produce unusual motile cells (either asexual zoospores or male gametes) with an anterior ring of flagella (stephanokont). The flagellar apparatus of these cells has been studied extensively (Pickett-Heaps, 1975) and is clearly unlike the swimming cells in other groups of green algae. Given the strikingly unusual ultrastructure of the swimming cells, homology assessment with flagellar characters found in the other groups is difficult. Pickett-Heaps (1975) hypothesized that this group represents a ''basal'' lineage that gave rise to other filamentous forms. Analyses of molecular data (18S and 26S rDNA) have indicated that the order is clearly monophyletic (Booton et al., 1998b; Buchheim et al., 2001) and is often placed sister to the rest of Chlorophyceae. However, this placement varies, often with differing resolutions that include the Chaetopeltidales and Chaetophorales. Because all three orders represent ''long branches'' in the tree, a robust placement is not often obtained or depends on method of analysis.

Chaetopeltidales—This order of four genera was proposed by O'Kelly (1994) to include taxa that produce quadriflagellate motile cells with a perfectly cruciate (DO) flagellar apparatus orientation. The vegetative morphologies found in this order include flattened thalli (*Chaetopeltis*) and a sarcinoid genus *Floydiella* (formerly *Planophila*; Friedl and O'Kelly, 2002). Analyses of 18S rDNA data place this group, albeit weakly, with the biflagellate DO taxa (Booton et al., 1998a; Krienitz et al., 2003). Analyses of combined 18S and 26S rDNA data were also inconclusive, but they often resulted in topologies with this order outside the two clades of biflagellate taxa, closer to the base of Chlorophyceae (Buchheim et al., 2001; Shoup and Lewis, 2003).

Chaetophorales—Members of the order have unbranched or branched filamentous vegetative bodies and produce quadriflagellate motile cells with upper and lower pairs of basal bodies

in a CW + CW arrangement. Phylogenetic analysis of 18S rDNA data by Nakayama et al. (1996a) placed *Chaetophora incrassata* as sister to the rest of Chlorophyceae. With expanded sampling, Booton et al. (1998b) placed Chaetophorales nearest the biflagellate taxa with the CW orientation, although this placement was very weakly supported. As with Oedogoniales and Chaetopeltidales, the monophyly of the order is supported, but its relative placement remains unresolved.

Incertae Sedis—Topologies resulting from the analysis of two genes (18S and 26S rDNA) have indicated a distinctive chlorophycean clade, adjacent to Sphaeropleales, which includes the filamentous genus *Cylindrocapsa* and the unicellular *Trochiscia* and *Treubaria*. Previous hypotheses indicated an alliance of *Cylindrocapsa* with Sphaeropleales based on morphology of the pyrenoid (region within plastid containing a high concentration of the enzyme ribulose-1, 5-bisophosphate carboxylase [rubisco] and associated with starch synthesis), but this relationship is not supported with molecular data (Buchheim et al., 2001). Another unnamed group of freshwater picoplanktonic taxa, the *Mychonastes* clade (Krienitz et al., 2003), forms a sister group to Oedogoniales at the base of Chlorophyceae. A new order will need to be established eventually to accommodate this clade. In addition, several flagellate taxa, including species of *Carteria* and *Hafniomonas*, form a grade at the base of the CW clade and do not correspond to a named group (Nakayama et al., 1996a; Hoham et al., 2002).

Trebouxiophytes—In their 1984 classification, Mattox and Stewart proposed the class Pleurastrophyceae to accommodate freshwater algae with the CCW flagellar apparatus orientation, a metacentric spindle, and phycoplast-mediated cytokinesis. This class included the nonmotile unicells *Pleurastrum* and *Trebouxia*, the unicellular flagellate *Tetraselmis*, and *Microthamnion*, a filamentous alga with short branches. Conversely, Melkonian (1990a) chose to group these same taxa (excluding the flagellate *Tetraselmis*) into a separate order Microthamniales. Additional studies expanded membership of this group to include the terrestrial alga *Leptosira*, which produces unbranched uniseriate filaments (Lokhorst and Rongen, 1994). An early molecular analysis based on just a few taxa by Kantz et al. (1990) provided evidence for the nonmonophyly of Pleurastrophyceae.

Friedl and Zeltner (1994) and Friedl (1995), using 18S rDNA data, demonstrated the designated type of the class Pleurastrophyceae, *Pleurastrum insigne*, was actually a member of Chlorophyceae. In addition, Friedl provided evidence for a distinct clade (class Trebouxiophyceae) of many of the taxa that were formerly in Pleurastrophyceae (such as *Microthamnion*), but not related to Ulvophyceae or Chlorophyceae. In agreement with Melkonian (1990a), Friedl (1995) excluded the flagellate *Tetraselmis* from the group. Subsequently, through the use of 18S rDNA data, an increasing number of taxa have been added to this order, including some *Chlorellas* and *Oocystis*, autosporic taxa for which collection of data on motile cell ultrastructure is impossible. Although many members of Trebouxiophyceae (*Trebouxia*) participate in lichen symbioses and several workers defined the group primarily on lichen phycobionts, this class now includes a growing number of free-living planktonic or terrestrial species. *Prototheca*, a secondarily nonphotosynthetic coccoid alga, and picoplanktonic coccoids such as *Nannochloris* are members of this class.

Members of Trebouxiophyceae reproduce asexually by au-tospores or zoospores. Sexually reproductive stages have not been observed directly in any of the trebouxiophyte algae; however, one recent application of phylogenetic data collected for lichen photobionts sheds light on this topic. Kroken and Taylor (2000) used fine-grained sampling of isolates of *Trebouxia jamesii*, a photobiont of the fungal genus *Letharia*, to provide evidence of a recombining population structure.

The trebouxiophytes were shown to be monophyletic (Friedl, 1995), but because some molecular studies do not recover monophyly or recovered weak support for monophyly (Krienitz et al., 2003), this question will need further attention. Again, designation of this group was based on molecular data and a suite of morphological characters, many or most of which have the plesiomorphic character state. For example, the CCW flagellar orientation is shared with Ulvophyceae. At least five distinct lineages are recovered with 18S rDNA data (e.g., Krienitz et al., 2003), four of which correspond to named groups (Fig. 22).

Trebouxiales—This order represents the ''lichen algae group'' and includes zoosporic taxa, like *Trebouxia*, which are lichen phycobionts (Ahmadjian, 1993). Note that not all lichenized algae are in this group; other photobionts include cyanobacteria, *Trentepohlia* (Ulvophyceae), and *Heterococcus* (tribophytes). Several genus-level taxonomic changes have been made within this order. For example, *Friedmannia* was shown to be nested within the genus *Myrmecia* and was renamed (Friedl, 1995).

Microthamniales—The highly branched filamentous *Microthamnion* and the unicellular *Fusochloris* (former member of *Neochloris*, Chlorophyceae) are supported as a clade, related to but distinct from Trebouxiales. Both taxa are zoosporic and have the characteristic biflagellate swimming cells of Trebouxiophyceae. A close phylogenetic relationship between such morphologically different taxa once again illustrates the rapid evolution of vegetative morphology.

Prasiolales—This group includes zoospore-forming taxa, which are unicellular, short filaments, or small sheetlike thalli. These algae are commonly found in a great range of environmental conditions, including freshwater, marine, and terrestrial habitats such as on moist concrete walls and rocks, and tree bark (Rindi et al., 1999; Handa et al., 2003; Rindi and Guiry, 2003). Members of this group are also often encountered in cold deserts (e.g., *Pabia signiensis* from Antarctica; Friedl and O'Kelly, 2002). *Prasiola* was formerly in Ulvophyceae, but analyses of molecular data support its membership in Trebouxiophyceae (Friedl and O'Kelly, 2002). Handa et al. (2003) also resolved a close phylogenetic relationship of a corticolous species of *Stichococcus* with *Prasiola*. Naw and Hara (2002) identified isolates of *Prasiola* with a vegetative body that resembles *Enteromorpha* but are distant from Ulvophyceae and can be distinguished on the basis of the stellate plastids.

Chlorellales—Molecular data have had a great impact first on placing members of Chlorellales in Trebouxiophyceae rather than Chlorophyceae and then on clarifying relationships within Chlorellales. This order includes two well-supported families of autosporic species, Chlorellaceae and Oocystaceae. *Chlorella* (previously classified in Chlorellales, Chlorophyceae) was shown to be paraphyletic with many (or most) members in Trebouxiophyceae (Huss and Sogin, 1990; Huss et al.,

1999). Yamamoto et al. (2003) investigated the genus *Nannochloris* using 18S rDNA and actin genes and determined nonmonophyly of these small planktonic, coccoid forms. Their molecular phylogenetic lineages corresponded to cell division patterns. The nonphotosynthetic *Prototheca* (Huss and Sogin, 1990; Ueno et al., 2003), the Oocystaceae (Hepperle et al., 2000), and the spiny *Lagerheimia* (Krienitz et al., 2003) are now additional members of this order. Clearly, major rearrangements are still possible for algae referred to with some frustration as LRGTs, or "little round green things" (Graham and Wilcox, 2000a).

Charophyte algae and land plants—Charophyte algae are relatively poor in species diversity but are paraphyletic to a hugely diverse group, the land plants (~500 000 species). Smith (1950) recognized Charophyceae as one of two classes of green algae (the other was Chlorophyceae), but he included only Charales (stoneworts) in the group. Mattox and Stewart (1984) revised the composition of the group greatly by including four other orders. Charophyceae sensu Mattox and Stewart shared features of glycolate metabolism, cytokinesis (phragmoplast or similar structures in more-derived forms), and motile cell structure (asymmetrical, with an MLS associated with flagella) that were recognized as both distinct from that in other green algae and shared by embryophytes. Early on, Stewart and Mattox (1975) and colleagues (Pickett-Heaps and Marchant, 1972; Pickett-Heaps, 1975) recognized this assemblage of algae as members of one of two divergent green algal lineages. These early workers recognized the implication of the findings: an assemblage of green algae living today are direct descendants of an ancestor shared with land plants (Pickett-Heaps, 1969, 1972). Phycologists still tended to separate the charophycean green algae from land plants, but other botanists pointed out the monophyly of the group (Mishler and Churchill, 1984, 1985; Bremer, 1985; Bremer et al., 1987).

Using data on biochemistry (Stewart and Mattox, 1972), flagellar structure (Melkonian, 1984), and cytokinesis, Mattox and Stewart (1984) codified Charophyceae to comprise five orders. In general, molecular studies have supported the monophyly of individual orders, with some modifications. In addition, one or more prasinophycean taxa may be members of the charophyte lineage (i.e., *Mesostigma*, Melkonian, 1989; Karol et al., 2001; Turmel et al., 2002c). Figure 18 summarizes the overall relationships among charophyte algae based on molecular data.

Mesostigmatophyceae—This asymmetrical unicell is singular in more ways than one. Its flagellar structure first indicated that it was a member of the charophyte clade (Melkonian, 1989). Subsequent molecular analyses of 18S rDNA led to the hypothesis that *Mesostigma* is sister to *Chaetosphaeridium*, and these two genera were placed in a new class, Mesostigmatophyceae (Marin and Melkonian, 1999). Later molecular studies indicated that this grouping was artifactual and that *Chaetosphaeridium* was a member of Coleochaetales, in which the genus had long been classified (Delwiche et al., 2002). This left *Mesostigma* as an unusual alga for which phylogenetic placement is still a subject of contention. Delwiche et al. (2002) using *rbcL* sequences and Karol et al. (2001) using a four-gene analysis (*rbcL*, *atpB*, *nad5*, and 18S rDNA) found *Mesostigma* to be sister to other charophyte algae and embryophytes. In contrast, other studies have found *Mesostigma* to be sister to the rest of the Chlorobionta, i.e., sister to Chlo-

rophyta and Charophyta. These studies, based on chloroplast small and large subunit rDNA sequences (Turmel et al., 2002a) and multiprotein sequences from genome sequences of the mitochondrion (Turmel et al., 2002c) and plastid (Lemieux et al., 2000), are consistent with pigment studies. Yoshii et al. (2003) identified 9′-cis neoxanthin in *Mesostigma* although all other green plants have trans-neoxanthin. The authors interpreted this result as an indication of the early divergence of *Mesostigma*, prior to the evolution of the cis-isomerases necessary to convert trans to cis (in all other green plants). In contrast, Martin et al. (2002) analyzed 274 protein-coding genes in analysis of 16 plastid genomes, including *Mesostigma*, two other noncharophyte green algae, and six embryophytes, and found a topology congruent with nesting of *Mesostigma* within the charophyte algae-embryophyte clade as in Karol et al. (2001). Clearly, genomic sampling is just beginning and should clarify these positions.

Chlorokybales—This order is monotypic, with thalli of sarcinoid packets of cells that grow subaerially (Rogers et al., 1980). Biflagellate scaly zoospores are produced, with hairy flagella that possess an MLS in the root. The four-gene analysis of Karol et al. (2001) and Delwiche et al. (2002) placed this species in the charophyte algae, near the base of the lineage.

Klebsormidiales—These algae are simple unbranched filaments with parietal laminate or lobed chloroplasts. Zoospores have an MLS-type root and two asymmetrical flagella. *Klebsormidium* was proposed by Silva et al. (1972) to remove confusion attending the name *Hormidium* when the alga was considered a member of Chlorophyceae. *Klebsormidium* contains approximately 24 species, and Lokhorst (1996) monographed European taxa. Two genera (*Stichococcus* and *Raphidonema*) thought to be members of the order are not, but *Entransia fimbriata*, originally placed by Hughes (1948) in Zygnematales, is now allied with *Klebsormidium* (McCourt et al., 2000; Karol et al., 2001; Turmel et al., 2002a). Cook (2004) studied morphology of this species, and although she did not find zoospores, she found empty zoosporangia, which distinguish it from Zygnematales.

Zygnematales and Desmidiales—Mattox and Stewart (1984) combined these two orders in one, Zygnematales, but wall ornamentation and structure has led to the demarcation of placoderm desmids in Desmidiales (Gerrath, 2003). Thalli are unicellular or filamentous, with one colonial genus. Two morphological synapomorphies unite the group: (1) flagella are entirely lacking at any stage in the life cycle, and (2) sexual reproduction involves conjugation, the fusion of nonflagellate gametes that move actively or passively through a tube or within a gelatinous envelope. Gametes are usually isomorphic but sometimes exhibit differences in motility (i.e., one amoeboid male gamete moves; Hoshaw et al., 1990). These algae represent the most species-rich group of charophyte algae, with approximately 4000 species (Gerrath, 2003). Family classification based on ultrastructure of the cell wall (Mix, 1972; Brook, 1981) has been supported by molecular studies (Bhattacharya et al., 1994; Surek et al., 1994; Besendahl and Bhattacharya, 1999; McCourt et al., 2000; Gontcharov et al., 2003). The derived condition of highly ornamented walls with elaborate pores is found in four families of placoderm desmids. Desmidiales and placoderm desmid families appear to be

monophyletic (McCourt et al., 2000; Gontcharov et al., 2003). However, two families with a plesiomorphic cell wall condition (smooth, unornamented) are either paraphyletic in their original definition or members of the sister of placoderm desmids (McCourt et al., 2000; Gontcharov et al., 2003). These molecular analyses indicate that more complex forms evolved from simple filaments and morphological switching from unicells to filaments occurred several times.

Coleochaetales—This group contains two genera (*Coleochaete* and *Chaetosphaeridium*) and about 20 species, which have a morphology and life history that makes them important as model systems for understanding the evolution of embryophytes (Graham, 1993, 1996; Graham and Wilcox, 2000b). *Coleochaete* thalli are mostly tightly arranged discs of cells, although some are made of more loosely branching filaments. *Chaetosphaeridium* thalli are filamentous. Characteristic of the order are distinctive sheathed hairs that are extensions of the cell wall containing a small amount of cytoplasm. Zygotes of *Coleochaete* are retained on the maternal gametophyte in some species, and placental transfer cells may transfer nutrients to the zygote (Graham and Wilcox, 1983; Graham, 1985, 1996).

Studies of 18S rDNA sequences cast doubt on the monophyly of Coleochaetales and indicated that *Chaetosphaeridium* was sister to the rest of the charophyte lineage (Sluiman and Guihall, 1999), a conclusion that held up despite the discovery of a fungal artifact in the sequence (Cimino et al., 2000; Sluiman, 2000). As discussed in the section on *Mesostigma*, an 18S rDNA analysis placed *Chaetosphaeridium* as sister to the flagellate *Mesostigma* (Marin and Melkonian, 1999), but subsequent analyses of *rbcL* (Delwiche et al., 2002), chloroplast small and large subunit rDNA (Turmel et al., 2002a), and a four-gene analysis of plastid, nuclear, and mitochondrial genes (Karol et al., 2001) presented convincing evidence that *Chaetosphaeridium* is sister to *Coleochaete*. Relationships of *Coleochaete* have been studied using *rbcL* (Delwiche et al., 2002), including endophytic species that live within the cell wall of *Nitella* (Cimino and Delwiche, 2002).

Charales—This group contains six extant genera with several hundred species, collectively called stoneworts or charophytes in the paleontological literature (Grambast, 1974; Feist et al., in press). Thalli are attached by rhizoids to sandy or silty substrates in quiet freshwater habitats and range from a few centimeters to several decimeters in height. The thallus consists of a central axis of large multinucleate internodal cells, with whorls of branchlets radiating from nodes of uninucleate cells; a single meristematic cell initiates growth at the apex of the axis and branchlets. This whorled branching habit is convergent with some aquatic angiosperms such as *Ceratophyllum* and *Myriophyllum*. Calcium carbonate accumulates on the surfaces of many species (hence the name stoneworts), which partly accounts for the rich fossil record of more than 80 genera and 10 families stretching back to the upper Silurian (Feist and Grambast-Fessard, 1991; Gensel and Edwards, 1993). Reproduction is oogamous, and sperm morphology is complex (Garbary et al., 1993).

Molecular studies have supported monophyly of the sole extant family, Characeae (McCourt et al., 1996, 1999; Meiers et al., 1997, 1999). This family is distinguished morphologically from extinct taxa but clearly sister to them (Feist et al., in press). *Chara* species appear to be monophyletic, although the results indicate that conventional taxonomy (Wood and Im-

ahori, 1965) of tribes, sections, and subsections within *Chara* are in need of revision (Meiers et al., 1997, 1999; McCourt et al., 1999). Species of *Nitella* have been studied using *rbcL* (Sakayama et al., 2002) and *atpB* sequences (Sakayama et al., 2004), and results indicate that taxonomy within this genus is also in need of revision. Oospore surface morphology appears to hold promise for such revision (Sakayama et al., 2002, 2004).

THE ORIGIN OF LAND PLANTS

A clade of their own—Standard textbooks on botany and phycology acknowledge a world view in which the green algae as a whole are no longer monophyletic to the exclusion of land plants (embryophytes; Raven et al., 1999; Graham and Wilcox, 2000a; Graham et al., 2002; Judd et al., 2002). The molecular data on this point support the already strong case from ultrastructural data and are overwhelming, coming from the nuclear ribosomal repeat unit, mainly the small (18S) subunit, but including 5S and large subunit (26S) rDNA sequences as well (reviews in Chapman and Buchheim, 1991; McCourt, 1995; Melkonian and Surek, 1995; Chapman et al., 1998). Several plastid genes sampled broadly enough to address this question yield similar results: *rbcL* (Manhart, 1994; McCourt et al., 1996, 2000; Delwiche et al., 2002) and small and large subunit rDNA (Turmel et al., 2002a). Other molecular synapomorphies supporting the charophyte clade (though not uniformly present or sampled in all groups) are genomic features not found in the chlorophyte clade: transfer of the *tufA* gene from the plastid to the nucleus in the common ancestor of some charophyte algae (Zygnematales, Coleochaetales, Charales) and embryophytes (Baldauf and Palmer, 1990), patterns of insertion of introns in two plastid tRNA genes (Manhart and Palmer, 1990), an ORF for *matK* in a group II intron of the *trnK* exon of Charales and possibly some other charophyte algae (Sanders et al., 2003), and insertion of an intron into the VATPase A gene in Coleochaetales (Starke and Gogarten, 1993).

Despite the convincing evidence for monophyly of charophyte algae and embryophytes, the topology of the charophyte tree has shifted dramatically. Specifically, the exact identity of the group sister to embryophytes has proven to be elusive. Various groups or combinations of groups received support from different sources of data. For example, several analyses of 18S rDNA sequences yielded a topology in which Charales was placed with strong support as sister to the other four orders plus land plants (Friedl, 1997; Huss and Kranz, 1997). In contrast, *rbcL* data and some 18S rDNA data indicated that Charales plus Coleochaetales (McCourt et al., 1996, 2000) or Charales alone (Melkonian and Surek, 1995; Delwiche et al., 2002) were sister to land plants. Such conflicts usually indicate a dearth of data in terms of taxa or characters or both. Another recent study sampled small and large subunit plastid rDNA genes broadly across all major charophyte and chlorophyte groups and five bryophytes (Turmel et al., 2002a) and found strong support for monophyly of the major groups, with the exception of *Mesostigma*, which, as mentioned, was positioned sister to both chlorophyte and charophyte clades. However, the analysis could not resolve with confidence the sister taxon of land plants.

Karol et al. (2001) sampled one mitochondrial (*nad5*), one nuclear (18S rDNA), and two plastid (*rbcL*, *atpB*) genes (total of 5147 nucleotides) across the major charophyte groups (26

species), plus eight land plants and six chlorophyte outgroups. Bayesian, maximum parsimony, and maximum likelihood analysis all supported monophyly of Charales and land plants. Turmel et al. (2003) sequenced the entire mitochondrial genome of *Chara vulgaris* L., and their analyses were congruent with the conclusions of Karol et al. (2001). Gene loss from the mitochondrion, and group I and II intron distributions in the *Chara* mtDNA clearly allied this alga with that of land plant mtDNA. Maximum likelihood analysis of 23 protein sequences also strongly supported monophyly of *Chara* with land plants, although the number of taxa in the analysis (six) adds a note of caution to the result. Clearly, more genes and more taxa are needed to evaluate this result (Rokas et al., 2003).

Extreme greens and the multiple origins of terrestrial green plants—One of the exciting insights of the last few years has been the discovery of diverse green algae that can inhabit "extreme" habitats. More intensive sampling, coupled with culture-based methods and "environmental sampling," have expanded our understanding of green algal diversity and of the conditions necessary for survival and growth of green algae. For example, although we have known about the occurrence of green algae in saline environments for a long time, recent studies provide an expanded view of the extent to which green algae can exist in extreme environments. The chlorophycean alga *Dunaliella salina*, one of the major species used in carotenoid production, can exist at supersaline conditions (in waters that are >10% salt, Padwahl and Singh, 2003). *Dunaliella acidophila*, another extremophilic species, grows at extremely low pH (<2). The ability to do so depends on active proton elimination (Gokhmann et al., 2000). Zettler et al. (2002) reported both charophyte and chlorophyte green algae, among other eukaryotes, living in acidic (pH 2) waters with high levels of heavy metals. Edgomb et al. (2002) used environmental sampling to uncover deep-sea hydrothermal vent eukaryotes related to the prasinophyte, *Mantoniella*.

Besides being found in highly saline and acidic waters, green algae are also common on land. Colonization of terrestrial environments by green plants is one of the most important events in the history of the Earth. We have learned a great deal about the adaptations that allowed for growth in terrestrial environments by comparing green algae to embryophytes. However, the embryophtye transition to land is just one of numerous such transitions in the green plastid-bearing lineage. Mapping the distribution of known terrestrial lineages on a generalized green plant cladogram (Fig. 18) illustrates that the evolution to terrestrial environments is neither uncommon nor phylogenetically limited. Terrestrial green algae are found in at least six major clades (classes or orders) exclusive of the embryophytes. Terrestrial lineages seem to be absent in the prasinophyte algae but are found in both chlorophyte and charophyte clades. Multiple terrestrial lineages exist within Chlorophyceae, Trebouxiophyceae, and charophytes. All of the known terrestrial algae are inferred to have aquatic ancestors, and, in fact, some are closely related to aquatic taxa (Hoham et al., 2002; Lewis and Flechtner, 2002).

Terrestrial algae are morphologically similar to aquatic taxa and include unicellular coccoids to filaments. Many spend a majority of the vegetative life out of water, although their reproductive stages cannot be completed without water. Members of Trentepohliales (Ulvophyceae) are known from aerial habitats, often as pathogens in and on the leaves of citrus plants. They are also common on bark and rocks, developing a characteristic brilliant orange color from secondary pigments. Within Trebouxiophyceae, many members are known lichen symbionts, and others are known from moist soils. Terrestrial members of Charophyceae include *Klebsormidium*, *Chlorokybus*, *Mesotaenium*, and *Cylindrocystis*; terrestrial Chlorophyceae include the filamentous genus *Fritschiella* and coccoid genera *Bracteacoccus*, *Chloromonas*, *Chlamydomonas*, and *Scenedesmus*.

Terrestrial algae grow in some of the most difficult habitats on earth, such as desert soils. Here, algae experience lengthy periods of desiccation and extremes in temperature and light levels. The earliest studies enumerating the green algae from deserts were based on vegetative morphology and recovered only a few genera (e.g., Cameron, 1960). However, morphological studies involving examination of alternate life cycle stages or studies using molecular analyses of green algae isolated from deserts of western North America have indicated a much larger number of genera than found previously and multiple transitions to arid habitats from aquatic ancestors within Chlorophyceae, Trebouxiophyceae, and charophytes such as *Klebsormidium* and *Cylindrocystis* (Flechtner et al., 1998; Lewis and Flechtner, 2002).

Psychrophilic algae are defined as those taxa having optimal growth below 10°C. Cold-loving taxa are found in Chlorophyceae and charophytes, including *Chloromonas*, *Chlamydomonas*, *Chlorosarcina*, *Mesotaenium*, and *Cylindrocystis*. Many cold-tolerant algae occur in numbers large enough to form red, pink, or green zones easily visible in snow. The unicellular biflagellate genus *Chloromonas* is one of the most common of the snow algae (Ling, 2001, 2002). *Chloromonas* is distinguished from *Chlamydomonas* by the absence of a pyrenoid, and the lack of pyrenoids and cold tolerance was thought to be correlated. However, molecular phylogenetic studies (Buchheim et al., 1990, 1997a, b; Hoham et al., 2002) concluded that neither *Chlamydomonas* nor *Chloromonas* is monophyletic, but rather that *Chloromonas* species were derived numerous independent times from *Chlamydomonas*. Hoham et al. (2002) showed that cold tolerance developed at least three times in the *Chlamydomonas* clade and that it was not correlated with the presence of a pyrenoid (Borkhsenious et al., 1998). At the deepest taxonomic levels, presence of a pyrenoid seems to be phylogenetically meaningful; all embryophytes (except for hornworts) lack pyrenoids. The utility of pyrenoid presence as a taxonomic character among closely related algae is now questionable because pyrenoids were shown to be under the regulation of a single gene in *Chlamydomonas* (Fukuzawa et al., 2001), and the transition between a pyrenoid-containing and a pyrenoid-less state might not be complex.

Documented cases of independent transitions to terrestrial habitats provide important contrasts to what is known about physiological adaptations in embryophytes. Examples of the transition from aquatic to a variety of terrestrial habitats within the green algae are also exciting because they provide detailed information about the phylogenetic relationships of extreme greens to their relatives in more moderate habitats, thus offering an opportunity to study the timing and mechanisms of the transitions to these specific environments. Such studies were made possible only recently because of molecular phylogenetic analyses and the ability to separate taxa with confusingly similar vegetative morphology. These investigations will become increasingly feasible and interesting as more taxon-dense sampling covers a wider range of habitats.

CONCLUSIONS

The classification is, of course, not without faults, and some are serious. Nevertheless, we think it is as good as can presently be devised (Mattox and Stewart, 1984, p. 45).

In 1984, Mattox, Stewart, and others working at that time no doubt felt that the accumulating body of new data had produced significant advances in green algal systematics and dramatically modified classification from a strictly vegetative basis to a focus on ''evolutionarily conserved'' ultrastructure. With this, we must agree. Progress in the molecular systematics of green algae rests on hundreds of ultrastructural and biochemical investigations that paved the way for evaluation of these explicit hypotheses. Many broad-scaled ultrastructure-based hypotheses have been corroborated by molecular data, yet many relationships now revealed by molecular data were never predicted or predictable based on ultrastructural data. In this review, we have tried to illustrate ways that molecular data have corroborated hypotheses from ultrastructural data and also the numerous important exceptions for which these data were completely ineffective at establishing relationships.

Molecular data have provided greater resolution than classifications based on ultrastructure. For example, the paraphyly of the charophytes was already indicated from cladistic analysis of nonmolecular data, but molecular data further clarified the relationships by providing additional evidence for the inclusion of *Mesostigma* in the charophyte lineage (or placing it as the sister taxon to all green algae and plants) and for resolving Charales as sister (among extant lineages) to embryophytes. Sequence data have also provided valuable insight into the phylogenetic placement of taxa that were difficult to place based on morphology because they contain characters representative of more than one group (e.g., Trentepohliales, *Entransia*).

There are many striking differences between molecular and ultrastructurally based classifications. Only two of the five classes established from ultrastructural data (Chlorophyceae and Ulvophyceae) are recovered with molecular data, although the ulvophytes are often not resolved as monophyletic with molecular data. Many of the taxa currently included in Trebouxiophyceae would not have been placed in this class on the basis of morphology alone. Two other classes based upon ultrastructure (Prasinophyceae, Charophyceae) are grades that should be reclassified. Another important impact of molecular data has been resolution of relationships among the problematic coccoid green algae (especially of the strictly autosporic taxa without swimming cells). No doubt there will be many more profound changes to come in the classification of these groups.

Molecular data are also expanding our view of diversity, both in familiar taxa and in newly revealed, previously unsampled taxa. The distinct lineages of paraphyletic *Chlamydomonas* were not distinguished because they possess nearly identical flagellar structure. Culture-based or ''environmental sampling'' studies that include molecular data have documented novel lineages (Phillips and Fawley, 2000; Lewis and Flechtner, 2003) that are now being characterized. If these new taxa correspond to critical or early diverging lineages, their inclusion in future analyses could have a profound impact on our understanding of relationships among the major groups of green algae.

Despite these advances, molecular data thus far have failed to resolve firmly some of the deeper branches of the green plant tree of life, including the monophyly of the ulvophyte algae, the relationships among lineages in the Chlorophyceae, and the topology of the prasinophyte algae. A contributing factor to this lack of resolution is a paucity of data compared to that from other plant groups. Only a small proportion of the known taxa have been included in molecular studies, and with few exceptions, only data from a single gene are available. A new focus on the collection of multiple genes or even genomes is underway (Qui and Lee, 2000; Chapman and Waters, 2002). The work of Karol et al. (2001), Lemieux et al. (2000), and Turmel et al. (2002a, b, c) have illustrated the power of resolving critical nodes with ever increasing amounts of data. In addition, C. J. O'Kelly (Bigelow Laboratory for Ocean Sciences, personal communication) and colleagues, funded through the Assembling the Tree of Life program of the National Science Foundation (USA), are collecting plastid and mitochondrial genome-level data for 30 green algae, selected to represent some of the ''difficult'' regions of green plant phylogeny (http://ucjeps.berkeley.edu/TreeofLife/). There is optimism that, with enough data, the more difficult regions of the tree and incongruence among single-gene data sets will be resolved (see Rokas et al., 2003), although nodes representing rapid radiations may require data that are less clocklike (e.g., informative changes in morphology or genome structure that track divergence of short branches).

Morphological and ultrastructural studies will still have a role in the systematics of green algae in the future. Previous studies (e.g., Sluiman, 1985; Bremer et al., 1987; Garbary et al., 1993; Mishler et al., 1994) used explicit homology assessment and cladistic analysis of morphological, ultrastructural, and molecular data to uncover major early diverging lineages of green plants. Scotland et al. (2003, p. 545) claim that morphological data are being superceded by molecular data because ''much of the useful morphological diversity has already been scrutinized.'' This is clearly not the case for groups such as the green algae, for which new morphological data are still being obtained, albeit at a much slower rate than molecular data. Novel molecular lineages are prime targets for future ultrastructural studies, but molecular diversity is not always predictive of ultrastructural diversity (O'Kelly et al., 2004). If our goal is to circumscribe natural and recognizable groups, understand morphological evolution, and study adaptation, the collection of disembodied sequence data will be of little use without ultrastructural data. In the case of green algae, discovery and characterization of unsampled diversity could have an immense impact in recovering the green plant tree of life.

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