MOLECULAR PHYLOGENY

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INTRODUCTION

Molecular phylogeny provides a test of the homology of morphological characters used in construction of evolutionary hypotheses for fossil and extant taxa. Even though molecular data are not directly available for fossils, gene-sequence data for those taxa within a group such the charophytes with an ancient fossil record can tie together branches that include fossil and living taxa. Moreover, relating charophytes, a group with such distinctive morphology, to extant sister taxa with little in the way of shared morphology is very difficult. But doing so may be possible with molecular data, and this approach has yielded significant insights into phylogenetic relationships of charophytes, land plants, and other green algae (McCourt, Karol, & others, 1996; McCourt, Meiers, & others, 1996; McCourt & others, 1999; Chapman & others, 1998; Karol & others, 2001; Sanders, Karol, & McCourt, 2003).

For the charophytes, molecular research has used data from several different genes or nonprotein-coding DNA (reviewed by McCourt, Meiers, & others, 1996). Phylogenetic hypotheses for extant charophyte genera and species have been tested against phylogenies based on the morphology of gyrogonites for the Characeae and related families. These comparisons have provided information that has been considered critical to understanding charophyte evolution, i.e., convergent evolution of some characters. This chapter describes the methods used to obtain molecular data from living charophyte genera also reported as fossils and methods of analysis of these data.

METHODS OF MOLECULAR PHYLOGENETIC STUDIES

COLLECTION OF MATERIAL

Molecular samples must be free of contaminating epiphytes or endophytes to avoid spurious amplification and sequencing of genes from other than those of the target taxa (e.g., Sluiman & Guihal, 1999; Cimino, Karol, & Delwiche, 2000). Because charophytes are often collected in association with other green algae, careful examination of thalli prior to extraction is essential. Even when no epiphytes are evident, endophytes may exist within charophyte cells (Jost, 1895; Cimino & Delwiche, 2002). This source of possible contamination is all the more problematic when the endophyte is a species of *Coleochaete,* such as *C. nitellarum,* which is related relatively closely to charophytes and may be similar enough genetically to confound phylogenetic analysis. The diversity of such endophytes may be greater than previously thought (Cimino & Delwiche, 2002).

Culturing of material in soil water medium (microcosms of ponds, in glass jars of water over a sterilized soil and sand mixture, Hoshaw & Rosowski, 1973) can yield fresh growing tips that are often the best material for DNA sampling. Fresh or flash-frozen material (using liquid nitrogen) is best for sampling, and as little as 0.1 g of material is sufficient, using slightly modified CTAB (Cetyltrimethylammonium Bromide) methods (McCourt, Karol, & others, 1996; McCourt & others, 1999) for extraction.

GENES USED FOR STUDY

Early phylogenetic research on many algal groups employed sequences of the small subunit ribosomal DNA present in all eukaryotes (SSU rDNA, also called 18S rDNA) (Hillis, Moritz, & Mable, 1996). This nuclear gene has been used in a wide variety of organisms and has been sampled in the Characeae as well (Kranz & Huss, 1996). The relatively large size (~1,800 bp) and slow rate of change make it more suitable for studies of deep branching within the

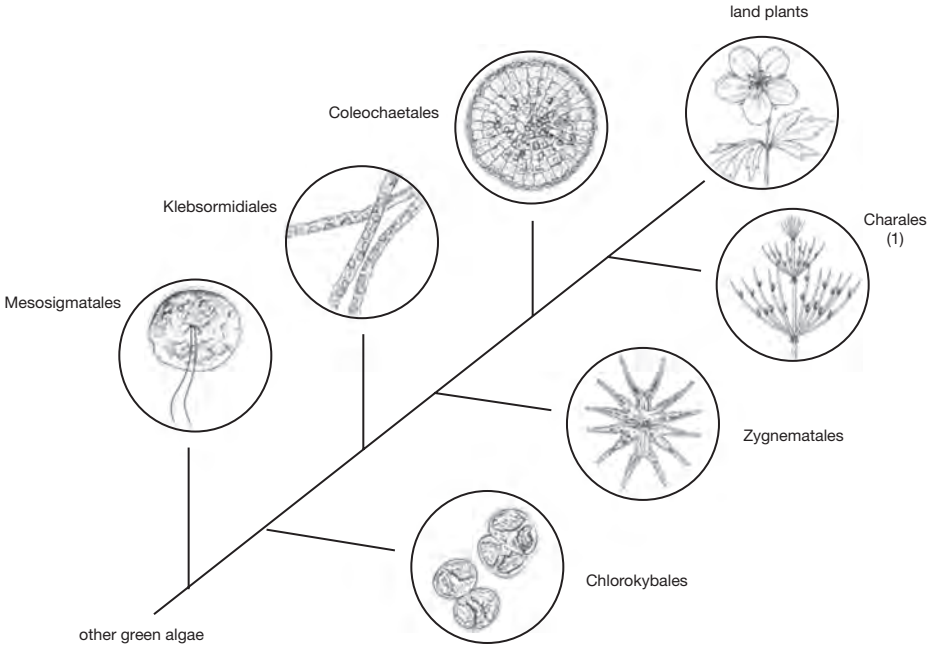

FIG. 41. Phylogeny of green algae and land plants based on four-gene analysis of Karol and others (2001); thumbnail sketches showing morphology of major groups of algae and one flowering plant in lineage including land plants or embryophytes (*i.e.,* liverworts, mosses, vascular plants, seed plants, and angiosperms); see Karol and others (2001) and Judd and others (2002); *1,* Charales, that is, Charophyta *s.s.* (adapted from Karol & others, 2001).

phylogeny of algae and green plants, although even at this relatively ancient level SSU sequences by themselves have been misleading (KRANZ & HUSS, 1996) or provided only weak resolution of phylogeny (KAROL & others, 2001). Internally transcribed spacer regions of DNA (ITS 1 and ITS 2) are transcribed but nontranslated regions located between the small subunit (SSU or 18S), 5.8S, and large subunit (LSU or 28S) of rDNA. The highly variable ITS regions are effective in studies of angiosperm species (BALDWIN & others, 1995), but they are apparently too variable to be equally informative in studies of more anciently diverged lineages in the Characeae (R. MCCOURT & K. KAROL, personal observation, 2002).

Sequences from other genomic compartments have also been sampled. The large subunit of Rubisco (see Glossary, herein p. 90) from the chloroplast *rbcL* is a protein-coding gene that exhibits greater sequence divergence than SSU sequences and is effective in deciphering sectional and generic relationships in the Characeae (MCCOURT, KAROL, & others, 1996). While informative at the interspecific level, *rbcL* alone did not resolve fully the relationships among species. The four-gene analysis of KAROL and others (2001) provided additional support for the *rbcL* results (see below). The finding of *matK* in the plastid of characean taxa (SANDERS, KAROL, & MCCOURT, 2003) provided an additional gene with more informative characters than other plastid genes normally sampled (MOHR, PERLMAN, & LAMBOWITZ, 1993; JOHNSON & SOLTIS, 1994, 1995; STEELE & VILGALYS, 1994; OOI & others, 1995; LIERE & LINK, 1995; GADEK, WILSON, & QUINN, 1996). This gene (~1,500 bp) resides within a group II intron of the *trnK* tRNA gene, which encodes the lysine tRNA. The level of divergence in *matK* holds promise for further species-level studies.

MOLECULAR PHYLOGENY AND IMPLICATIONS FOR FOSSIL CHAROPHYTES

RELATIONSHIP OF CHARACEAE TO OTHER GREEN ALGAE AND LAND PLANTS

The morphological complexity of charophytes compared to most other green algae has led workers to classify them in a distinct group, usually at the division (=phylum) level (i.e., Charophyta) or as a distinct class within the green algae in the broader sense (i.e., Charophyceae within the Chlorophyta) (Smith, 1950; Bold & Wynne, 1985). Smith (1950) preferred assigning the Characeae to the class Charophyceae because of the their distinctly different vegetative and reproductive features, such as verticillate branching and sheathing cells surrounding the reproductive structures. Mattox and Stewart (1984) expanded the taxon Charophyceae to include the Charales plus an assemblage of other green algae (listed below) that share a number of traits with land plants. These characters included features of cell division, structure of the flagella, and other features that indicated this assemblage of green algae, including the Charales and fossil relatives, is on the line of evolution leading to land plants (embryophytes; i.e., liverworts, bryophytes, and nonvascular and vascular plants). Moreover, the Charophyceae or at least one of its member groups shared a more recent common ancestor with land plants than with other green algae. Thus, charophycean algae plus land plants constituted one of two major lineages, and the other comprised the rest of what we commonly call green algae (Mishler & Churchill, 1985; McCourt, 1995). Bremer (1985) proposed to call the monophyletic group of charophycean green algae plus land plants the Streptophyta or streptophytes.

The hypothesis of Mattox and Stewart (1984), based primarily on ultrastructural morphology, has been verified by molecular studies in the past decade (McCourt, 1995; Chapman & others, 1998; Karol & others, 2001; Chapman & Waters, 2002). The Charophyceae of Mattox and Stewart (1984), however, included several groups in addition to charophytes *sensu stricto,* and the identity of the sister taxon of the land plants has proven elusive (Graham, 1993; McCourt, 1995; Chapman & others, 1998). These other groups of the Charophyceae *sensu* Mattox and Stewart include the Klebsormidiales (filamentous green algae), Chlorokybales (unicells arranged in packets), Zygnematales (conjugating green algae), and Coleochaetales (discoid or filamentous algae with sheathed hairs).

It is important to note that the advent of molecular data did not answer immediately the question of which group is the sister taxon of land plants. These new data from gene sequences occur not in a vacuum but in an arena of competing hypotheses on the relationships of green algae and land plants (see Graham, 1993; Chapman & others, 1998 for reviews). Analyses of the nuclear SSU gene suggested that the Charales were the earliest branch from the streptophyte lineage, with less complex filamentous forms (e.g., Klebsormidiales, Zygnematales, Coleochaetales, and Chlorokybales) forming an unresolved sister group of the land plants (Kranz & Huss, 1996). In contrast, data from the plastid gene *rbcL,* the large subunit of the photosynthetic enzyme Rubisco suggests that the Charales, the Coleochaetales, or a clade of both groups formed the sister taxon of the land plants (McCourt, Karol, & others, 1996; Chapman & others, 1998). The reason for this conflict between analyses based on two genes is not clear but is likely due to inadequate taxon sampling and insufficient sequence data.

A recent four-gene study of a broad range of algal and plant groups using sequence data from chloroplast (*rbcL, atpB*), nuclear (SSU rDNA), and mitochondrial (*nad5*) genes suggested that the Charales (and presumably extinct charophytes) form an exclusive group that is the sister taxon of land plants (Fig. 41) (Karol & others, 2001). The analysis and

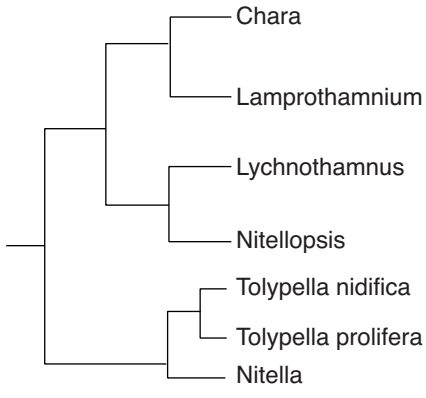

FIG. 42. Phylogenetic relationships of extant genera of Characeae based on analysis of four genes; sequences of four genes included 2 plastid genes (*rbcL, atpB*), 1 mitochondrial gene (*nad5*), and 1 nuclear-encoded gene (small subunit, or 18S, rDNA); an aligned dataset of 5,147 base pairs was subjected to Bayesian inference; same tree resulted from an analysis using maximum parsimony and minimum evolution. This tree represents a portion of phylogeny for green algae and land plants from Karol and others (2001), where more details may be found.

thorough taxon sampling of the latter study provides the strongest support to date of a sister-taxon relationship between charophytes and land plants. In other words, the Charales and their extinct relatives are descended from a unique, green-algal ancestor that was related to other streptophytes but distinct from them. These results are consistent with later analyses of *rbcL* in a large study of the Coleochaetales (DELWICHE & others, 2002) and SSU and LSU plastid rDNA in a broad survey of streptophytes *sensu* BREMER, 1985 (TURMEL & others, 2002).

The findings of KAROL and others (2001) raise intriguing questions regarding fossil charophytes, which were more diverse and abundant than their extant relatives. The oldest gyrogonites (order Sycidiales) are approximately the same age as the earliest-known fossils of land plants (GRAHAM, 1993; GENSEL & EDWARDS, 2001). Despite the diversity of fossil charophytes relative to living forms, charophytes never approached the ecological and evolutionary success of land plants. The reason for this disparity of success is not clear. Since charophytes are so different from land plants and other charophycean algae *sensu* MATTOX and STEWART, it is unlikely that the common ancestor of charophytes and land plants closely resembled either group. Some traits are shared by charophytes and the primitive land plants, however: a filamentous germling stage, gross sperm morphology, many discoidal chloroplasts per cell, absence of zoospores, and envelopment of fertilized oogonia by sterile cells (KAROL & others, 2001). In addition, this common ancestor no doubt possessed ancestral forms of the many genes common to charophytes and land plants. Further studies of the functional genomics of these groups may shed light on the changes that occurred in these derived green algae that led to the successful colonization of land.

RELATIONSHIPS WITHIN THE CHARACEAE BASED ON MOLECULAR AND FOSSIL STUDIES

The first molecular study of genera in the Characeae was that of MCCOURT, KAROL, and others (1996), who used *rbcL* sequences and morphology to construct phylogenetic hypotheses of the group. The phylogenetic relationships of genera in the Characeae conformed generally with the traditional view that the family is divided into two subfamilies, the Charoideae and Nitelloideae, although support for the monophyly of the latter group was weak. This study supported the monophyly of the Characeae relative to green plants and resolved some relationships within the family. The topology based on *rbcL* sequences alone was strongly supported by the analysis of KAROL and others (2001) using three additional genes (Fig. 42). This larger data set also supports the monophyly of both subfamilies.

Perhaps most interesting about the phylogeny derived from these molecular studies is the very strong support of the monophyly of the two sections of *Tolypella* (*sensu* WOOD

& IMAHORI, 1964–1965). Previous studies had suggested that the genus might be paraphyletic because one section exhibits a multipartite basal plate, as found in *Nitella*, and another section in the genus has a simple, one-piece basal plate, as found in *Chara* (SOULIÉ-MÄRSCHE, 1989; FEIST & GRAMBAST-FESSARD, 1991). Clearly, either this character evolved more than once in *Tolypella,* or the genus should be split, and taxa with a simple basal plate should be put into a new genus in the Charoideae. The *rbcL* data clearly support the former hypothesis, that *Tolypella* is monophyletic and that a multipartite basal plate evolved twice within the Characeae. A further implication is that a multipartite basal plate is not necessarily a synapomorphy in other fossil taxa. A multipartite basal plate may have evolved twice in the Porocharaceae and in the *Aclistochara-Lamprothamnium* lineage. Still, basal plate features may be synapomorphies for some groups.

MEIERS and others (1997) and MEIERS, PROCTOR, and CHAPMAN (1999) used SSU sequence data to determine phylogenetic relationships within the Characeae. Their findings were generally congruent with those based on *rbcL* data; however, the taxon sampling and slower rate of evolution of the SSU gene relative to *rbcL* make comparison difficult. For example, MEIERS, PROCTOR, and CHAPMAN's (1999) finding that *Lamprothamnium* may be a member of *Chara* is contradicted by *rbcL* data for a larger sample of *Chara* and *Lamprothamnium.* The relationships of genera of Characeae based on *rbcL* were supported by the four-gene analysis of KAROL and others (2001).

MCCOURT and others (1999) sampled a wider range of species in the Characeae, in particular species of *Chara* and *Nitella*. Genera of the family Characeae are strongly supported as monophyletic. Within *Chara* the traditional grouping of species into sections by WOOD and IMAHORI (1965 in 1964–1965) is very strongly refuted, although some of the subsections within these sections are monophyletic. WOOD's practice of combining monoecious and dioecious microspecies as forms of more inclusive, broader species is not supported, although monoecious and dioecious taxa believed to be closely related are supported as such by the *rbcL* data. Thus, sequence data in general support the monophyly of species (i.e., microspecies of WOOD & IMAHORI, 1965 in 1964–1965) and genera recognized on morphological grounds.

Branch length asymmetry between genera in the Characeae and Nitelleae of WOOD and IMAHORI (1965 in 1964–1965) was noted in the *rbcL* studies of MCCOURT, KAROL, and others (1996) and MCCOURT and others (1999). Branches in *Nitella* and *Tolypella* are much longer than those in *Chara* and the other genera of the family. The reasons for this asymmetry are difficult to discern because of the lack of a good fossil record for the noncalcifying *Nitella* and *Tolypella* (GRAMBAST, 1974). One explanation could be that sequence change is faster in the latter genera for some unknown reason. Alternatively, the rate of sequence change could be roughly equal in all genera of the family, but species lineages in *Nitella* and *Tolypella* may be more ancient, and branch length would be proportional to time since divergence. In other words, extant *Chara* species may be descended from a more recent common ancestor. One of the oldest fossils of Characeae is a *Nitella*-like thallus from the Lower Devonian (TAYLOR, REMY, & HASS, 1992). If *Nitella* or *Tolypella* are indeed the descendants of more ancient divergences and can be reliably dated in the fossil record, it will provide a paleontological test of a hypothesis derived from molecular data.

SUMMARY

Molecular and morphological data are complementary and may be mutually illuminating in studies of charophytes. Hypotheses derived from studies of fossil or extant taxa hold the promise of providing reciprocal tests that can further our understanding of

charophyte evolution. Data from fossils provide evidence of much greater diversity of charophytes in the past, but many taxa have become extinct. Molecular data are valuable for revealing relationships of charophytes to the rest of the green algae and plants.

NOMENCLATURAL NOTE

The terms charophyte and Charophyta have traditionally been applied to living and fossil members of the monophyletic group of green algae in the Charales, Moellerinales, and Sycidiales (see p. 88). We have continued this usage herein. MATTOX and STEWART (1984), however, employed the root charo- for their class Charophyceae, including the traditional Charophyta MIGULA plus several other orders (Chlorokybales, Klebsormidiales, Zygnematales, and Coleochaetales). Because the latter group is paraphyletic without the inclusion of embryophytes, BREMER (1985) proposed the name of Streptophyta for the group (from the Greek *strepto,* for twisted, i.e., the morphology of the sperm of some members). Given the historical use of the term Charophyceae (SMITH, 1950), KAROL and others (2001) implied that the larger, more inclusive group of Charophyceae plus land plants be termed the Charophyta (see also DELWICHE & others, 2002). The Charales and fossil relatives would thus be relegated to the subdivision rank of Charophytina. This modified use of the division Charophyta, while controversial, would recognize the monophyly of a major clade of green algae and plants.

CLASSIFICATION OF CHAROPHYTA

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EARLY WORKS IN THE HISTORY OF CHAROPHYTE CLASSIFICATION

The first step toward classifying the Charophyta dates to 1719 when Vaillant grouped several extant forms under the generic name *Chara,* taken from the memoir by Dalechamps (1587) and later validated by Linnaeus in 1753. This name is thought to be derived from the Greek, meaning joy of water. Agardh (1824) proposed the Characeae, based on the presence of verticillate branches bearing capsules (female) and globules (male) and including two genera, *Chara* and *Nitella;* the name Characeae had been previously mentioned by Kunth (1815), who attributed it to L. Cl. Richard.

Fossil forms were discovered in the second half of the 18th century. Schreber (1759) was the first to describe and illustrate thalli and gyrogonites as well as oospores from around Halle (Germany) but without recognizing their true nature. Until the first half of the 19th century, charophyte remains were attributed to different groups of animals such as worms (Schreber, 1759) and corals (Sandberger, 1849), and the first fossil charophyte species, *Gyrogonites medicaginula,* was described by Lamarck (1801, 1804) as a miliolid foraminifer.

Leman (1812) recognized the relationship between the fossil gyrogonite and the living genus *Chara.* Leman's viewpoint was generally accepted, and newly discovered fossil remains were attributed to *Chara* (Brongniart, 1822; Lyell, 1826; Prevost, 1826). The first subdivisions of the fossil forms were introduced by Stache (1889), who described several genera and erected two tribes, keeping them apart from the extant Chareae and Nitelleae: the Lagynophoreae for bottle-shaped gyrogonites and the Kosmogyrae for ornamented ones. Groves (1933) and Grambast (1957) have expressed doubts as to the reconstitutions proposed by Stache, and his pioneering classification has now been abandoned.

DEVELOPMENT OF CHAROPHYTE CLASSIFICATION

The first structured classification was established by Pia (1927), who added to Stache's subdivisions two new families, the Palaeocharaceae and the Clavatoraceae. The Kosmogyrae Stache that Pia considered as artificial were not included in this system.

CLASSIFICATION (Pia, 1927)

- Class Charophyta
  - Unquestioned Charophyta
    - Family Characeae
      - Subfamily Nitelleae
      - Subfamily Chareae
      - Subfamily Lagynophoreae
    - Family Palaeocharaceae, based on the Devonian *Palaeochara* Bell, 1922
    - Family Clavatoraceae, based on the Mesozoic *Clavator* Reid & Groves, 1916
  - Doubtful Charophyta remains
    - Genus *Palaeonitella* Kidston & Lang
    - Genus *Trochiliscus* Pander
    - Genus *Sycidium* Sandberger

According to the bibliography of his paper, Pia (1927) was not aware of the monograph by Karpinsky (1906), who had shown that *Trochiliscus* and *Sycidium* were distinct Paleozoic branches of the Charophyta that he placed into two new subdivisions, the Trochiliscidae and the Sycididae. These were renamed later by Peck (1934a) as Trochiliscaceae and Sycidiaceae Karpinsky. Karpinsky (1906) attributed *Trochiliscus* to Pander (1856), but as noted by Peck (1934a), Pander designated a group of species also including *Sycidium* under the name Trochilisken. Thus the attribution of the authorship of *Trochiliscus* to Pander should not be maintained.

In 1938, PECK erected the Atopocharaceae, but this family could not be maintained after the inclusion of *Atopochara* within the Clavatoraceae (HARRIS, 1939).

MÄDLER (1952) summed up the knowledge acquired to that date and erected new subdivisions at the ordinal level as well as the new subfamily Characeae Aclistocharae, defined later (MÄDLER, 1955). The Aclistocharae are characterized by a more or less distinct periapical dehiscence furrow. They include the genus *Porochara,* in which the apex is always open, and genera in which the apex is closed by the swollen terminal ends of the spirals, constituting a convex rosette. For MÄDLER (1955) this rosette was comparable to the opercule that falls during germination in the living forms.

CLASSIFICATION (MÄDLER, 1952)

Class Charophyta
- Order Sycidiales *nov. ord.*
  - Family Sycidiaceae (KARPINSKY, 1906) PECK, 1934a
- Order Trochiliscales *nov. ord.*
  - Family Trochiliscaceae (KARPINSKY, 1906) PECK, 1934a
- Order Charales *nov. ord.*
  - Family Palaeocharaceae BELL, 1922
  - Family Clavatoraceae REID & GROVES, 1916
  - Family Lagynophoraceae STACHE, 1880
  - Family Characeae RICHARD in KUNTH, 1815
    - Subfamily Aclistocharae *nov. subf.*
    - Subfamily Kosmogyreae STACHE, 1889
    - Subfamily Nitelleae VON LEONHARDI, 1863
    - Subfamily Chareae VON LEONHARDI, 1863

The classification proposed by GRAMBAST (1962b) includes four more families and three more subfamilies than that of MÄDLER, 1952. Within the Sycidiales, the Chovanellaceae were erected for gyrogonites with numerous, vertical cells that are undivided or subdivided only at their apical ends.

Within the Charales, the Eocharaceae GRAMBAST, 1959a include gyrogonites with numerous sinistrally spiralled cells. The Raskyellaceae are based on the presence of five apical cells closing the apex. Within the Characeae, the Gyrogonae (=Brachycharae) bring together gyrogonites in which the apex, bearing convex nodules, is surrounded by a clear periapical furrow (GRAMBAST, 1956c). Two subfamilies are not retained: the Kosmogyrae STACHE and the Aclistocharae MÄDLER. GRAMBAST (1957) has shown that the ornamentation that characterizes the Kosmogyrae is not a feature of great taxonomic value and may not be constant within a species, such as in *Peckichara varians* GRAMBAST. Even a single specimen may be only partially ornamented, as in *Nitellopsis* (*Tectochara*) *thaleri* (CASTEL & GRAMBAST) GRAMBAST & SOULIÉ-MÄRSCHE. For GRAMBAST (1961) the subfamily Aclistocharae, which is composed of two distinct groups, is artificial. He erected the Porocharaceae for species with an apical pore always open, with the apical region either truncated (Porocharoideae) or drawn into a neck (Stellatocharoideae).

CLASSIFICATION (GRAMBAST, 1962b)

Order Sycidiales MÄDLER, 1952
- Family Sycidiaceae PECK, 1934a
- Family Chovanellaceae *nov. fam.*

Order Trochiliscales MÄDLER, 1952
- Family Trochiliscaceae PECK, 1934a

Order Charales
- Family Eocharaceae GRAMBAST, 1959a
- Family Palaeocharaceae PIA, 1927
- Family Porocharaceae *nov. fam.*
  - Subfamily Porocharoideae GRAMBAST, 1961
  - Subfamily Stellatocharoideae *nov. subfam.*
- Family Clavatoraceae PIA, 1927
- Family Lagynophoraceae STACHE, 1889
- Family Raskyellaceae GRAMBAST, 1957
- Family Characeae RICHARD in KUNTH, 1815
  - Subfamily Charoideae BRAUN in MIGULA, 1897
    - Tribe Gyrogoneae GRAMBAST, 1956b
    - Tribe Chareae VON LEONHARDI, 1863
  - Subfamily Nitelloideae BRAUN in MIGULA, 1897

WANG Zhen (1978a) proposed two subfamilies: Cuneatocharoideae, which includes gyrogonites of Porocharaceae with a conical outline in their upper part, and Gyrogonoideae for gyrogonites of Characeae with a depression or a breaking line around the apical zone. The Gyrogonoideae include two tribes: Gyrogoneae and Raskyelleae. For WANG Zhen, the apex structure of the Raskyellaceae corresponds to a Gyrogonoidae in which the reduction of width and thickness of the spiral cells around the apex reaches a point where it breaks, so the apical cells are separated from the spirals by a fracture and not by a true wall.

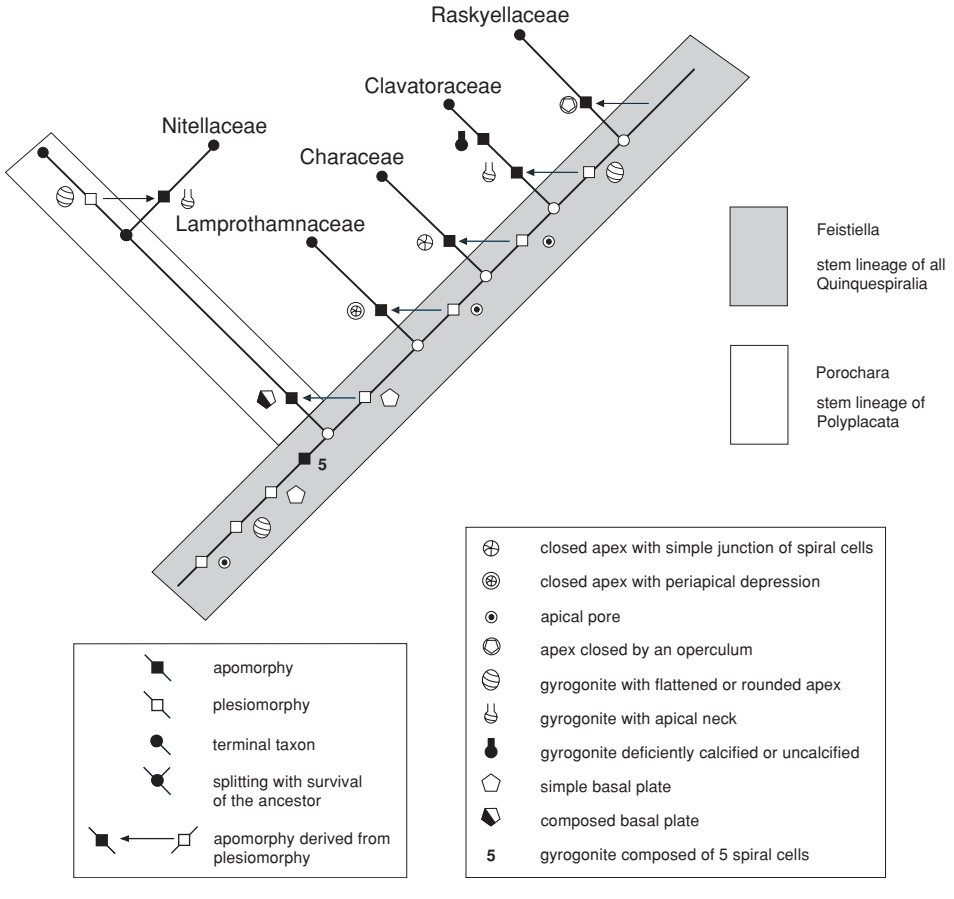

FIG. 43. Phylogenetic diagram of Quinquespiralia, with single-celled basal plate representing plesiomorphic character state (Martin-Closas & Schudack, 1991, fig. 2, hypothesis 2).

CLASSIFICATION (WANG, 1978a)

Family Porocharaceae GRAMBAST, 1962b
  Subfamily Stellatocharoideae GRAMBAST, 1962b
  Subfamily Porocharoideae GRAMBAST, 1961; *emend.*
  Subfamily Cuneatocharoideae *subfam. nov.*
Family Characeae RICHARD in KUNTH, 1815
  Subfamily Gyrogonoideae *subfam. nov.*
    Tribe Gyrogoneae GRAMBAST, 1956b
    Tribe Raskyelleae (L. & N. GRAMBAST) *comb. nov.*
  Subfamily Charoideae VON LEONHARDHI, 1863
  Subfamily Nitelloideae BRAUN in MIGULA, 1897
  Subfamily Aclistocharoideae MÄDLER, 1952

Additional families have been proposed, isolating one genus at a higher systematic level, without a new diagnosis. Thus the Nitellopsidaceae KRASSAVINA, 1971, the Primocharaceae ISHCHENKO and SAIDAKOVSKY, 1975, the Tectocharaceae MÄDLER and STAESCHE, 1979 and the Aclistocharaceae ZHOU, 1983 (in HAO & others, 1983) have not been retained in the classification adopted in the *Treatise.*

WANG Zhen and LU (1980) erected two new Paleozoic families. The Pinnoputamenaceae of the Sycidiales include gyrogonites with vertical ramified cells. The Trochiliscales are twofold, comprising the Trochiliscaceae emended, including gyrogonites with spiral cells segmented transversely and a basal pore with bilateral symmetry, and the Karspinskyaceae, for Trochiliscales devoid of these characters.

CLASSIFICATION (WANG & LU, 1980)
Class Charophyta
  Order Sycidiales MÄDLER, 1952
    Family Sycidiaceae PECK, 1934a
      Genus *Sycidium* SANDBERGER, 1849
    Family Chovanellaceae GRAMBAST, 1962b
      Genus *Chovanella* REITLINGER & JARWEZA, 1958
    Family Pinnoputamenaceae *fam. nov.*
      Genus *Pinnoputamen gen. nov.*
  Order Trochiliscales MÄDLER, 1952; *emend.*
    Family Trochiliscaceae PECK, 1934a; *emend.*
      Genus *Trochiliscus* (PANDER, 1856) KARPINSKY, 1906; *emend.*
    Family Karpinskyaceae *fam. nov.*
      Genus *Karpinskya gen. nov.*
      Genus *Moellerina* ULRICH, 1886

MARTIN-CLOSAS and SCHUDACK (1991) proposed a new classification of the mainly post-Paleozoic charophytes based on cladistic analysis. In that system, the chief character is the morphology of the basal plate (simple or multipartite), and the hypothesis of an ancestral position for a simple basal plate is preferred (Fig. 43). In this analysis, the genera *Porochara* and *Feistiella* are interpreted as paraphyletic taxa and written with quotation marks.

1. Quinquespiralia *nov. subord.* (apomorphy: five spiral cells), stem lineage formed by *"Feistiella"* and other traditional "Porocharoideae."

1.1. Family: Polyplacata *nov. fam.* (apomorphy: composed basal plate), stem lineage formed by *"Porochara."*

1.1.1. Subfamily: Nitellaceae *emend.* (apomorphy: apical neck), stem lineage formed by traditional "Stellatocharoideae" GRAMBAST; *emend.,* BREUER, recent terminals formed by *Nitella* and *Tolypella* (section *Tolypella*).

Genus: *"Porochara":* stem lineage of Polyplacata and primitive sister-group of Nitellaceae.

1.2. Family: Lamprothamnaceae *nov. fam.* (apomorphy: closed apex with periapical depression), *Lamprothamnium* and traditional synonyms (*Aclistochara,* etc.).

1.3. Family: Characeae *emend.* (apomorphy: closed apex with simple junction of the spiral cells), traditional Charoideae, except for *Lamprothamnium* and synonyms, but adding *Sphaerochara* (= *Tolypella* section *Rothia*).

1.4. Family: Clavatoraceae (apomorphy: apical neck, deficiently calcified gyrogonite) traditional Clavatoraceae.

1.5. Family: Raskyellaceae (apomorphy: apical operculum calcified), traditional *Saportanella*, *Raskyella*, (?) *Rantzienella*.

Genus: *"Feistiella":* stem lineage of Quinquespiralia and primitive sister group of taxa 1.2 to 1.5 (MARTIN-CLOSAS & SCHUDACK, 1991, p. 69–70).

In their classification of the Paleozoic forms, LU, SOULIÉ-MÄRSCHE, and Q. WANG (1996) considered the subdivision of the gyrogonite cells by transverse ridges as the most important character.

CLASSIFICATION
(LU, SOULIÉ-MÄRSCHE, & WANG, 1996)
Class Sycidiphyceae LANGER, 1976
  Order Sycidiales MÄDLER, 1952
    Family Sycidiaceae PECK, 1934a
      Genus *Sycidium* SANDBERGER, 1849
    Family Trochiliscaceae KARPINSKY, 1906; *emend.,* WANG & LU, 1980
      Genus *Trochiliscus* (PANDER, 1856) KARPINSKY, 1906
Class Charophyceae SMITH, 1938
  Order Chovanellales CONKIN & CONKIN, 1977
    Family Chovanellaceae (GRAMBAST, 1962b); *emend.*
      Genus *Chovanella* REITLIGER & JARZEWA, 1958; *emend.*
    Family Xinjiangocharaceae *fam. nov.*
      Genus *Xinjiangochara* YANG & ZHOU, 1990
  Order Moellerinales *ord. nov.*
    Family Moellerinaceae FEIST & GRAMBAST-FESSARD, 1991; *emend.*
      Genus *Moellerina* ULRICH, 1886; *emend.,* WANG, 1984
    Family Pseudomoellerinaceae WANG, 1984
      Genus *Pseudomoellerina* WANG, 1984
  Order Charales LINDLEY, 1836
    Family Eocharaceae GRAMBAST, 1959a
      Genus *Eochara* CHOQUETTE, 1956
    Family Palaeocharaceae PIA, 1927
      Genus *Palaeochara* BELL, 1922
    Family Porocharaceae GRAMBAST, 1962b
      Genus *Porochara* MÄDLER, 1955
    Family Pinnoputamenaceae WANG & LU, 1980
      Genus *Pinnoputamen* WANG & LU, 1980

For LU, SOULIÉ-MÄRSCHE, and WANG (1996), whether the Pinnoputamennaceae Z. WANG & LU, 1980, are charophytes is questionable.

CLASSIFICATION ADOPTED IN THE *TREATISE*

The classification adopted in the *Treatise* follows FEIST and GRAMBAST-FESSARD (1991) for the subdivisions of the Charales, but a new finding, the discovery of a utricle in most Paleozoic genera, led us to reconsider the concept of the Paleozoic orders and families.

As in previous classifications, Moellerinales and Charales are distinguished by the orientation of the gyrogonite cells, but for the Sycidiales, which contain all Paleozoic taxa with a utricle, this character cannot be used because the orientation of the gyrogonite cells is not preserved generally inside this organ. In the rare instances where gyrogonite cells are visible in thin section, their number is rather high, much greater than five. All other morphological evidence shows that it is not possible to classify the Sycidiales families together with the Clavatoraceae (Charales), which also present utricles but whose gyrogonites possess five sinistrally spiralled cells. Thus, it appears that the character of the utricle evolved independently in two groups of charophytes. The types of gyrogonites that may be inside the utricles of the Sycidiales are very likely to be found among the Moellerinales, the only charophytes devoid of a utricle that were in existence when the Sycidiales appeared during the late Silurian and Early Devonian. In our present state of knowledge, we keep the Sycidiales provisionally as a group apart but with close affinities to the Moellerinales.

The Sycidiales comprise four families, the Sycidiaceae, Trochiliscaceae, Chovanellaceae, and Pinnoputamenaceae, distinguished by the utricular characters. WANG and LU (1980) had already observed the similarities between Sycidiaceae and Trochiliscaceae, although they differ in cell orientation. The Chovanellaceae constitute a homogeneous group, characterized by utricles showing vertical undivided cells tending to spiral; the distinction of the Xinjiangocharaceae, which differ only by the cell number of what has been shown to be a utricle, has been abandoned. The attribution of the Pinnoputamenaceae to charophytes has been confirmed by the discovery of antheridia at the surface of the utricle of *Pinnoputamen* sp. (Fig. 48*e,* Systematics, herein p. 99) (FEIST & FEIST, 1997). In this family, the utricles are bilateral, and they bear ramified branches as in *Sycidium.*

In the Moellerinales, which do not present utricles, gyrogonites are spiralled dextrally. Within this group, a new name has been proposed for the Karpinskyaceae WANG & LU, the Moellerinaceae. The Moellerinaceae are based on the earliest genus *Moellerina* ULRICH, which also exhibits the most typical characters of the family (FEIST & GRAMBAST-FESSARD, 1991; LU, SOULIÉ-MÄRSCHE, & WANG, 1996). The two families Moellerinaceae and Pseudomoellerinaceae are distinguished by different numbers of gyronite cells.

Within the Charales two new suborders have been introduced in order to separate the families with more than five gyrogonite cells (Palaeocharinae) from the ones with five sinistrally spiralled cells (Charinae) (FEIST & GRAMBAST-FESSARD, 1991).

The Raskyellaceae is deemed a valid family after new observations with scanning electron microscopy that have confirmed the individuality of the apical cells (ANADON & FEIST, 1981). The Lagynophoraceae STACHE, which do not differ from the Characeae regarding the apex morphology (BIGNOT & GRAMBAST, 1969), have not been maintained. The apical neck typical of this family represents an external encrustation of coronula cells (CASTEL, 1969). The subdivision of the Charoideae into Chareae and Gyrogonae, which GRAMBAST (1962b, p. 76) thought already quite difficult to apply, was abandoned subsequently when further observations displayed the possible relationships between genera placed in the two different subfamilies, such as *Rhabdochara* and *Stephanochara,* as well as *Tolypella* and *Sphaerochara.*

During the past twenty years, new paleontological and biological data have shed a different light on the problem of the classification inside the Characeae family. The oldest representative of the Charoideae, *Aclistochara,* possesses a multipartite basal plate, whereas in the extant forms this character is present only in the Nitelloideae. The attribution of *Aclistochara* to the Charoideae is based on its clear resemblance with *Lamprothamnium* concerning the morphology of the apex (Soulié-Märsche, 1989). Martin-Closas and Schudack (1991) even considered both genera as synonyms. On the other hand, according to molecular data (McCourt, Karol, & others, 1996), the two sections of *Tolypella*, including section *Rothia* (=*Sphaerochara*) with a simple basal plate and the section *Tolypella* with a divided (multicellular) basal plate, are included in the same clade. This suggests that the character is not a synapomorphy distinguishing groups at the family and subfamily level (Soulié-Märsche, 1989; Feist & Grambast-Fessard, 1991; Martin-Closas & Schudack, 1991). The basal plate character is valuable, but it can be applied only to the generic or subgeneric levels, and high-level taxa based only on it, such as the Monoplacata and Polyplacata Martin-Closas & Schudack (1991), are no longer justified.

In this classification, the criteria for the distinction of the different categories of taxa are as follows.

ORDERS

Distinction at the order level is based on the orientation of the gyrogonite cells, whether dextrally spiralled (Moellerinales) or sinistrally spiralled (Charales); and presence of a utricle and a high number of gyrogonite cells for the Sycidiales.

FAMILIES

Distinction at the family level is based on the number of spiral cells, the apical structure of the gyrogonite, or the presence of a special character such as the utricle of the Clavatoraceae and of the four Sycidiales families; the characters of the apical zone of the gyrogonite predominate also in the separation of the subfamilies. The classification of the extant forms, which are all included in the family Characeae, comprises two tribes, Chareae and Nitelleae. In the systematics of the fossil forms adopted in the *Treatise,* however, families are divided into subfamilies; and we do the same for the Characeae, which have been subdivided into Charoideae and Nitelloideae.

GENERA

Genera are distinguished on the basis of particular characters of the gyrogonite apex, the basal plate, and the general outline of the gyrogonite.

SPECIES

Distinction of species is based on special characters of gyrogonite shape, ornamentation, and dimensions; in the Sycidiales and Clavatoraceae characters of the utricle are also taken into account.

Thallus remains, which are not connected to gyrogonites, are not included in this classification; they are treated separately (see Morphology, herein p. 12).

In the *Treatise* charophyte volume, only the generic attributes in accord with this classification have been considered for deter-

mining the ranges and distributions of genera. Descriptions of genera are presented not as diagnoses but as brief decriptions that emphasize comparative characteristics.

CLASSIFICATION
(Feist & Grambast-Fessard, herein)

Phylum Charophyta Migula, 1897
- Class Charophyceae Smith, 1938
  - Order Moellerinales Lu, Soulié-Märsche, & Wang, 1996
    - Family Moellerinaceae Feist & Grambast-Fessard, 1991; *emend.,* Lu, Soulié-Märsche, & Wang, 1996
    - Family Pseudomoellerinaceae Wang, 1984
  - Order Sycidiales Mädler, 1952; *emend.,* herein
    - Family Sycidiaceae Karpinsky, 1906; *emend.,* herein
    - Family Trochiliscaceae Karpinsky, 1906; *emend.,* herein
    - Family Chovanellaceae Grambast, 1962b; *emend.,* herein
    - Family Pinnoputamenaceae Wang & Lu, 1980; *emend.,* herein
  - Order Charales Lindley, 1836
    - Suborder Palaeocharineae Feist & Grambast-Fessard, 1991
      - Family Eocharaceae Grambast, 1959a
      - Family Palaeocharaceae Pia, 1927
    - Suborder Charineae Feist & Grambast-Fessard, 1991
      - Family Porocharaceae Grambast, 1962b
        - Subfamily Porocharoideae Grambast, 1961; *emend.,* Wang & Huang, 1978
        - Subfamily Clavatoritoideae Kozur, 1974
        - Subfamily Stellatocharoideae Grambast, 1962b
      - Family Clavatoraceae Pia, 1927
        - Subfamily Clavatoroideae Pia, 1927; *emend.*, Grambast, 1969
        - Subfamily Atopocharoideae Peck, 1938; *emend.*, Grambast, 1969
      - Family Raskyellaceae L. & N. Grambast, 1955
      - Family Characeae Agardh, 1824
        - Subfamily Charoideae Braun in Migula, 1897
        - Subfamily Nitelloideae Braun in Migula, 1897

GLOSSARY

MONIQUE FEIST and MICHELINE GUERLESQUIN

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This glossary explains special terms used in this volume. Some of these definitions follow CORILLION (1975) and MOORE (1986).

AND. Distance from apex to widest portion of gyrogonite (LED), as measured along polar axis.

antheridium (pl., antheridia). Male reproductive organ producing motile spermatozoids; does not secrete calcium carbonate, therefore seldom preserved.

apex (=summit). Distal end of gyrogonite, opposite pole of attachment to thallus.

apical neck. Protruding ends of spiral cells on apex, which form elongated, constricted neck.

apical pore (=apical opening). Opening in apical end of gyrogonite.

axial nodes. Short nodes of main axis and branches of unlimited growth.

basal depression. At basal pole, when distal opening of basal pore is of smaller diameter than proximal opening; a crater-shaped depression present when viewed externally.

basal opening. See basal pore.

basal plate (=basal plug). Plate at distal end of basal pore, formed as a result of calcification of sterile sister cell of oosphere.

basal pore (=basal opening). Opening.

bract cells. Single-celled processes growing out from peripheral cells of branchlet nodes (Chareae).

bracteoles. Pair of single-celled processes (similar to bract cells) originating from basal node below oogonium, one growing on each side of oogonium (Chareae).

bractlet. Single-celled process subtending oogonium in females of dioecious species of *Chara* replacing the antheridium.

branchlets (=phylloids). Laterals of limited growth produced in whorls at stem (axial) nodes.

bulbils. Agglomerations of starch-containing cells developing on rhizoids and at stem nodes of some charophytes.

capitula. Small cells within antheridium from which filaments develop that produce spermatozoids.

calcine. Calcium carbonate deposited in enveloping cells.

cellular ridges. Ridges down center of spirals.

cladom. In phycology, designates an axis issued from unlimited activity of an initial apical cell that generates alternating nodes and internodes. The pluricellular nodes produce phylloids in turn (=branchlets) having structure similar to main axis, but of definite growth; one or more connected cladoms constitutes thallus.

conjoined. Having antheridium and oogonium adjacent at same branchlet node.

coronula. Small, crownlike structure at apex of oogonium in one row of five cells (recent Chareae) or two rows of five cells each (recent Nitelleae), at tops of spiral cells.

cortex. Outer covering of longitudinally arranged cells, giving thallus axes a striped or ridged appearance.

corticate. Thallus having a cortex.

cortication. See cortex.

dichotomous branching. Typical of *Nitella;* phylloid (=branchlet) subdivided into two identical parts, which further subdivide themselves in two and so on; process results in formation of rays of 1st, 2nd, 3rd orders.

dioecious. Having male and female gametangia produced on separate male and female individuals of species.

diplostephanous. Having a double ring of stipulodes at base of each whorl of branchlets.

diplostichous. Having cortex arranged in alternate primary and secondary rows, there being two cortical rows corresponding to each branchlet, e.g., *Chara vulgaris.*

ecorticate. Lacking a cortex.

enveloping cells. External cells of gyrogonite or utricle.

equator. Widest portion of gyrogonite.

equatorial angle. Acute angle made between equatorial line (LED) and suture of spiral cell.

eutrophic. Water that is nutrient rich, thus supporting a large plankton population so transparency may be reduced.

eutrophication. Process of artificial enrichment, particularly by excessive level of phosphates from domestic and agricultural sources.

furcate. Forked.

gametangia. Gamete-producing sexual reproductive organs.

gymnophyllous. Having naked branchlets, i.e., branchlets without a cortex in species of *Chara* where main axes are corticate, as in *Chara gymnophylla* (recent).

gyrogonite. Fossil calcified oogonium.

haplostephanous. Having single ring of stipulodes at base of each whorl of branchlets.

haplostichous. Having cortex of primary cells only, i.e., one cortical row corresponding to each branchlet, as in *Chara canescens* (recent).

intercellular suture. Line marking junction between enveloping cells.

internode. Elongated portion of specimen stem between nodes consisting of single, elongated central cell.

ISI. Isopolarity index (LPA/LED), ×100.

LED. Largest equatorial diameter of gyrogonite.

LPA. Longest polar axis of gyrogonite.

manubria. Stalklike cells within antheridium that support capitula cells.

monoecious. Male and female gametangia produced on same individual of a species.

monogenetic (life cycle). In the Characeae, life cycle includes only one generation (haplobiontic); individuals generated by an oospore are haploid gametophytes, and meiosis occurs at germination of zygote (=fertilized egg).

monopodial. Having a main axis not supplanted by any lateral branch.

nodules. Swollen apical ends of spiral cells at center of apex.

oligotrophic. Water that is nutrient poor, does not support a large plankton population, and is therefore transparent.

oogamy. Female gamete (oosphere, egg) differentiated from large central cell of oogonium; motile sperm produced on cells of antheridium.

oogonium (=oosporangium). Female reproductive organ that encloses egg cell.

oosporangium. See oogonium.

oosphere. Female cell differentiated from large central cell of oogonium.

oospore. Fertilized egg cell (zygote).

parthenogenetic. Producing viable oospores without fertilization by male gametes: *Chara canescens* (recent).

phylloids. See branchlets.

proembryo. See protonema.

protonema. Small, rudimentary cladom issued directly from germination of oospore; gives rise to secondary erect cladom from which thallus develops.

ray. Internode of branchlet in Nitelloideae.

rhizoids. Colorless, hairlike filaments growing from charophyte base into substrate, with dual function of absorption and attachment.

rosette. Central apical swellings of ends of spirals on specimens with well-developed peripheral grooves.

Rubisco: Abbreviation of ribulose bisphosphate carboxylase/oxygenase, which is the critical enzyme in photosynthesis that takes carbon dioxide from the atmosphere and incorporates it into sucrose.

sejoined. Having antheridium and oogonium produced at separate branchlet nodes of same individual.

shield cells. Eight platelike cells that make up outer, protective layer of antheridium (8 shield cells = octoscutate; 4 = tetrascutate in some microspecies of recent *Chara zeylanica*).

sister cell of oosphere. See basal plate.

spine cells. Single-celled processes growing out from primary cortical cells.

spiral cells. Enveloping cells of gyrogonites; 5 in Charales, may be up to 12 in some Paleozoic genera.

sporostine. Two inner, suberized layers of oospore.

stipulodes. Single or double ring of single-celled processes growing out from base of branchlet whorls.

summit. See apex.

sympodial. Having branches that supplant and seemingly continue their parent branches so there is no one main axis.

taxon. Recognizable entity that may be separated from related entities at any level of classificatory hierarchy.

thallus. Vegetative system without stem and true leaves.

triplostichous. Cortex having two secondary rows alternating with each primary row, with three cortical rows corresponding to each branchlet, as in *Chara globularis* (recent).

tubercles. Rounded, obtuse, or acute protuberances distributed either at random or regularly over spiral cells of gyrogonites.

utricle. Outer covering of gyrogonite, made up of calcified segments of thallus.