MORPHOLOGY

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MORPHOLOGY OF LIVING CHAROPHYTA

The extant charophytes belong to a single family, the Characeae, which includes seven genera with more than 400 microspecies (*sensu* WOOD & IMAHORI, 1964–1965). Species have a wide range of geographic distributions from local endemics to cosmopolitan. All the extant genera are also known as fossils; however, the fossil record of *Nitella,* whose fructifications are uncalcified, is quite limited.

Living charophytes possess highly specialized morphological features that distinguish them from other green algae, especially in the complexity of the thallus and gametangia. These and other such features as the phragmoplast type of cell division and molecular phylogenetic analyses suggest a close relationship with higher plants.

THALLUS

Structure

The oospore germinates into a short filamentous structure called the protonema, a cladom approximately 1 mm long, which gives rise to the adult (Fig. 1.1). The thallus consists of one or more main axes with regularly alternating nodes and internodes (Fig. 2*a*–2*b*). Each node consists of several small cells that give rise to lateral branchlets. The internodes are elongated, multinucleate single cells generally 1 to 4 cm long, reaching 15 cm in the largest species.

Cortication

Some species have an external layer of narrow cells (cortex) surrounding the internodal cells and at the base of the nodal branchlets (phylloids). Such cortication (Fig. 3) is well developed in most species of *Chara,* incomplete in *Lychnothamnus,* and absent from *Chara* section *Charopsis, Lamprothamnium,* and the subfamily Nitelloideae. Some corticate species possess spine cells, short pointed cells projecting from the cortical cells. Spine cells occur singly or in clusters on the main axes. In *Chara, Lamprothamnium, Lychnothamnus,* and *Nitellopsis sarcularis* ZANEVELD, single-celled stipulodes originating from nodal cells occur in one or two tiers at the base of the branchlet whorls (Fig. 2b). Stipulodes are absent from *Tolypella* and *Nitella.* These appendages are very useful in the classification of living forms.

The cortex consists of an external layer of 6 to 14 thin cortical cells surrounding the internodal cells and at the base of the nodal branchlets. The cortication (Fig. 3.1–3.3) is **haplostichous** when the primary cortical cells are arranged so as to correspond one-to-one to the branchlets. In this instance, they may be **contiguous** (*Chara canescens* DESVAUX & LOISELEUR-DESLONGCHAMPS) or **noncontiguous** [*Lychnothamnus barbatus* (MEYEN) LEONHARDI]. When the cortical cells are subdivided, the cortication is **diplostichous** if the primary tubular cells under the phylloids alternate with one row of secondary tubular cells; spine cells, which occur only on primary tubes, are a useful character to distinguish both kinds of cortical cells.

Cortication is called **triplostichous** when two rows of secondary cortical cells are intercalated between the primary tubular cells; in this instance, the cortication is styled **isostichous** (as in *Chara globularis* THUILLER) if primary and secondary tubes have the same size and **anisostichous** [as in *Chara globularis* var. *virgata* (KÜTZ) R. D. WOOD] when the primary tubes have a larger diameter in section. The diameter of the cortical cells is also taxonomically significant. The cortex is said to be **aulacanthous** when the primary tubular cells are smaller in diameter than the secondary ones. It is **tylacanthous** in the reverse instance. In the extant forms, subdivision of the cortical cells occurs only in the genus *Chara.*

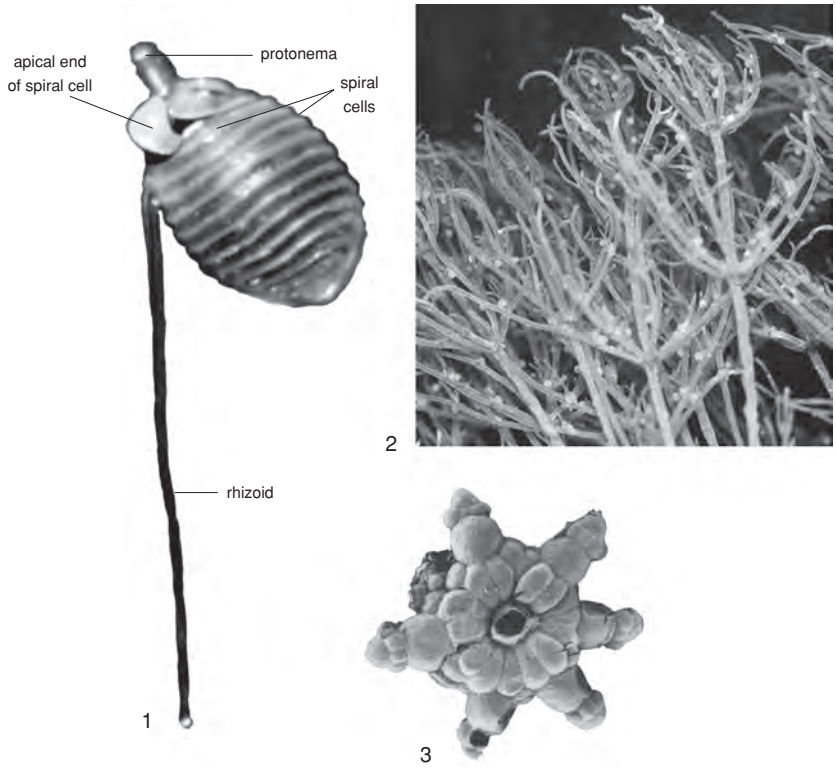

FIG. 1. *1,* Germination of oosporangium of a *Chara* species, ×70 (Kiss & Staehelin, 1993); *2, Chara vulgaris* f. *contraria* (A. BRAUN *ex* KÜTZING) R. D. WOOD; general view of several specimens bearing antheridia, ×4 (Corillion, 1994); *3, Nitellopsis obtusa* (DESVAUX in LOISELEUR-DESLONGCHAMPS) J. GROVES; stellate bulbil, ×92 (Feist & Grambast-Fessard, 1991, fig. 4b).

Branching

In the extant subfamily Charoideae, axes as well as branchlets are divided into nodes and internodes (Fig. 4). The branchlets are not divided dichotomously. As is shown by transverse sections, nodes have two central cells surrounded by two layers of numerous peripheral cells (Fig. 3.4–3.6). In *Lamprothamnium* the central cells are subdivided. In the extant subfamily Nitelloideae transverse sections of nodes have four central cells surrounded by only one row of a small number of peripheral cells (BHARATHAN, 1980) (Fig. 3.6). In *Nitella* branchlets bifurcate one to five times. Only main axes are subdivided into nodes and internodes. In *Tolypella* the branching is more complex, with divided primary and secondary rays. Bract cells, originating from nodal cells of branchlets, are more or less elongate elements that occur around the gametangia but are rudimentary on or absent from the sterile nodes. Bracteoles are single cells, quite similar to the bract cells but derived from antheridial primordia. Two bracteoles adjoin the oogonium (Fig. 2*c*–2*d*). Bracteoles are sometimes represented as casts at the base of encrusted fossils of the family Characeae.

Rhizoids

Charophytes are fixed on the substratum by the rhizoids. These are very thin and colorless filaments (Fig. 1.1, 4.3), and they are irregularly organized in nodes (with ramifications) and internodes. Besides attachment, the rhizoids are involved in the absorption of nutrients.

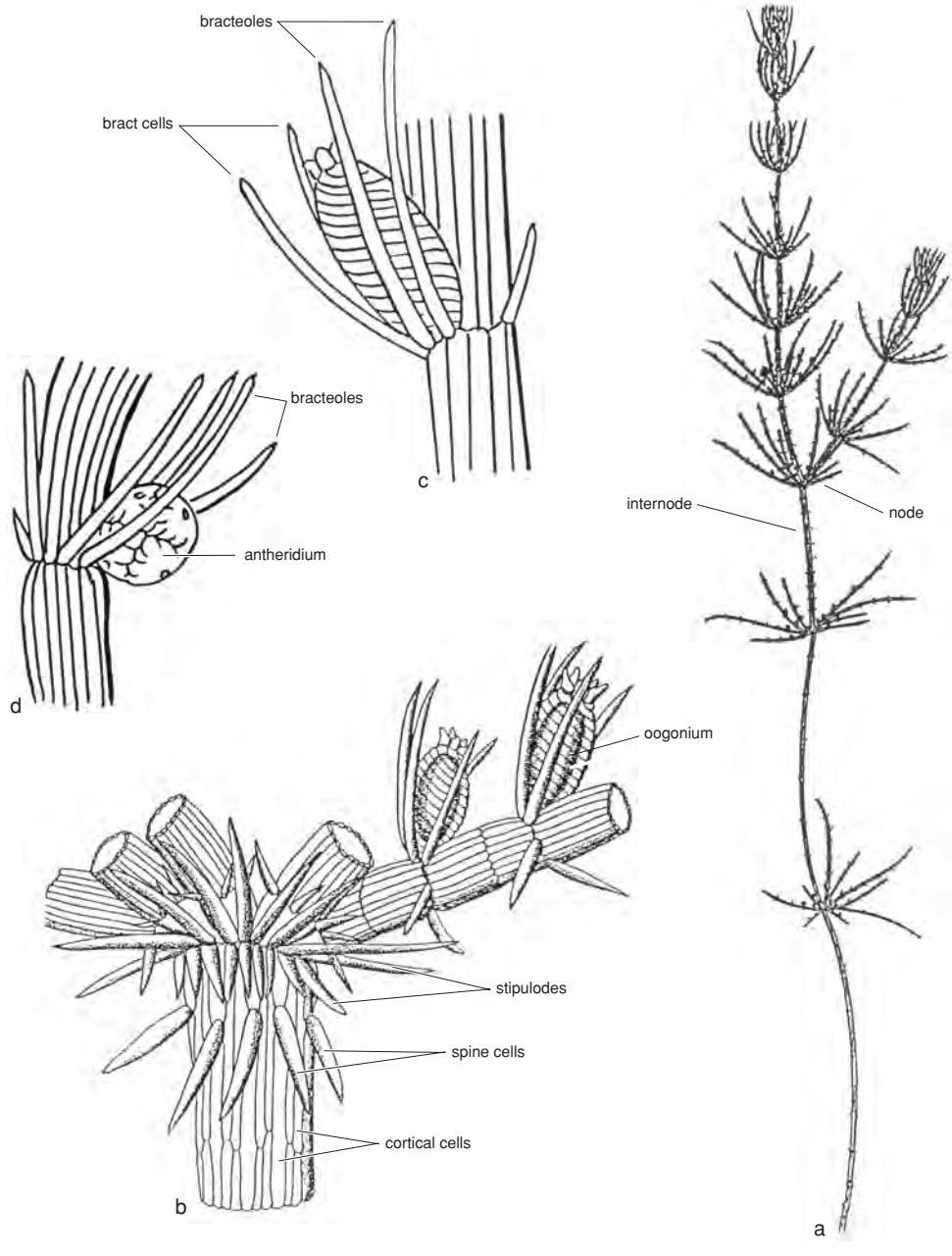

FIG. 2. *a–b, Chara zeylanica* f. *elegans* (A. BRAUN *ex* T. F. ALLEN) H. & J. GROVES; *a,* habit, ×1; *b,* axial node with stipulodes in 2 tiers on the main axis, corticated axis, base of branchlets and solitary spine cells, ×23 (Wood & Imahori, 1964 in 1964–1965, pl. 95,*1,4*); *c–d, Chara* sp., fertile nodes of a dioic species, ×40 (Corillion, 1975, pl. III, f, g).

Bulbils

Bulbils are multicellular tuberous growths, either isolated or aggregated on the rhizoids. In *Nitellopsis obtusa* (DESVAUX in LOISELEUR-DESLONGCHAMPS) J. GROVES, stellate bulbils are modified branchlet whorls situated on the lower axial nodes (Fig. 1.3). Bulbils function in vegetative propagation.

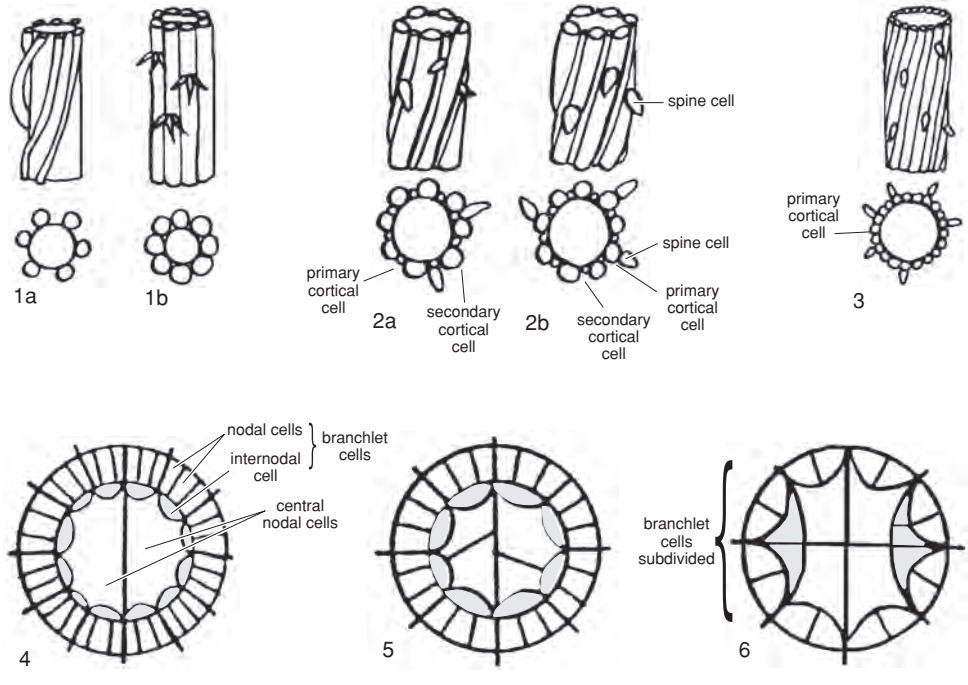

FIG. 3. *1–3,* Different types of cortication; *1,* haplostichous; *a,* incomplete (noncontiguous), *b,* complete (contiguous); *2,* diplostichous and anisostichous; *a,* aulacanthous, *b,* tylacanthous; *3,* triplostichous and isostichous (Corillion, 1957, pl. 1,*4–8*); *4–6;* transverse sections of nodes showing origin of branchlets by subdivisions of central nodal cells; *4, Chara (C. zeylanica); 5, Lamprothamnium (L. papulosum); 6, Nitella (N. acuminata)* (adapted from Bharathan, 1980, fig. 20, sections 1, 5, 9).

Calcification

It has been shown that the thallus and gyrogonite have two types of calcification (FLAJS, 1977; SOULIÉ-MÄRSCHE, 1989). For the thallus, the calcium deposit is external and generally not resistant. Incrustation of thalli occurs independently of the biocycle, provided the temperature is high enough for precipitation of calcium carbonate. In corticated forms calcite may be included in the elongated filaments around the internodal cells; this external calcified layer strengthens the thallus and allows its preservation, usually in the form of unconnected fragments in the sediments.

REPRODUCTIVE ORGANS

Oosporangium

Development.—The oosporangium (female gametangium, oogonium, Fig. 5, 6.1–6.2) originates from a nodal cell on the lowest nodes and sometimes at the base of the branchlets. The nodal cell divides into three: the upper cell enlarges to form the oosphere and its sister cell(s); the lowest cell often forms a short stalk. The central cell divides to produce five elongate cells that spiral sinistrally around the oosphere and subdivide apically to produce the coronula (one tier of five cells in the subfamily Charoideae and two tiers in the subfamily Nitelloideae).

Morphology.—In the family Characeae, which includes the seven extant genera, the oosporangium consists of five spiral cells enclosing the oosphere or, after fertilization, the oospore (Fig. 6.1–6.2). The spiral cells are joined at the apex along a broken line, and the base of the oosporangium is obturated by one to three sister cells of the oosphere, which constitute the basal plate. The apex is surmounted by one (in the

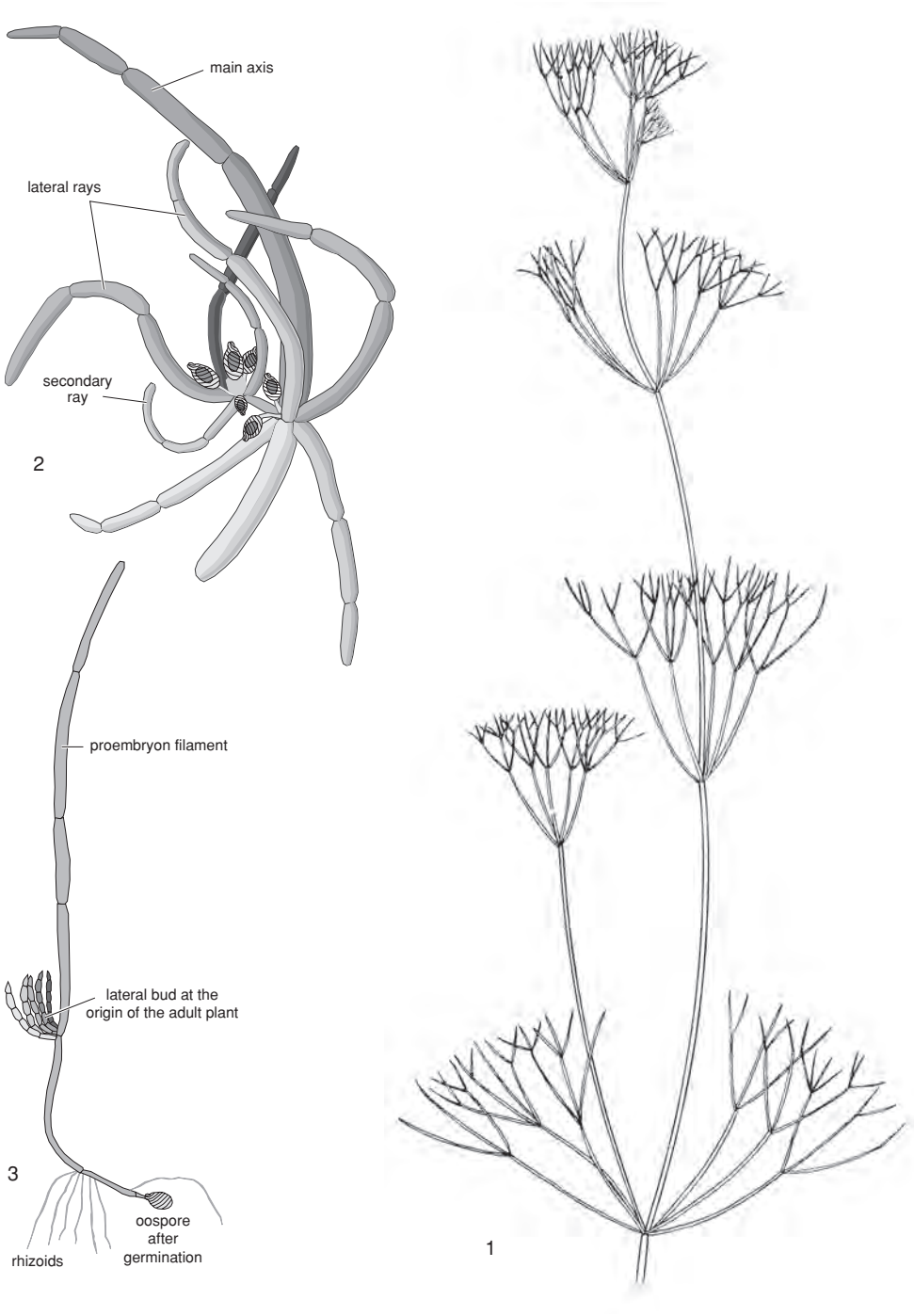

FIG. 4. *1. Nitella bifurcata* subsp. *mucronata* (A. BRAUN) R. D. WOOD; habit, ×1 (Wood & Imahori, 1964 in 1964–1965, pl. 224,*6*); *2, Tolypella nidifica* var. *glomerata* (DESVAUX in LOISELEUR-DESLONGCHAMPS) R. D. WOOD, ×12; fertile branchlet with 1 ray node bearing 5 lateral rays, the left one bearing 3 secondary rays (adapted from Wood & Imahori, 1964 in 1964–1965, pl. 382,*8*); *3, Chara* sp., proembryon (Corillion, 1975, pl. I,*C*).

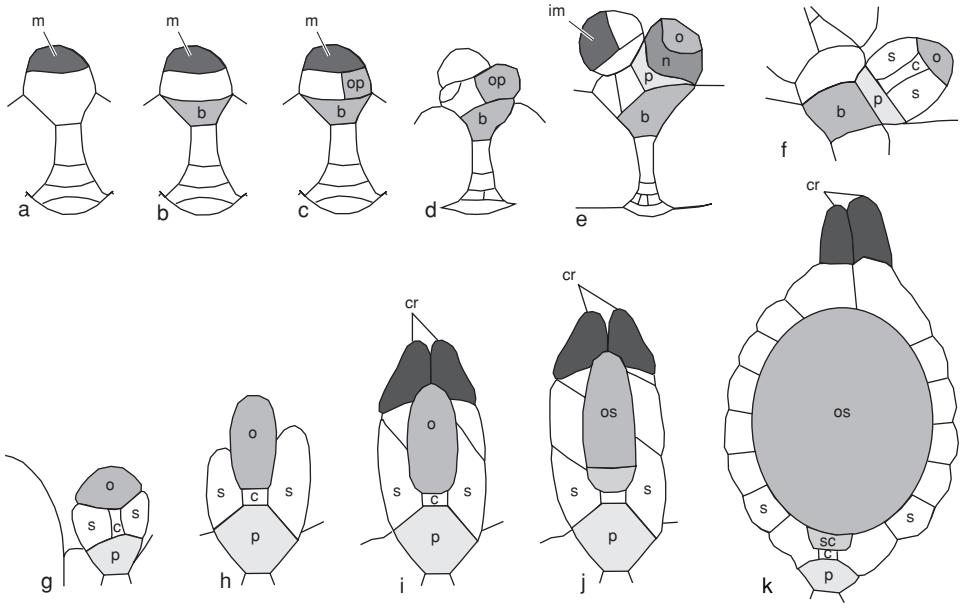

FIG. 5. Ontogenesis of an oosporangium of *Chara; b,* basal cell; *c,* central cell; *cr,* coronula cell; *im,* immature male gametangium; *m,* male gametangial primordium; *n,* oosporangial node; *o,* oosphere mother cell; *op,* oosporangial primordium; *os,* oosphere; *p,* pedicell cell; *s,* spiral cell; *sc,* sterile cell (adapted from Leitch, John, & Moore, 1990, fig. 1).

subfamily Charoideae) or two (in the subfamily Nitelloideae) rows of five coronula cells (Fig. 6.3). In most representatives of the subfamily Charoideae, the spiral cells and the basal plate become calcified; in all species, the oospore is included in an organic wall containing sporopollenin.

The basal plate.—The oosphere sister cell remains undivided in the Charoideae and in *Sphaerochara;* it subdivides into three (rarely two) cells in *Nitella* and *Tolypella.* The sister cell (or cells) becomes the basal plate in calcified species. Basal-plate morphology provides useful characters in the classification of fossils.

The gametangial wall.—After fertilization, the walls ensheathing the oosporangium thicken and undergo biochemical changes to form a hermetic and resistant envelope around the newly formed oospore. This complex wall is composed of three groups of layers (Fig. 7; HORN AF RANTZIEN, 1956b).

(1) In contact with the oospore is an organic transparent layer, the **sporine**, split into endosporine and ectosporine.

(2) Closely united with the ectosporine is the **sporostine**, a pigmented layer that is composed mainly of sporopollenin and cellulose (SHAW, 1971) and colored by a melanin-like compound (DYCK, 1970). The sporostine includes two layers, often indistinguishable, the endosporostine and the ectospostine, called by LEITCH (1989) a pigmented helicoidal layer and an ornamented layer respectively (Fig. 8.1). There is a lack of unanimity about the origin and position of the sporostine. HORN AF RANTZIEN (1956b), DYCK (1970), and LEITCH (1989) judged the sporostine to be produced by the inner walls of the spiral cells, whereas SOULIÉ-MÄRSCHE (1989) considered this layer to be an intercellular substance that covers all the parts of the oospore as well as its sister cells or basal plate and is independent of the spiral cells.

The surface of the sporostine may be smooth or marked by ornamentation of different shapes and disposition. GROVES and BULLOCK-WEBSTER (1920) defined three types of decoration that were also recognized

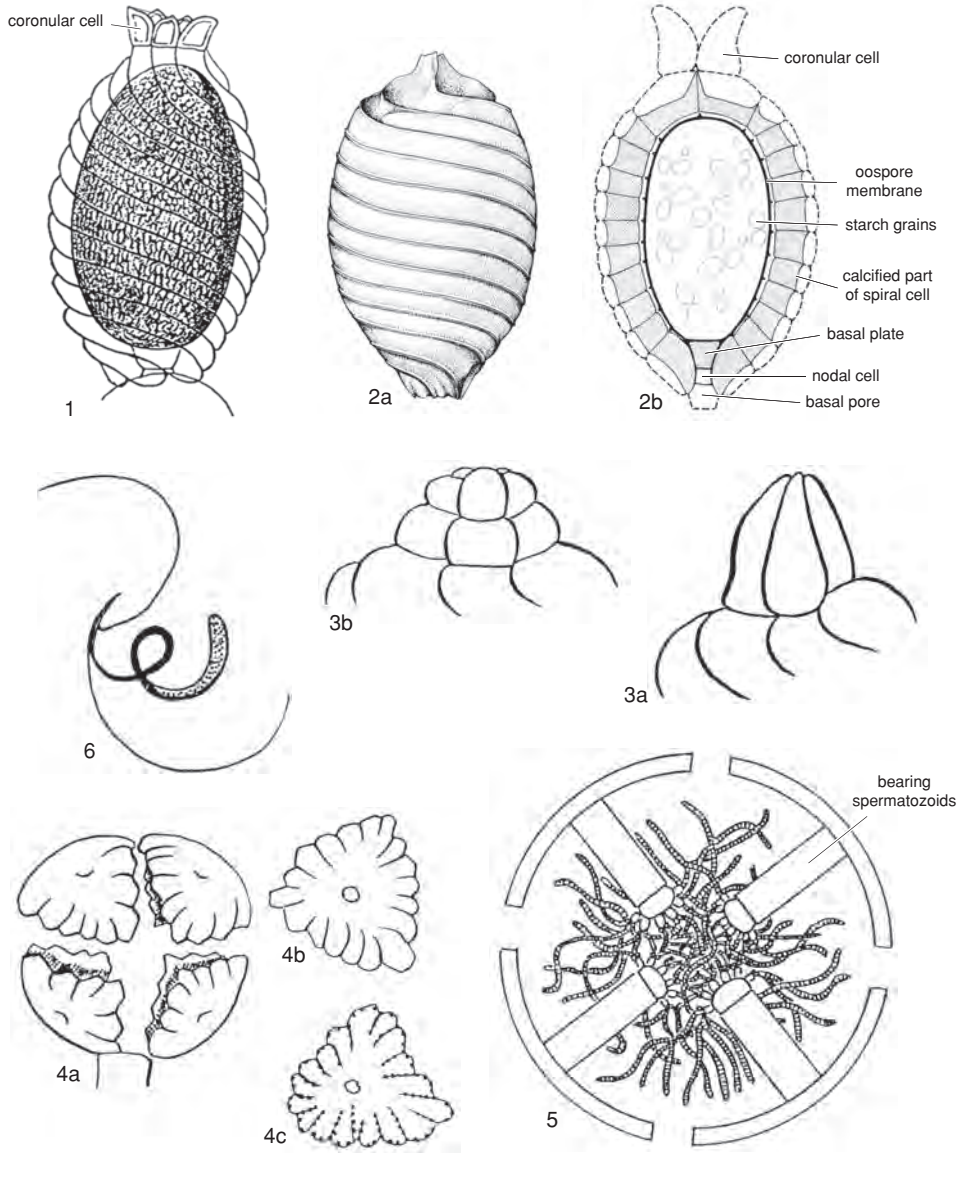

FIG. 6. Reproductive organs of the Characeae. *1,* Oosporangium, ×50 (Migula, 1897, fig. 27-7); *2, Chara hispida* L. *emend.* R. D. WOOD; *a,* gyrogonite (calcified oosporangium), *b,* same, longitudinal section; *dotted lines,* uncalcified part of spiral cells and of coronula cells, not preserved in fossils, ×40 (Feist & Grambast-Fessard, 1991, fig. 1A–1B); *3,* coronula cells; *a,* in *Chara, b,* in *Nitella* (Corillion, 1975, pl. IV,*d, f*); *4,* antheridium; *a,* external view of an antheridium open, showing 4 shield cells, *b,* isolated shield cells in *Chara, c,* in *Nitella; 5,* equatorial transversal section of antheridium showing 4 shield cells with their manubrium bearing spermatozoids; *6,* isolated biflagellate spiraled spermatozoid (Corillion, 1975, pl. V,*c,d,f,j*).

by HORN AF RANTZIEN (1959b). Electron microscopy has induced a renewal of investigations of the oospore membrane. JOHN and MOORE (1987) established a key to the *Nitella* species based on 21 categories of oospore. In *Chara,* which is less diversified, only seven categories have been determined (JOHN, MOORE, & GREEN, 1990). SOULIÉ-MÄRSCHE (1989) presented a synopsis of the oospore in the seven extant genera as well as

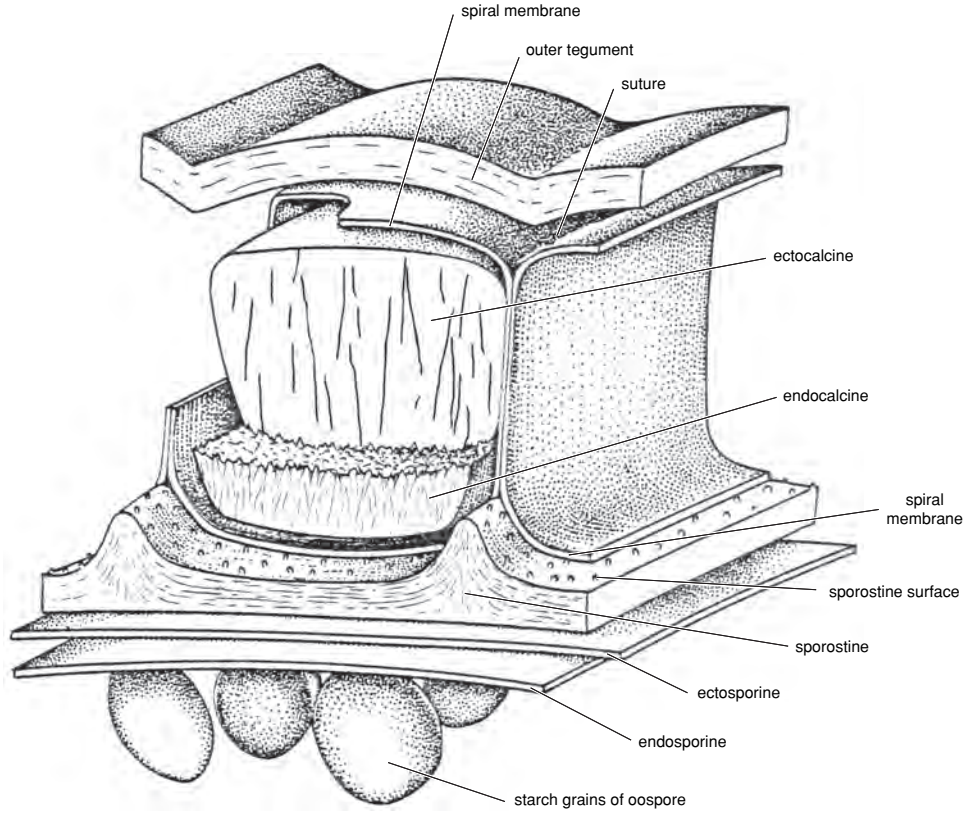

FIG. 7. Schematic representation of calcified oosporangial wall (Soulié-Märsche, 1989, fig. 8A).

in two Oligocene species, *Chara* sp. and *Stephanochara ungeri* FEIST-CASTEL. Features of the oospore were thought as early as the second half of the last century to be useful in recognizing species (DE BARY, 1875; NORDSTEDT, 1889). Although new investigations have revealed the usefulness of these characters in several taxonomic problems at the specific or infraspecific levels, their use is limited: the ornamentation varies according to the degree of maturity of the oospore, which is difficult to recognize; and the same ornamentation can occur in different species (JOHN & MOORE, 1987; SOULIÉ-MÄRSCHE, 1989; JOHN, MOORE, & GREEN, 1990). Following are some types of oospore ornamentation in extant species that also occur, more or less similarly, in fossil forms.

(a) Granulate, when the diameter of the ornamentation elements is less than 1 μm (*Chara globularis* var. *aspera* f. *galioides,* Fig. 8.2).

FIG. 8. Oospore membrane; *1, Chara hispida* L. *emend.* R. D. WOOD; section of compound oosporangial wall, ×4,000; labels: spiral cells: 1, spiral cell primary wall, 2, crystine, 3, pigmented helicoidal layer, 4, ornamentation layer; labels: oospore: a, oospore primary wall, b, amorphous layer, c, helicoidal layer, d, microfibrillar layer (Leitch, 1989, fig. 3); *2–7,* ornamentation of ectosporostine; *2, Chara globularis* var. *aspera* f. *galioides* (DE CANDOLLE) R. D. WOOD; granulate ornamentation (new); *3, Nitella syncarpa* var. *capitata* (NEES) KÜTZING; bristling tuberculate ornamentation, ×600 (Soulié-Märsche, 1989, pl. VI,*3*); *4–5, Nitellopsis obtusa* (DESVAUX in LOISELEUR-DESLONGCHAMPS) J. GROVES; lamellate endocalcine masking ectosporostine, ×240, ×1,200 (Soulié-Märsche, 1989, pl. IV,*3–4*); *6, Nitella gracilis* subsp. *gracillima* f. *robusta* (IMAHORI) R. D. WOOD; granulate-perforate ornamentation (Soulié-Märsche, 1989, pl. VI,*8*); *7, Lamprothamnium papulosum* (WALLROTH) J. GROVES; tuberculate ornamentation, ×600 (Soulié-Märsche, 1989, pl. V,*3*).

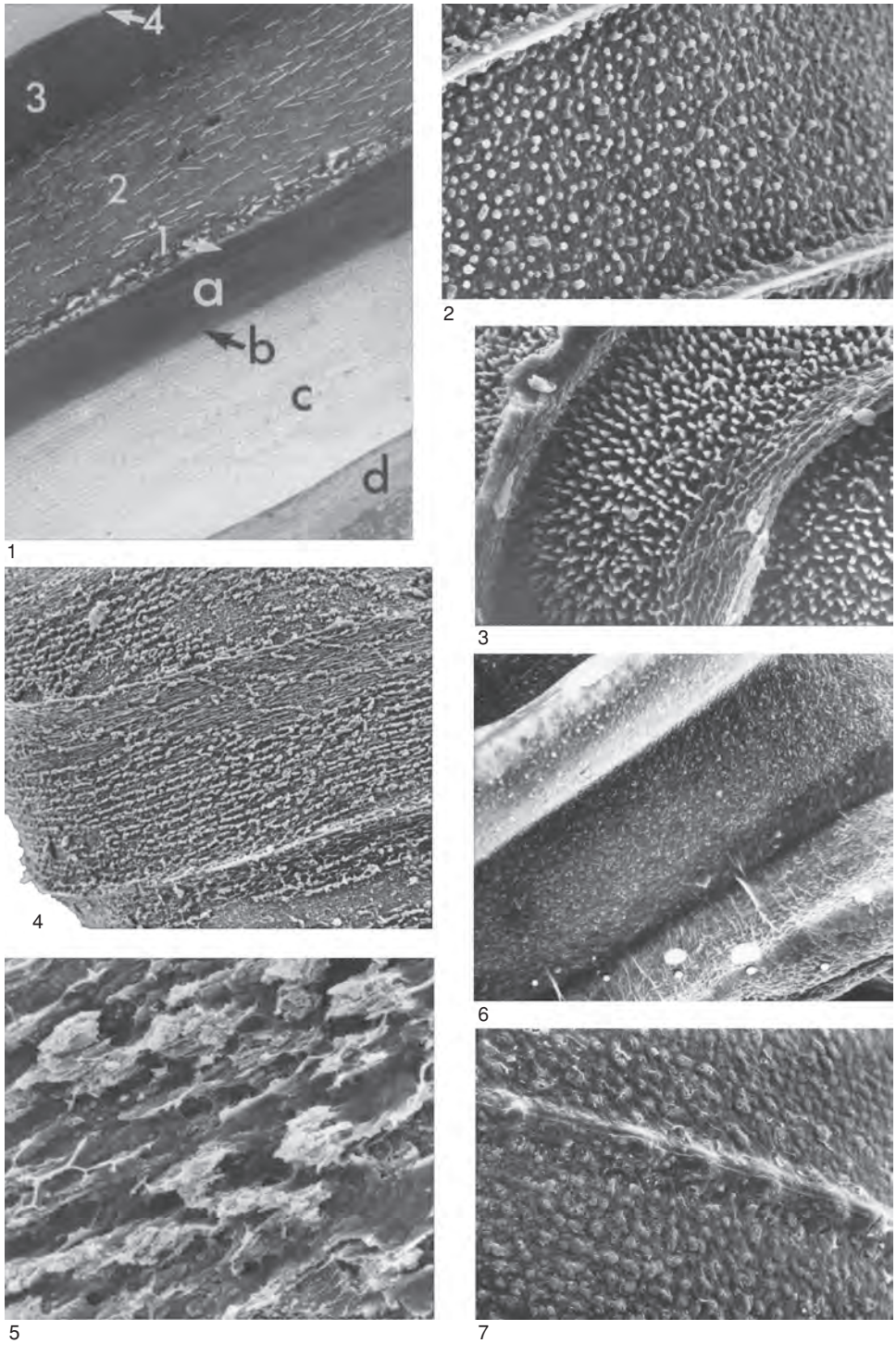

FIG. 8. *For explanation, see facing page.*

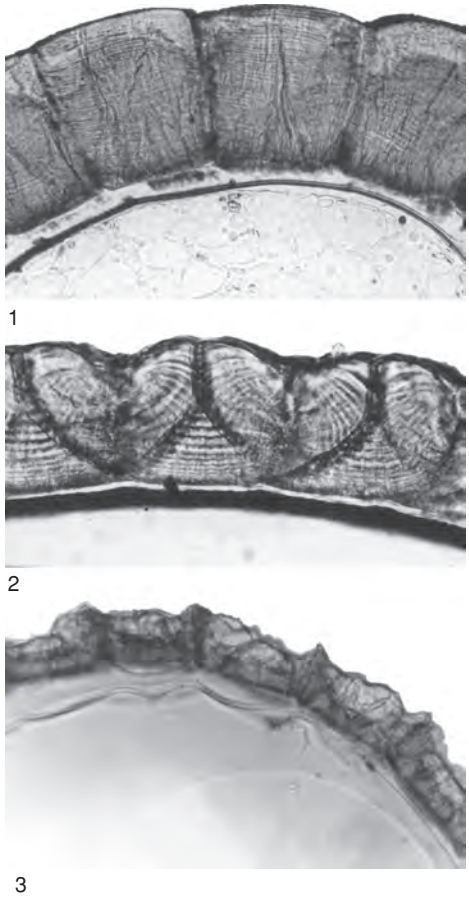

Fig. 9. Structure of calcified wall in axial longitudinal sections; *1,* parallel lamination in *Nitellopsis obtusa* (Desvaux in Loiseleur-Deslongchamps) J. Groves, Gouwzee Lake, The Netherlands, ×120; *2,* Y-lamination in *Lamprothamnium papulosum* (Wallroth) J. Groves, Marseillan, Hérault, France, ×210; *3,* nitelloid calcification in *Tolypella intricata* f. *prolifera* (Ziz *ex* A. Braun) R. D. Wood, Juignée Lake, Maine-et-Loire, France, ×250 (new).

(b) Tuberculate, when the diameter of the ornamentation elements is greater than 1 µm [*Lamprothamnium papulosum* (Wallroth) J. Groves, Fig. 8.7]. Granules and tubercules can be perforated [*Nitella gracilis* subsp. *gracillima* f. *robusta* (Imahori) R. D. Wood, Fig. 8.6].

(c) Reticulate (see Fig. 78,*1d,* Systematics, herein p. 144).

(d) Vermiculate, as in *Nitella syncarpa* (Soulié-Märsche, 1989, pl. VI,*2*).

In *Nitellopsis obtusa,* the sporostine is masked by lamellate ectocalcine (Fig. 8.4–8.5).

Lamprothamnium and *Lychnothamnus* have the same types of ornamentation as *Chara;* in *Tolypella* the surface of the oospore is smooth (Soulié-Märsche, 1989). In uncalcified forms (*Nitella, Tolypella* section *nidifica,* and some species of *Chara*) the spiral cells are not preserved, and oospores are propagated covered only with the ectosporostine, which is, however, particularly resistant. Proctor (1962) has shown that such oospores retain their vitality when passing through the digestive tract of migratory water birds.

(3) The **calcine** (Horn af Rantzien, 1956b) is the main constituent of calcified oosporangia. The crystals are disposed according to three types, discussed below.

Parallel lamination is the most common (Fig. 9.1). The calcine is differentiated into two zones, the internal one or **endocalcine**, having concentric organic lamellae, and, toward the exterior of the cell, the **ectocalcine**, massive and generally devoid of lamination. The lamination may occupy all the thickness of the cell.

Y-calcification (Fig. 9.2) occurs in some halophilic species, such as *Lamprothamnium papulosum.* Here, a system of radial convergent lines occurs in addition to the usual concentric lamination. These lines depart from the sutures and from the adaxial wall, going toward the median part of the spiral cell. In the middle of the cell the limit of these radial stripes outlines a letter Y, the fork of which is directed toward the center of the gyrogonite. Species with Y-calcification are found preferentially in somewhat saline environments, but it cannot be said that there is a causal relationship between these two facts. The meaning of this peculiar structure remains enigmatic (Feist & Grambast-Fessard, 1984; Leitch, 1989; Soulié-Märsche, 1989).

Nitelloid calcification (Fig. 9.3) of the wall of the extant nitelloid gyrogonites of *Sphaerochara* Wood has a characteristic powdery aspect, the origin of which is not

clear. It has been observed generally that the outer part of the calcareous shell consists of a granular layer of loosely connected calcium carbonate particles, different in its structure from the calcine of the other Characeae (Migula, 1897; Horn af Rantzien, 1959a). The more complete calcification of the fossil representatives of the genus does not differ fundamentally from the types of calcification in the extant forms.

Sporostine and calcified spiral cells form a thick and resistant envelope that protects the egg during unfavorable conditions, which may last from a few months to several years. After disintegration of the living cells, only the calcified walls remain, sometimes with the internal sporostine and basal plate intact. All these resistant parts of the oogonium constitute the gyrogonite, which can be preserved as a fossil.

Antheridium

The antheridium is usually composed of eight shield cells, which are closely joined together to form a sphere (Fig. 6.4). A stalklike cell (manubrium) protrudes into the sphere from the inner surface of each shield cell. Each manubrium bears from one to four filaments that produce about 200 biflagellate spermatozoids (Fig. 6.5). The complex structure of the antheridium is unique in the biologic world. Antheridia are not calcified, but casts of them have been found in fossil representatives of the Clavatoraceae and Pinnoputamenaceae. These casts have internal features quite similar to those of extant species.

Monoecy and Dioecy

The presence of both sex organs on the same individual (monoecy) or on different individuals (dioecy) is a species-specific character. In the monoecious species, the male and female gametangia may be present at the same nodes (conjoined species) or at different nodes (sejoined species). The distinction between monoecious and dioecious forms cannot be detected generally in fossils except among the Clavatoraceae and Pinnoputamenaceae, which display clear examples of conjoined monoecy.

REPRODUCTION AND LIFE CYCLE OF CHAROPHYTA

Oogamy

The large oogonium (up to 1,400 μm) fixed on the thallus is fertilized by a small biflagellate spermatozoid. The spermatozoids share morphological characters that clearly ally them with other advanced green algae (Charophyceae *sensu* Mattox & Stewart, 1984) and higher plants (Garbary, Renzaglia, & Duckett, 1993). The zygote, filled with reserves and surrounded by resistant walls, falls to the bottom where it remains dormant until germination.

Parthenogenesis

Parthenogenesis is known in only one living species, *Chara canescens* Desvaux & Loiseleur-Deslongchamps, the females of which are more widely distributed than the males.

Vegetative Reproduction

As in most cryptogams, vegetative reproduction plays an important role in the Charophyta. Bulbils and axillary nodes enable widespread and rapid dispersion of vegetative propagules. Such structures seem likely to have played the same role in fossil taxa as suggested by the co-occurrence of calcified nodes and gyrogonites in sediments.

Development and Biocycle

It is generally recognized that after fertilization, meiosis occurs at the first division of the zygote. After a variable period of dormancy, the egg germinates into a haploid protonema on which the thallus develops. The diploid phase is restricted to the egg, and the green thallus is haploid. Thus the life cycle is monogenetic haplophasic. According to an alternative hypothesis (Tuttle, 1926; Soulié-Märsche, 1989), the four cells (oospore and sister cells) issuing from the first divisions during the development of the

oogonium of *Nitella* were suggested to be the products of meiosis. If so, meiosis occurred in the diploid thallus before the formation of gametes. Recent cytological and chromosome studies (Guerlesquin, 1984; Michaux-Ferrière & Soulié-Märsche, 1987), however, have confirmed the haploidy of the thallus.

Polyploidy

Polyploids occur in most living species. The multiplication of the euploid chromosome number may increase the possibilities of adaptation of the species to new biotopes and thus favor the widening of their geographic distribution.

SPECIAL ASPECTS

Charophytes have three unique features. They have a vegetative apparatus with regular alternation of a giant polynucleate cell (produced by endomitosis) and a multicellular node made of several small uninucleate cells; the giant cells make charophytes useful as model systems for cell biology. They have a fairly large female gametangium made of an oosphere surrounded by a multicellular wall (five elongated cells) of vegetative origin. A complex male gametangium produces numerous helicoidal spermatozoids with two flagella inserted at the anterior end; their ultrastructure is similar in some respects to that of bryophyte spermatozoids.

Use of the charophytes by humans has varied during different periods. Silicified and calcified thalli were used in the past as natural abrasives. Crushed charophytes favoring colloid flocculation were used formerly to clarify fruit juices. Fresh or naturally dried specimens are used as green fertilizer in Africa and Asia.

The thallus is used as a support by epiphytes, as food by aquatic herbivores and waterbirds (ducks and moorhens), and as a calcium source by crayfish during ecdysis (molting of the carapace). Their dense vegetation provides sites for spawning by animals as well as shelter from predators.

MORPHOLOGY OF FOSSIL CHAROPHYTA

MORPHOLOGY OF FOSSIL VEGETATIVE REMAINS

Small fragmentary nodes and internodes of thalli are frequently found in nonmarine sediments, generally together with gyrogonites. Their state of preservation varies, and as a result the stem material may have different external characters. The most important in number, size, and diversity occur among the Clavatoraceae. Preservation adequate to provide information on the organism's habit is rare.

Calcified Species

Calcified thallus remains consist of nodes and portions of axes having the axial canals surrounded by small tubes (cortical cells) parallel to the main axis or coiled around it (Fig. 10). In fossil Characeae, the cortex is similar to those of the extant forms (see p. 1 herein), but the Clavatoraceae have a more complete cortication in which spine cells often cover the characean cortex. Just as in living species, different types of cortication may be present within a single fossil species (Corillion, 1975). The following types of cortication have been reported so far from the fossil record: haplostichous, including isostichous noncontiguous (Fig. 10.2) and isostichous contiguous (Fig. 10.1, 10.4); anisostichous diplostichous (Fig. 10.6); and isostichous diplostichous (Fig. 10.7). The incomplete preservation of nodes does not allow the use of the cortication characters in systematics as it does for extant species.

Uncalcified Species

Vegetative parts of uncalcified taxa are rarely preserved as fossils. Silicified remains are the best preserved, such as in *Palaeonitella cranii* (Kidston & Lang) Pia; thalli enclosed in the silicates of the Rhynie Chert are visible in relief in thin sections (Fig. 11.1). These uncorticated thalli, bearing whorls of branchlets separated by internodes devoid of any appendages, are similar to the extant

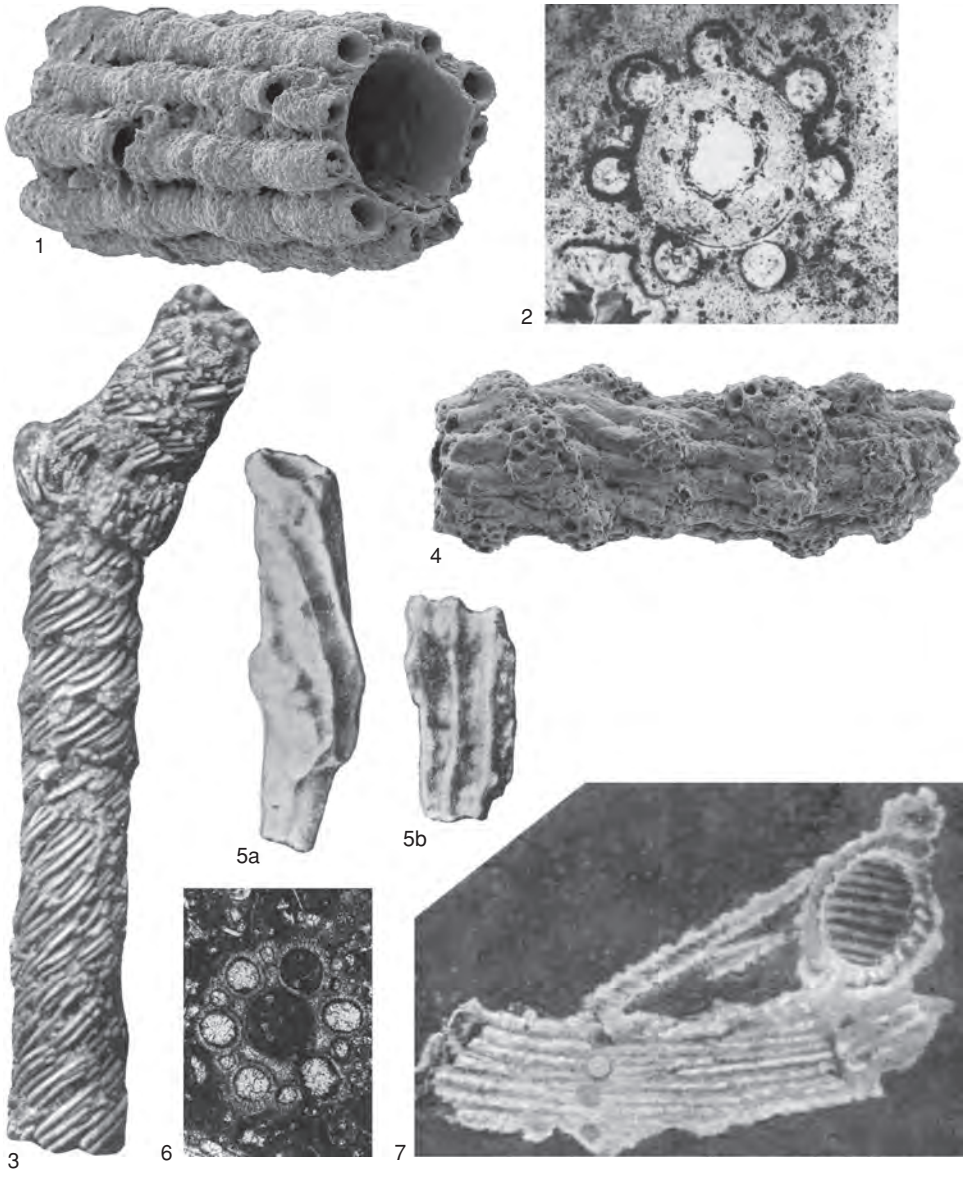

FIG. 10. *1,* Thallus internode showing central axis surrounded by cortical cells; associated with several species of Characeae, Bartonian, Paris basin, France, UMP C105-15, ×75 (new); *2,* thallus fragment associated with *Gyrogona medicaginula* LAMARCK, Oligocene, Ireland; transverse section of node, showing 7 noncontiguous isostichous cortical cells, ×65 (Corillion, 1994, fig. 4); *3, Echinochara spinosa* PECK; node and part of internode showing arrangement of spine cells, Upper Jurassic, Colorado, USA, ×5 (Peck, 1957, pl. I,*17*); *4,* thallus internode showing cortical cells partly covered by spiralized swollen belt bearing numerous spine-cell scars, Berriasian, Germany, ×18 (new); *5a–b,* thallus fragments associated with *Sycidium panderi minor* KARPINSKY, Devonian, Russia, external views showing incomplete cortication; *a,* slightly spiralized cortical cells and uncorticated portion of thallus, ×25, *b,* straight cortical cells, ×30 (Karpinsky, 1906, fig. 63, 65, 67); *6,* thallus fragment associated with *Clavator reidi* GROVES, Lower Cretaceous, Jura Mountain, Switzerland; transverse section of node, showing alternating large and small cortical cells, ×30 (Mojon & Strasser, 1987, fig. 10D); *7, Chara sausari* SAHNI & RAO; Deccan Intertrappean Beds, early Paleogene, Chindwara, C.P., India; external view of connected thallus and gyrogonite, showing contiguous isodiametrical cortical cells, ×30 (Sahni & Rao, 1943, fig. 2).

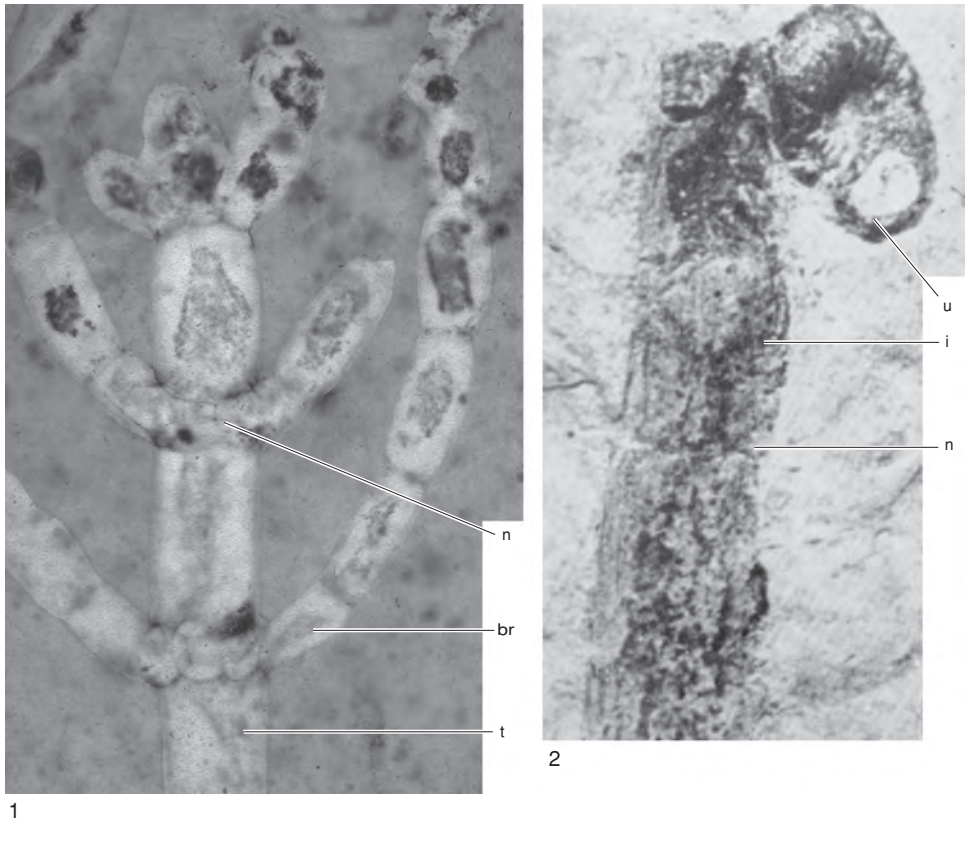

FIG. 11. *1, Palaeonitella cranii* (KIDSTON & LANG) PIA, Pragian, Lower Devonian, Rhynie Chert, Scotland; thin section showing uncorticated thallus (*t*) with branchlets (*br*) originating from nodes (*n*), ×125 (new); *2, Praesycidium siluricum* T. & A. ISHCHENKO, Ludlow, Silurian, Slasky Formation, Ukraine; cast showing corticated thallus with nodes (*n*) and internodes (*i*), bearing a utricle (*u*) at top right, ×5 (Ishchenko & Ishchenko, 1982, pl. 5*g*).

Nitella, although no characters typical of the genus have been observed so far.

Preservation as casts may provide images of thalli in connection with gyrogonites or utricles. A cast of *Praesycidium siluricum* T. & A. ISHCHENKO, one of the oldest representatives of Charophyta, shows a haplostichous isostichous cortication (Fig. 11.2). Similarly, casts of the Eocharaceae from the Upper Devonian of South Africa (GESS & HILLER, 1995) have verticillate thalli bearing structures presumed to be gyrogonites.

Fossil remains of the thallus are too scarce and fragmentary to legitimize a formal taxonomy, although species names have been given to some of them. In most cases, isolated fragments of the thallus are simply mentioned without names being given.

MORPHOLOGY OF FOSSIL REPRODUCTIVE ORGANS

Gyrogonite

The term gyrogonite was used for the first time by LAMARCK (1801) for fossil shells of undetermined nature that LEMAN (1812) recognized later as remains of charophytes. The classification of fossil charophytes is based on characters of the gyrogonite or of the utricle. The **gyrogonite** consists of calcium carbonate that is deposited in both the enveloping cells (i.e., **spiral cells**, **spiral units**, **spirals**), which spiral around and enclose the oospore, and the **basal plate**, representing the calcified sister cell of the oosphere. In the Clavatoraceae and the Sycidiales, a calcareous outer covering or **utricle** covers the gyrogonite, and many types of disposition may result

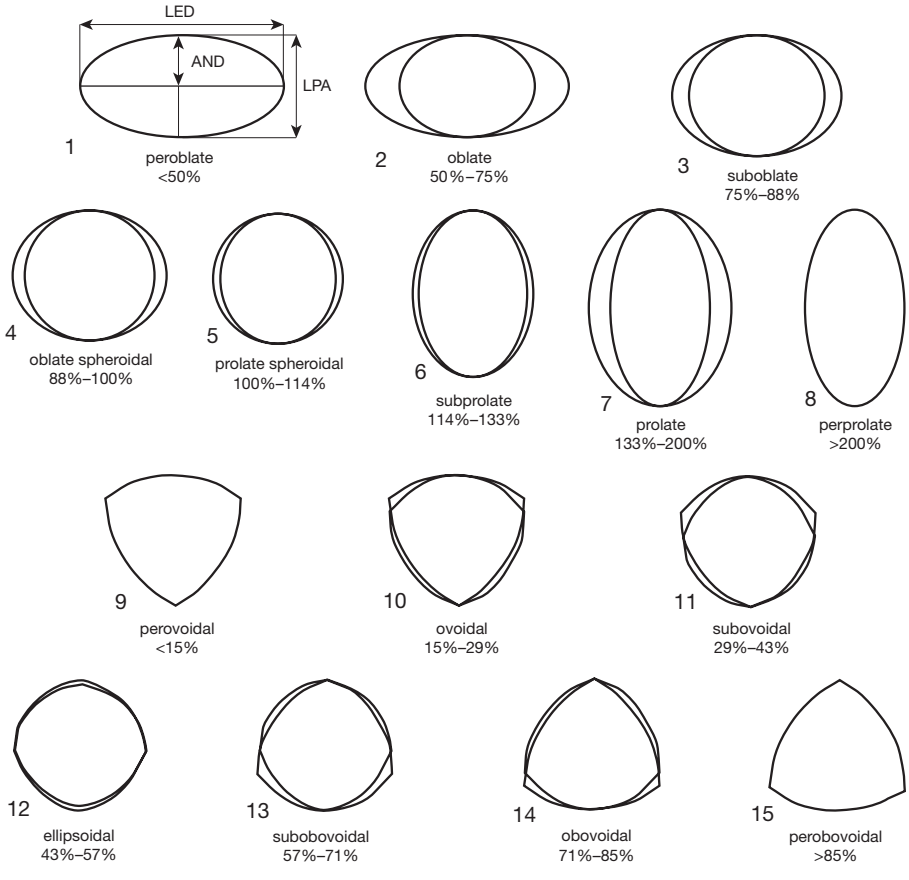

FIG. 12. Terminology of charophyte shapes; *1–8,* isopolar specimens, LPA/LED, ×100; *9–15,* anisopolar specimens, AND/LPA, ×100; *LPA,* length of polar axis; *LED,* largest equatorial diameter; *AND,* distance from apical pole to LED (adapted from Peck & Morales, 1966, text-fig. 2).

(*Atopochara, Globator, Flabellochara, Sycidium*).

General shape and number of convolutions.—Gyrogonites that have similar morphological characters are placed into the same species. To define gyrogonite shapes mathematically, HORN AF RANTZIEN (1956b) has provided a useful set of descriptive terms based on the relationships of the **polar axis** (LPA, length) to the **equatorial diameter** (LED, width) (Fig. 12). The **isopolarity index** (ISI) represents the value of (LPA/LED) ×100. The number of **convolutions** visible on lateral views of gyrogonites and to a lesser extent their thickness are useful characters for definitions of species. A relationship exists between the number of convolutions and the general shape. Peroblate to oblate spheroidal gyrogonites of *Gyrogona* and *Maedleriella* have a low number (4 to 8) of relatively thick convolutions (Fig. 66,*2a,2g,* Systematics, herein p. 126), whereas *Chara* has generally a prolate to perprolate shape and numerous, thin convolutions, often more than 10 (Fig. 64,*1a–1b,* Systematics, herein p. 122).

Dimensions.—Gyrogonites generally range from 200 μm to 2 mm maximum diameter. The giant of the group is the Devonian *Sycidium xizangense* Z. WANG, whose utricles reach 3.2 mm in diameter. As the process leading to calcification commences only after fertilization of the oosphere, all the gyrogonites correspond to the same mature stage, and the differences in dimensions must reflect natural populational variation.

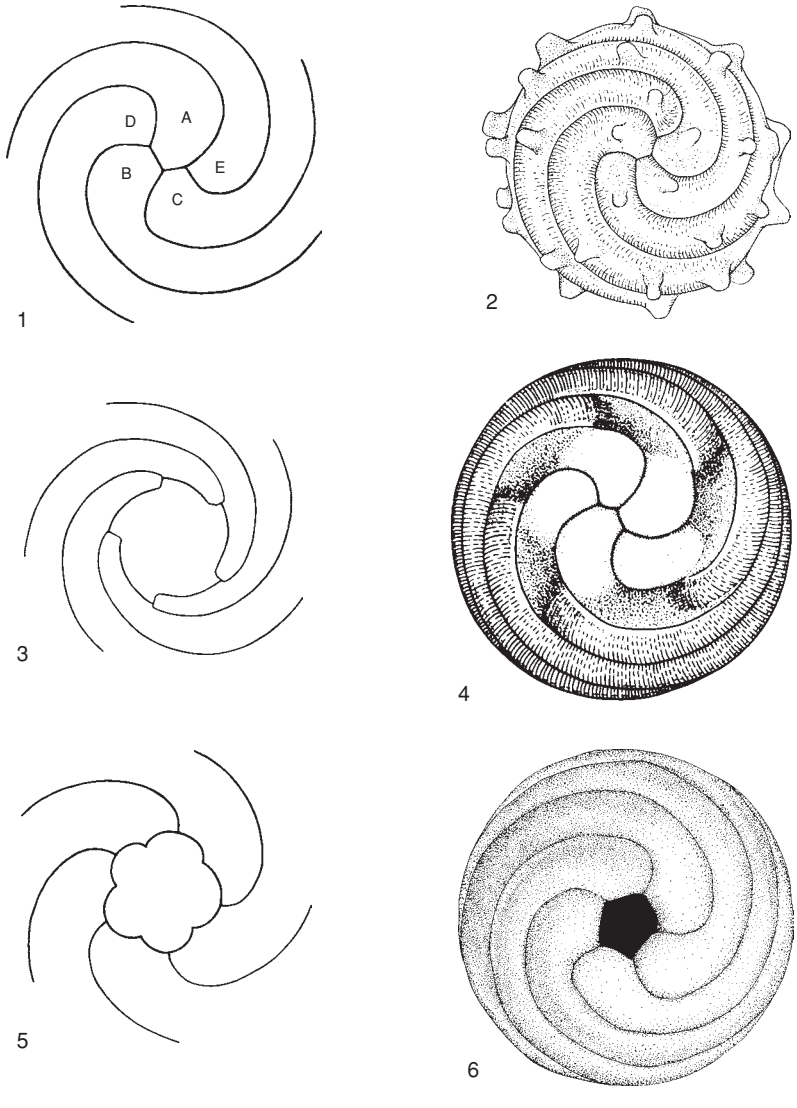

FIG. 13. *1,* Apical view showing junction line of spiral cells (Grambast, 1958, p. 34); *2, Maedleriella mangenoti* GRAMBAST; apical view showing thin tubercles at ends of spiral cells, ×70 (Grambast, 1957, fig. 4); *3,* pore of dehiscence of a Characeae in shape of a toothed wheel (Grambast, 1958, p. 201, fig. c); *4, Gyrogona lemani capitata* GRAMBAST; apical view showing rosette in center, ×50 (Grambast & Grambast-Fessard, 1981, fig. 5b); *5,* pore of dehiscence of a Raskyellaceae in shape of a rose (Grambast, 1958, fig. d); *6,* apical pore of a Porocharaceae, ×50 (new).

Within a population of a given species the variation of dimensions has a Gaussian distribution provided the sample is large enough (>100 specimens) and chosen randomly from a larger population. Sampled gyrogonites from one or a few specimens, as can be measured in modern species, are expressed by non-Gaussian histograms reflecting individual allometric variation (SOULIÉ-MÄRSCHE, 1989).

Apex.—The characters of the gyrogonite summit have great importance for the systematics of fossil charophytes as well as for extant charophytes: the primary subdivision of the living Characeae is based on the number of coronula cells (Fig. 6.3). The coronula

is not preserved generally in fossil forms, but other features allow the assignment of fossil characean genera to subfamilies. Characters of the apex have revealed also their usefulness for distinguishing taxa of various ranks (GRAMBAST, 1956a, 1958; HORN AF RANTZIEN, 1959b; FEIST & GRAMBAST-FESSARD, 1982).

Characeae.—**Junction line:** The spiral cells are contiguous at the gyrogonite apex. The junction consists of a broken line (Fig. 13.1) where one of the spiral cells (A) is in connection with the four others: two (B and C) are contiguous to three cells, and two (D and E) are adjacent to only two cells (GRAMBAST, 1958; MASLOV 1966). In some genera (e.g., *Nitellopsis* and *Gyrogona*) the junction line may be hidden by the voluminous nodules at the spiral apical ends, but it corresponds to the same basic pattern.

Periapical and apical differentiation: The periapical region corresponds to the dehiscence zone during germination. At this level the spiral cells generally become narrow or thinner. This differentiation delimits the so-called apical part. When convex, the apical part is called a **rose** or **rosette**. The apical ends of the spirals may bear more or less developed apical nodules that are either isolated or coalescent. For example, nodules are convex, massive, and jointed in *Gyrogona lemani capitata* GRAMBAST (Fig. 13.4), or thin and prominent, similar to those ornamenting the lateral parts of the spirals in *Maedleriella mangenoti* GRAMBAST (Fig. 13.2).

The characters of the apical part of the gyrogonite are useful to determine subdivisions in the family Characeae, which includes half the described charophyte genera (see Systematics section, herein p. 119).

Pore of dehiscence: In a characean population some specimens with an apical opening subsequent to germination are found together with closed gyrogonites. The breaking off of the apical part leaves an aperture in the shape of a regularly toothed wheel that is characteristic of the family (Fig. 13.3).

Coronula cells: Only a few coronula cells have been reported so far in fossil Characeae:

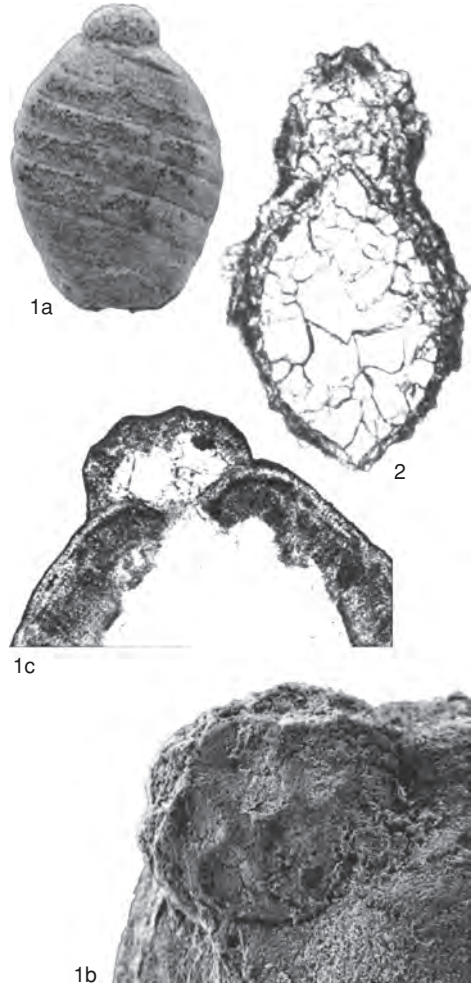

FIG. 14. Coronula cells; *1a–c, Feistiella* sp., El Koubbat, Morocco, Paleocene (new); *a,* specimen with coronula surmounting apex, ×35; *b,* detail showing 3 coronula cells, ×120; *c,* section of same specimen showing apical pore surmounted by coronula cells, ×75; *2, Microchara vestita* CASTEL; longitudinal section showing junction of spiral cells at apex, surmounted by coronula cells, lower Eocene, France, ×100 (Castel, 1969, fig. 5).

in *Chara* (e.g., *C. sausari* SAHNI & RAO; Fig. 10.7) and *Microchara* (e.g., *M. vestita* CASTEL; Fig. 14.2).

Raskellaceae.—In this family, the apex is closed by an operculum made of five supplementary apical cells that leaves, after it falls off, a dehiscence opening in the shape of a rosette with rounded lobes (GRAMBAST, 1957) (Fig. 13.5). In *Saportanella,* the apical

opercular cells are in the prolongation of the spiral cells (Fig. 62,*1b,* Systematics, herein p. 118), but in *Raskyella* the two kinds of cells alternate (Fig. 13.5).

Forms with an apical pore always open.—In numerous fossil forms (Sycidiales, Moellerinales, and, among Charales, the Porocharaceae and Clavatoraceae) the calcified spiral cells are interrupted at the periphery of the apex, leaving an opening varying in shape and size according to different taxa. Its diameter is generally smaller than in the family Characeae. The pore of these ancient forms represents the dehiscence opening that, during life, must have been obstructed by an organic, uncalcified part that is generally not fossilized (Croft, 1952). Gyrogonites with a flat summit generally have a relatively wide apical pore of various shapes: star shaped (e.g., *Stomochara moreyi,* Fig. 50,*1b,* Systematics, herein p. 102), rounded (e.g., *Feistiella bijuescencis* Schudack, Fig. 50,*2b,* Systematics, herein p. 102) or rose shaped (e.g., *Porochara douzensis* (Feist & Grambast-Fessard) Schudack, Fig. 50,*3c,* Systematics, herein p. 102). The apical pore is smaller in the forms with an apical neck (e.g., *Leonardosia langei* Sommer, Fig. 52,*3a,* Systematics, herein p. 105).

Coronula cells: *Karpinskya* (family Trochiliscaceae) has seven to ten coronula cells corresponding in number and position with the spiral cells that form an erect broad ring around the large apical pore (Fig. 46,*1b,* Systematics, herein p. 96). In only one instance has a coronula been found preserved in a member of the family Porocharaceae (Fig. 14.1).

Base.—**Basal pore:** Spiral cells are not joined at the base of the gyrogonite; their terminal ends delimit a pentagonal space, the basal pore, which is closed to the interior by the basal plate. The basal pore may be superficial at the same level as the external surface of the spirals (e.g., in *Peckisphaera verticillata* (Peck) Grambast; Fig. 74,*1b,* Systematics, herein p. 138) or at the bottom of a funnel made of the truncation of the spirals (e.g., in *Amblyochara begudiana* Grambast; Fig. 64,*2c,* Systematics, herein p. 122).

Basal plate.—In the fructification of living as well as fossil charophytes, the basal orifice is closed by a pentagonal piece termed the basal plate (Fig. 15). Grambast (1956b) has shown that this element corresponded to the sister cell of the oosphere and that its morphology is useful in the characterization of genera and species. The basal plate is simple when comprising only one piece in the Characeae subfamily Charoidea, *Aclistochara* excepted; it is multipartite (Fig. 15.1) when comprising two or three pieces in the Characeae subfamily Nitelloideae, *Sphaerochara* excepted, as well as in such Porocharaceae as *Porochara, Latochara,* and some *Stellatochara* species. The multipartite basal plates are generally relatively thin (e.g., *Tolypella*), whereas the thickness varies considerably in the simple basal plates. When the calcification is limited to its upper face in contact with the oospore, the basal plate is very thin as in *Nitellopsis* and *Harrisichara* (Fig. 15.2); in contrast, the basal plate can be higher than wide (e.g., *Gyrogona lamarcki* Grambast; Fig. 15.3*a*) or nearly as high as wide (e.g., *Gyrogona medicaginula* Lamarck; Fig. 15.3*b*). In *Rhabdochara* (Fig. 15.6), the basal plate is conical with a hollow lower face. In *Chara* (Fig. 15.5), the height is typically greater than half the width. In *Sphaerochara,* the basal plate, in the form of a short column, has an upper face that is rounded and pentagonal; lateral faces are slightly concave, and the lower face is stellate and visible from the exterior (Fig. 15.4).

Enveloping cells.—**Orientation and number of spiral cells:** The orientation of the enveloping cells of the gyrogonite is the feature on which the subdivisions of higher rank are founded: they are dextrally spiralled in the order Moellerinales and sinistrally spiraled in the order Charales. Their number is significant for families; initially high (up to 13), it is reduced to 5 in the upper Carboniferous (see section on Evolutionary History, herein p. 60).

Calcification.—Except for ornamentation, which is unknown in modern species, the structure of the enveloping cells is quite comparable in extant and fossil forms. All types of calcification occur in fossils.

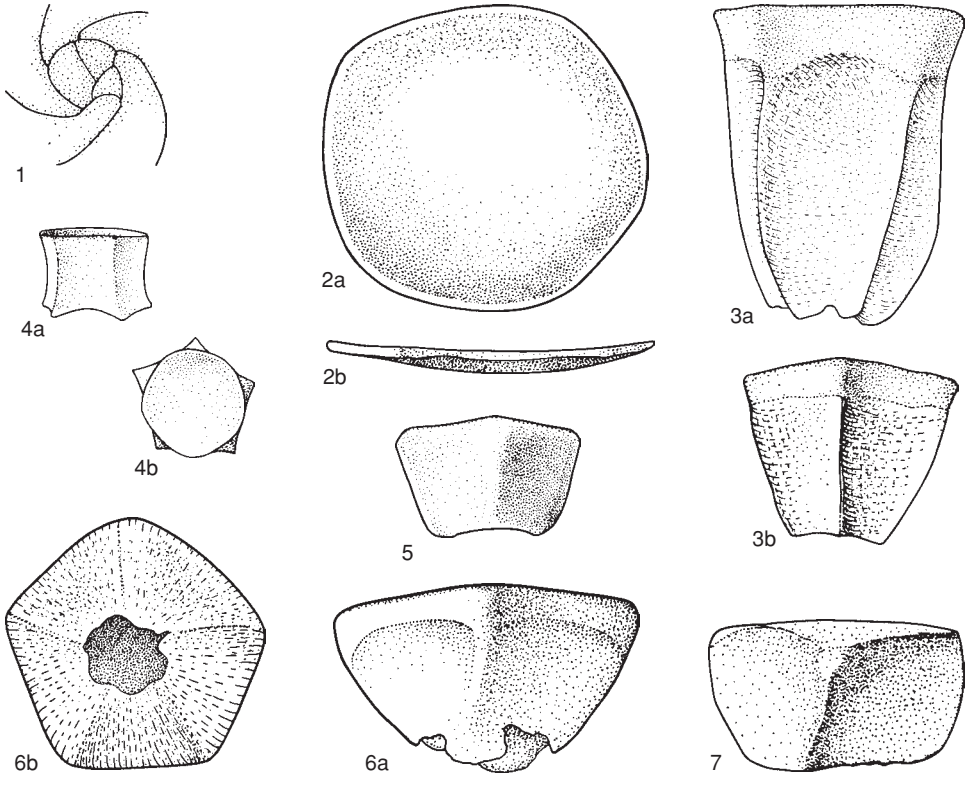

FIG. 15. Different types of basal plates; *1, Tolypella* sp., multipartite basal plate, upper side, ×280 (Grambast, 1956a, fig. 8); *2a–b, Harrisichara tuberculata* (LYELL) GRAMBAST, *a,* lower side, *b,* lateral side, ×250 (Grambast, 1957, fig. 2a–2b); *3a, Gyrogona lamarcki* GRAMBAST (*Brachychara archiaci*), lateral view, ×190 (Grambast, 1956a, fig. 5); *3b, Gyrogona (Brachychara) medicaginula* LAMARCK, lateral view, ×190 (Grambast, 1956a, fig. 4); *4a–b, Sphaerochara granulifera* (HEER) MÄDLER, lateral and lower sides, ×190 (Grambast, 1956a, fig. 6–7); *5, Chara hispida* L., lateral view, ×190 (Grambast, 1956a, fig. 2); *6a–b, Rhabdochara langeri* (ETTINGS) MÄDLER, hollow basal plate; *a,* lateral view, *b,* basal view, ×250 (Grambast, 1957, fig. 7a, 7c); *7, Feistiella (Porochara) globosa* (GRAMBAST & GUTIÉRREZ) SCHUDACK, lateral view, ×210 (Grambast & Gutiérrez, 1977, fig. 2a).

Calcification with parallel lamination (Fig. 16.3) is the most frequent. The Y-calcification (Fig. 16.2) is known in the halophilic genera *Lamprothamnium, Porochara,* and *Stellatochara*. The typical calcification of the extant Nitelloideae occurs in a more complete state in their fossil representatives. In *Sphaerochara ulmensis* (STRAUB) GRAMBAST, from the Oligocene of southern France (Fig. 16.1), the lamellar endocalcine is surmounted by a crest that must represent the ectocalcine. It is made of rows of crystals that are borne by thin lamellae diverging from points localized in the endocalcine. These crystals weather easily, giving a characteristic powdery aspect to the spiral surface.

According to the degree of calcification, which may vary within a population, the spiral cells may be concave, planar, or convex, providing gyrogonites of different outlines; but intermediate specimens allow one to recognize the homogeneity of a taxon. In such instances, the diagnostic criteria are those of the most calcified specimens.

Ornamentation.—Ornamentation consists of reticula, tubercles, punctuations, rods, and midcellular crests that are continuous or tend to split into individual nodules (Fig. 17). A specimen may be ornamented partly, usually at the upper part of the gyrogonite, as in *Nitellopsis* (*Tectochara*) *thaleri* (CASTEL & GRAMBAST) GRAMBAST &

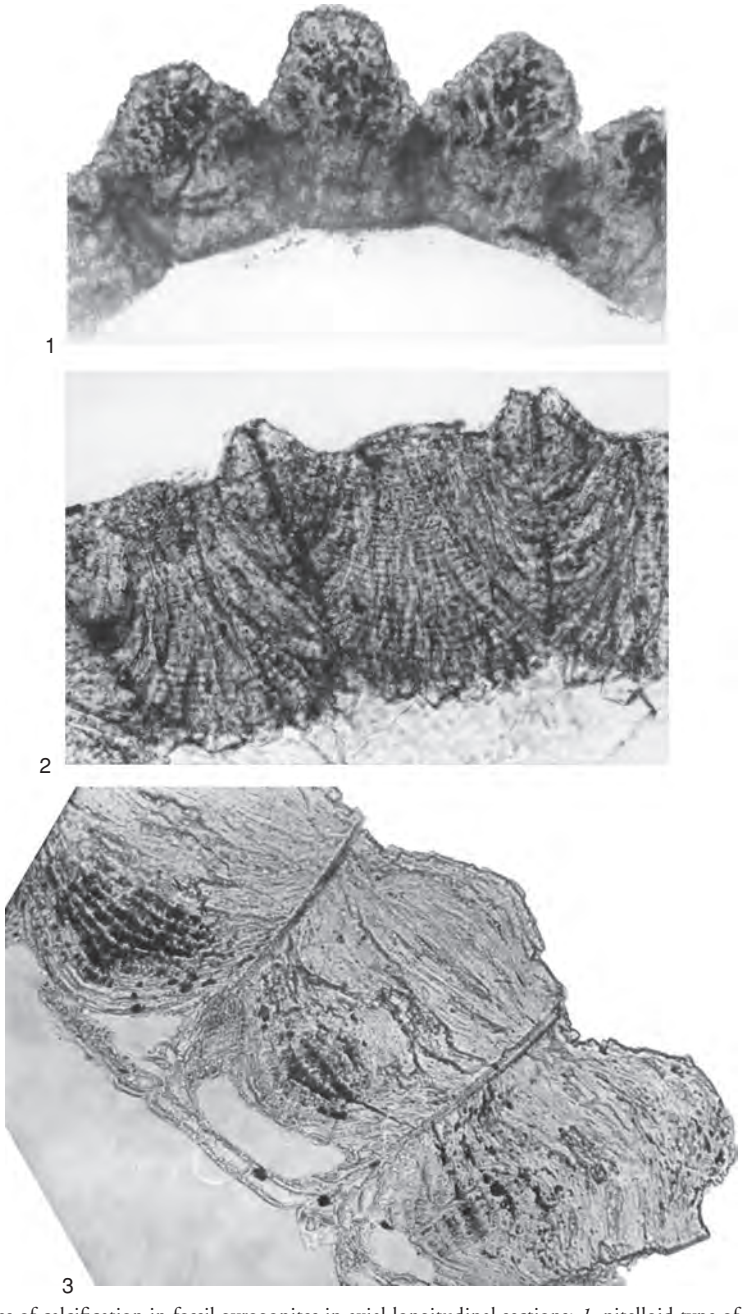

FIG. 16. Types of calcification in fossil gyrogonites in axial longitudinal sections; *1,* nitelloid type of calcification, *Sphaerochara ulmensis* (STRAUB) GRAMBAST, lower Oligocene, France, ×280; *2,* Y-calcification, *Porochara douzensis* (FEIST & GRAMBAST-FESSARD) SCHUDACK, Middle Jurassic, France, ×450; *3,* parallel lamination, *Nitellopsis (Tectochara) meriani* (L. & N. GRAMBAST) GRAMBAST & SOULIÉ-MÄRSCHE, upper Oligocene, France, ×310 (new).

SOULIÉ-MÄRSCHE, where this feature is one of the identifying criteria of the species. On the other hand, a species may include both typically ornamented and completely smooth specimens (e.g., *Rhabdochara praelangeri* CASTEL). Such examples have led GRAMBAST (1957) to recognize only a limited systematic value in the characters of the ornamentation.

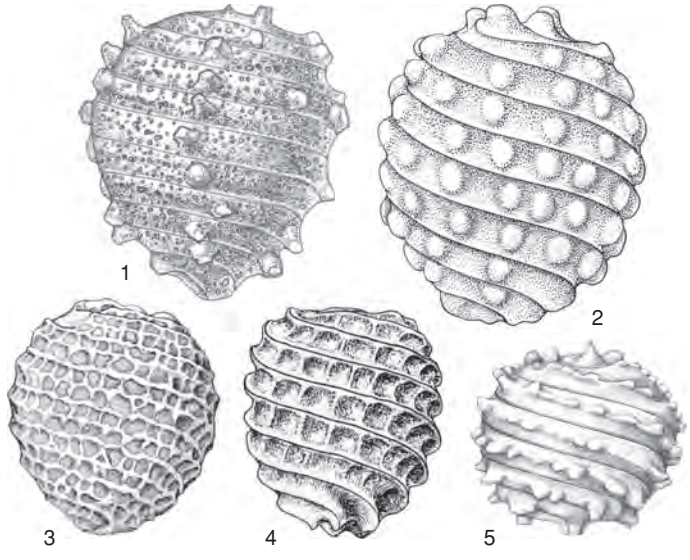

FIG. 17. Different types of ornamentation; *1, Harrisichara sparnacensis* GRAMBAST; small punctuations and tubercles, ×40 (adapted from Grambast, 1977a, pl. II,*3a*); *2, Sephanochara compta* GRAMBAST; tubercles, ×40 (Grambast, 1959b, fig. 3a); *3, Peckichara cancellata* GRAMBAST; reticulum, ×40 (Grambast, 1971, fig. 12a); *4, Rhabdochara praelangeri* CASTEL; rods, ×40 (new); *5, Maedleriella cristellata* GRAMBAST; midcellular crest tending to subdivide into tubercles, ×50 (Grambast, 1977a, fig. 11a).

We see below (in the section on Classification, herein p. 83) that opinions on this may have differed fundamentally in the past. In general, ornamentation is characteristic of species and more rarely of such genera as *Harrisichara* and *Maedleriella,* whose representatives nearly always have well-developed ornamentation. Among fossils, ornamentation has been reported only in the families Raskyellaceae and Characeae.

Oospore membrane.—Remains of oospores are relatively common in the fossil record, but preservation of the ornamentation of the sporostine is exceptional. Silicified rock (chert and flint) and gypsum are the most favorable for preservation of this organic membrane (Fig. 18). The sporostine appears as a translucent, brown or black layer lining the calcified (or secondarily silicified) gyrogonite wall. In noncalcified species, the oospores are isolated in the rock. Different types of ornamentation have been reported from fossil oospores. Among oospores inside gyrogonites, there is the granulate type in the Devonian *Trochiliscus podolicus* CROFT, where granules occur together with crateriform wounds (Fig. 18.4), and in the Oligocene Characeae *Stephanochara ungeri (Chara escheri)* (CROFT, 1952); the vermiculate type in the Oligocene *Rhabdochara praelangeri* CASTEL (Fig. 18.1), in which the vermiculations look much more intricate than in the extant *Nitella syncarpa* that also has this type of ornamentation; and the tuberculate perforated type in an Oligocene *Chara* species similar to the extant *C. zeylanica* (SOULIÉ-MÄRSCHE, 1989) (Fig 18.2). Among oospores isolated in the sediments, the well-preserved oospores of the Jurassic *Nitellites,* occurring in chert, have a fine reticulate ornamentation very similar, if not identical, to that of the extant *Nitella tenuissima* (HORN AF RANTZIEN, 1957) (Fig. 18.3).

These few examples show that the ornamentation of fossil oospores is relatively diverse; however, the scarcity of well-preserved remains does not allow presently the use of this character in recognition of fossil species.

Utricle

The utricle is an envelope made of vegetative appendages investing the gyrogonite. The most complex structure occurs in the

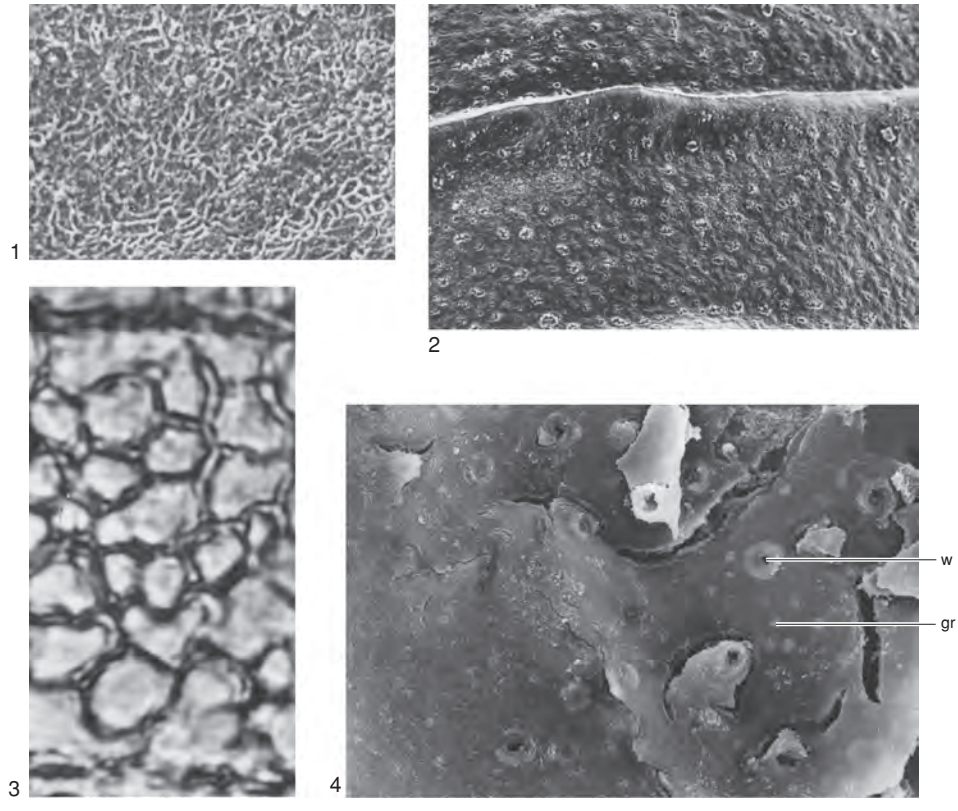

FIG. 18. Fossil oospore sculpturing; *1, Rhabdochara praelangeri* CASTEL; vermiculate sculpture, upper Oligocene, Marseille, southern France, ×600 (Feist & Grambast-Fessard, 1991, fig. 4ac); *2, Chara* sp. tuberculate perforated sculpture, upper Oligocene, Portel, southern France, ×800 (new); *3, Nitellites sahnnii* HORN AF RANTZIEN; reticulate sculpture; Middle Jurassic–Upper Jurassic, Rajmahal Hills, Bihar, India, ×2500 (Horn af Rantzien, 1957, pl. II, fig. 2); *4, Trochiliscus podolicus* CROFT; granulate sculpture, Lower Devonian, Ukraine, *gr,* granules, *w,* wounds, ×900 (new).

Clavatoraceae Clavatoroideae and in the Sycidiales where the utricle is composed of three layers: an internal layer, smooth or made of thick nodules; a middle layer, represented by a system of canals; and an external layer, constituted by whorls of branchlets. The internal nodulose layer may be represented alone, with possibly a whorl of leaflets in the basal region of the utricle; this structure exists in the oldest Clavatoraceae (*Nodosoclavator*) as well as in the incompletely calcified, more complex utricles that have been named nodosoclavatoroid utricles by SCHUDACK (1989). The canals of the middle layer, which correspond to the cavities of long cells, originate in a basal chamber; they could have been occupied by a gas, which lightened the fructifications and could thus benefit their dispersion (GRAMBAST, 1966b). In the Clavatoraceae Atopocharoideae and in the Pinnoputamenaceae, the utricle has only the external layer, and the internal layer is reduced to an amorphous surface covering the gyrogonite, sometimes visible between the external whorls. The structure of the utricles in most Sycidiales is at present still incompletely known, but they seem to have a complexity similar to that of the Clavatoraceae Clavatoroideae; two layers are visible in *Trochiliscus podolicus* CROFT (Fig. 46,2*h,* Systematics, herein p. 96), and a system of canals occurs in *Sycidium xizangense* Z. WANG (Fig. 45*k,* Systematics, herein p. 95).

Inside utricles, gyrogonites are preserved only rarely. The presence of gyrogonite cells may be recognizable in thin sections (Fig. 55,*2b,* Systematics, herein p. 109), but these do not provide information on gyrogonite morphology. The orientation and number of gyrogonite cells are detectable in casts on the internal utricular surface (Fig. 58,*2e,* Systematics, herein p. 114) or on the surface of the internal mold of the oospore and egg (Martin-Closas, 1988, fig. 12). These observations were made in the Clavatoraceae. For the present, the exact gyrogonite morphology of the Sycidiales is still unknown.

The external layer of the utricle in the Clavatoraceae has undergone a high degree of diversification, which has been illustrated in particular by Grambast (1974) and Martin-Closas (1996).

The morphology of the utricle in Sycidiales and Clavatoraceae is relatively well documented. In contrast, very little about its nature and origin is known from the fossil record. Data from morphogenesis studies in extant species give some information on the process by which the utricles may have been formed in the geological past. After placing female buds of *Chara vulgaris* Linnaeus in an artificial growth medium, Ducreux (1975, p. 270, pl. VIII,*3*) observed that the oogonia have two whorls of spiral cells, the external being composed of loose cells and the internal forming the oogonial wall (see Fig. 63, Systematics herein, p. 120); the spirals eventually become ramified and bear antheridia. Ducreux (1975) noticed that these modifications recall the Cretaceous *Perimneste-Atopochara* phylogenetic lineage (Grambast, 1967), where the utricle cells of ancestral species are ramified and carry antheridia at the nodes of the ramifications. Supernumerary whorls and unusual features never appear during the normal development of living species; however, the occurrence of these features in culture suggests potentialities that are expressed only in special conditions. The laboratory experiments conducted by Ducreux (1975) are evidence of the developmental remnants of utricular cells in Characeae. The morphology of *Lagynophora* Stache and *Coenoclavator* Wang & Lu may indicate that this family has already expressed the tendency to develop utricles. This observation might indicate that possible development of a utricle is a phenomenon inherent in Charophyta that could have developed at any time and could presumably also develop in the future.

Antheridia

Antheridia are not calcified, but antheridial casts of some genera have been recorded. These casts have internal features quite similar to those of the extant species (M. Feist & R. Feist, 1997). In *Pinnoputamen* sp. (Fig. 48*e,* Systematics, herein p. 99) and *Perimneste horrida* Harris (Fig. 60*b,* Systematics, herein p. 116), the antheridia lie on branches of the external layer of the utricle, whereas in *Diectochara andica* Musacchio (Fig. 59,*2b,* Systematics, herein p. 115) they occur below the fructifications, as in many living forms.

Chiralization

Chirality, the pattern of spiraling of structures around vegetative axes, has been recognized in Charophyta as a fundamental feature, not only of oogonia and gyrogonites but also of thallus morphology and architecture in general. Soulié-Märsche (1999) stated that the first-formed axillary buds of branchlets are arranged in a helical pattern toward the apex and around the axis. The divergence angle between two successive buds is 144°; the phyllotaxis index is thus 2/5th, the same as that often encountered in land plants. Martin-Closas, Bosch, and Serra-Kiel (1999) modelled biomechanically the Early Cretaceous *Globator* species and concluded that spiralization tends to accentuate the globular shape of utricles by increasing the resistance of the calcified wall to internal pressure caused by the accumulation of reserves. They hypothesized that the resulting accumulation of storage material devoted to supplying young germlings has been a driving force in charophyte evolution.

MINERALIZATION

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MINERAL COMPOSITION

Charophytes are aquatic and metabolize CO_2 dissolved in water by photosynthesis. Being fixed by rhizoids to the bottoms of lakes, they absorb HCO_3^- and minerals (mainly $CaCO_3$) from the substratum. Calcification of the oosporangium is initiated after fertilization by a process yet to be elucidated. Calcification starts at the internal surface of the spiral cells on the adaxial and lateral faces, sometimes at the surface of the ectosporostine (Soulié-Märsche, 1989; Leitch, 1989) and continues as long as the cell is living. Timing of maturation of the oosporangium is influenced by altitude and latitude and generally completed by middle to late summer. Calcite is the major constituent of the calcareous shell, although acid etching shows very thin organic lamellae (3 to 5 μm in thickness) between the layers of calcite. Chemical analysis of the whole organism (Bathurst, 1971; Strauss & Lepoint, 1966) as well as microprobe and X-ray analysis of oosporangia have revealed traces of several additional elements, ions, and compounds: magnesium, strontium, silica, chloride, and barium. In some species that live in saline environments, the percentage of magnesium can reach one percent of the calcified oosporangia (Soulié-Märsche, 1989). Diagenesis of the mineral composition of fossil forms has not yet been studied in detail and may be related broadly to the disappearance of the organic parts and to diagenetic processes at work in the surrounding rock.

ISOTOPIC COMPOSITION

Jones and others (1996) established that the composition of oxygen and possibly carbon isotopes of living characean gyrogonites are in equilibrium with the surrounding water; consequently, these shells can be used to reconstruct the geochemical properties of the ancient water bodies in which they grew. In isotopic analyses, however, account should be taken of the relationships to the substratum and the fact that gyrogonites calcify during the highest temperatures of the year (i.e., the time window of Jones & others, 1996).

There have been few investigations of the isotopic composition of fossil charophytes. Berger (1990) and Becker, Picot, and Berger (2002) analyzed the variations in stable isotopes of ^{13}C and ^{18}O in late Cenozoic charophytes of western Switzerland (Fig. 19). According to J.-P. Berger (personal communication, January 2001), an isotope excursion lower than 2‰ is not significant for paleoecological or paleoclimatical interpretation; the general covariance between $\delta^{13}C$ and $\delta^{18}O$ excursions is typical for closed lakes, and the important excursions observed in the isotopic record are probably due to an increase of seasonality during the Cenozoic, the negative values indicating a more humid period. This seasonality could have resulted from different climatic, paleogeographic, and tectonic events occurring during this time. Also, the erosion of marine Mesozoic carbonate could produce more positive excursions in the $\delta^{13}C$. The isotopic curves for

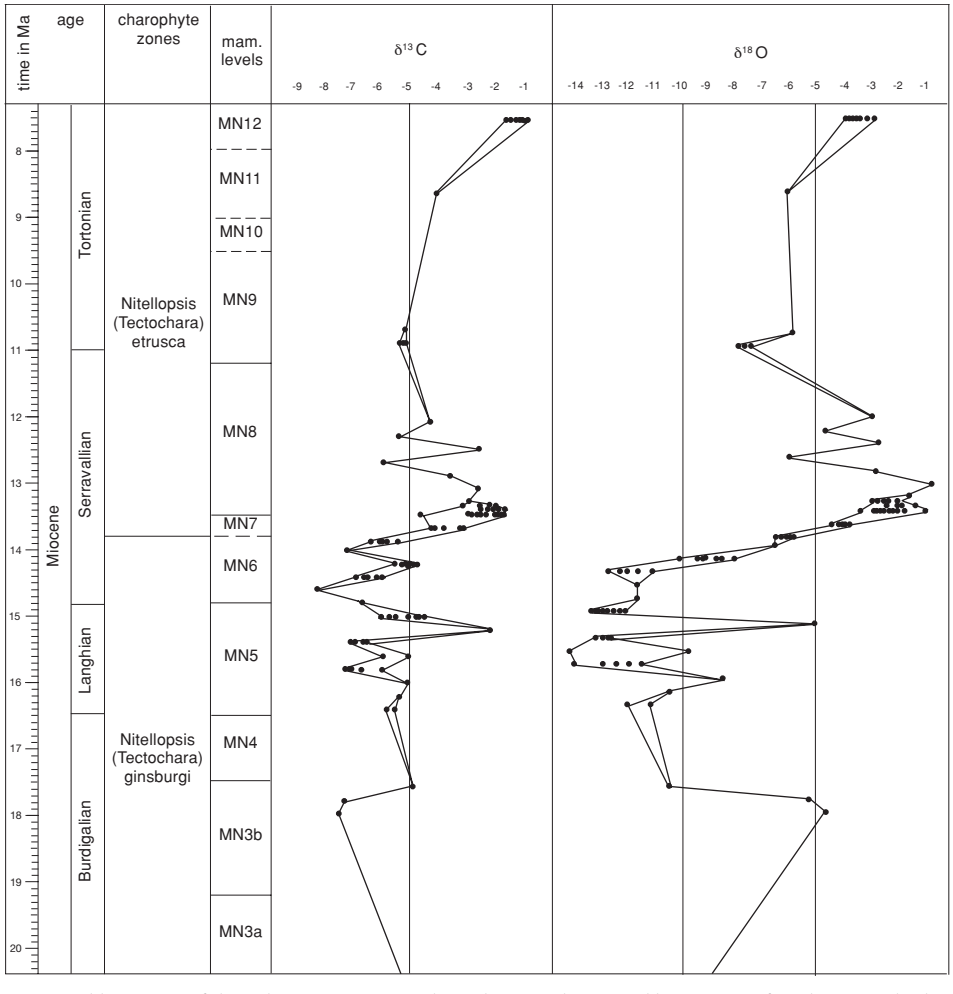

FIG. 19. Stable isotopes of charophyte gyrogonites and correlation with mammal biozonation, from lower Burdigalian to upper Tortonian; *mam. levels,* mammal standard levels, after Schmidt-Kittler, 1987 (adapted from Becker, Picot, & Berger, 2002).

the time interval between early Burdigalian and late Tortonian show several warming and cooling phases that are correlated on the whole with the variations observed in oceanic DSDP records (WOODRUFF, SAVIN, & DOUGLAS, 1981). In particular a cold phase occurred approximately 12 Ma, which is thought to be related to the formation of the Antarctic ice cap. The latter correlations do not include consideration of the new calibration of the inferred geological time scale (BERGGREN & others, 1995).

TECHNIQUES FOR PREPARATION AND STUDY OF FOSSIL CHAROPHYTA

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The first step in studying fossil charophytes is to release the gyrogonites from the matrix. One to five kilograms of sediment are necessary to obtain a high enough number of specimens to allow the study of populations, to make oriented thin sections, and for photography. Nondetrital lacustrine or nonmarine brackish-water deposits are the most likely to yield charophytes. The specific methods used for the treatment of charophytes are much the same as those used for other small fossils, the ostracodes in particular (SOHN, 1961).

RELEASE FROM MATRIX

Depending on the lithology, two different methods can be used to release charophytes from the matrix. Washing and sieving are used to extract charophytes from such loose sediment as argillaceous or lignitic marls. Found with charophytes are such various small fossils as foraminifers, molluscs, ostracodes, dinosaur eggshell fragments, microscopic teeth of mammals, and, rarely, seeds, conodonts, and scolecodonts. For one kilogram of sediment, 50 g of sodium carbonate (Na_2CO_3) and one liter of 30-percent hydrogen peroxide are required. The dry sediment is poured into a plastic jar, mixed with sodium carbonate, and covered with water. Add the hydrogen peroxide and allow the mixture to react for 12 to 48 hours. Sieve with running water, pouring the mixture through a set of three sieves of meshes 2 mm, 500 μm, and 150 μm. The sieves can be air dried or placed in a drying cupboard.

Well-preserved gyrogonites may be extracted from more indurated sediments such as marls and marly limestones by using copper sulfate. For 100 g of rock, 100 g of anhydrous copper sulfate, 250 ml of acetic acid, and 500 ml of ammonium hydroxide are needed. In a fume hood, mix a solution of acetic acid and copper sulfate in a glass jar two hours before use. Into the solution, place the completely dry rocks, cut into pieces approximately 1 cm in size. Let the mixture react for 12 to 24 hours until most of the limestone pieces have been dissolved. Neutralize the remaining solution with ammonium hydroxide, then wash and sieve with water.

Extremely hard, recrystalized limestones can be studied only with thin sections. These provide information as to the microfacies, which, in turn, furnishes ecological information but generally few details of gyrogonite morphology.

CONCENTRATION OF FOSSILS

Once the sediment has been washed and sieved, the charophytes can be concentrated by using a number of methods.

Kerosene can be used to eliminate the argillaceous fraction of the sediment. The dry sediment is soaked in kerosene. Once it is thoroughly impregnated, it is mixed with water and left to soak for 4 to 12 hours, then washed and sieved again. Some people prefer to use gasoline instead of kerosene, but because of the highly volatile, inflammable, heavy vapor and the consequent danger of fire or explosion, such work should be done out of doors or in a fume hood with a strong exhaust fan.

Bromoform, with a specific gravity of 2.8, is appropriate to separate gyrogonites from other sediment matrix. This procedure is to be used only in open air or using a fume hood. The dry sediment is poured into a fine-meshed sieve inside a large container without any trace of water. Bromoform is added. Then pure ethyl alcohol is added

gradually until flotation of gyrogonites occurs. Gyrogonites are removed from the top of the liquid with a fine wire mesh.

To facilitate the picking process, the dry sediment is subdivided into similarly sized fractions by using a column of six metallic sieves. Generally, these sieves are 10 cm in diameter and their meshes range from 65 μm to 1.25 mm.

To bring about mechanical separation on an inclined plane, thin lines of sediment are placed around the periphery of a rectangular, flat container with shallow edges. By inclining and agitating the container, gyrogonites will roll down to the bottom where they can be collected with a wet brush. This procedure, which relies on the broadly spherical shape of the gyrogonites, is not appropriate for the angular utricles of the Clavatoraceae.

Gyrogonites may also be picked from the sediment with a fine wet brush (number 00 to 1) at powers 5× to 40×, depending on their size. Charophytes may be stored in small plastic boxes or in hollowed slides.

CLEANING

Boiling in water with addition of a detergent removes argillaceous dust from the spirals. For hardened incrustations, a more efficient method is immersion in EDTA (ethylenediaminetetraacetic acid) disodium salt dihydrate followed by use of an ultrasonic cleaner. A six-percent solution is used for well-calcified specimens; a three-percent solution is used for fragile material. Gyrogonites are immersed for five minutes in the EDTA disodium salt dihydrate solution; a slight release of bubbles from the disaggregation of the crust indicates that the process is complete. The gyrogonites are then rinsed with freshwater and placed into an ultrasonic cleaner for three minutes (power 50W, frequency 40 kHz).

COLORATION

Coloration can be applied to the gyrogonite to enhance definition when viewed with a binocular microscope. An alcohol solution of methyl green outlines the external structures without covering them. In contrast, volatile correcting fluid mixed with alcohol, acetone, and chrysoidine covers the specimens entirely. The process is simple but delicate: a gyrogonite or a utricle is placed into a watch glass and covered with a drop of coloring liquid and shifted rapidly until dry. The liquid covers the hollows evenly with an orange coating and underlines the relief in red. This coloration, which was used for optical photography during the 1960s, is still useful for showing complex structures of clavatoracean utricles. An apex of *Septorella brachycera* Grambast (Fig. 56,*1b,* Systematics, herein p. 111) provides an example of this coloration.

PREPARING THIN SECTIONS OF GYROGONITES

Axial sections are used most often. They provide valuable information about the calcified wall and the basal plate; more rarely, when revealing the structure of the summit, sections allow a taxonomic assignment, generally at the family level (Castel, 1969). Axial sections also allow examination of the oospore wall, if the latter has been preserved.

The procedure involves embedding the specimen in plastic. It is then thinned on both sides by polishing in the selected orientation. The product used for the embedding is a clear epoxy resin. A mixture of resin and hardener is put into a container, preferably of cardboard, and stirred for two minutes on a hot plate. The specimen is fastened to the external side of a small plastic box whose borders are fringed with paraffin or wax from a candle; the specimen is then covered with a few drops of the mixture and left to polymerize.

To polish the specimen, first remove the wax fringe with a razor blade. The resin block including the specimen is abraded directly on the lapidary disk with 600-mesh grit, during which the process is carefully monitored using a binocular microscope. The abrasion should stop when the level of

the axial plane of the gyrogonite is nearly reached. Then, a drop of the plastic mixture is applied to the ground surface of a glass slide, and another drop is applied to the worn side of the specimen. The specimen is then attached to the slide, and the solution is allowed to polymerize. Then the second side of the section is ground down and covered with Canada balsam or with the resin mixture.

OBSERVATION AND PHOTOGRAPHY

REFLECTED LIGHT

Charophytes are usually examined with a binocular microscope using reflected light at 20× to 100×. Gyrogonites are held in position on a glass slide smeared with an adhesive (rubber cement). The use of glue or gum tragacanth, which seeps inextricably into the spiral sutures, is not recommended.

The basal plate may be extracted by crushing the gyrogonite carefully between two glass slides. The basal plate is recognizable by its pentagonal shape among the fragments of spiral cells.

Photographs may be taken directly with the microscope at low magnification. Use of a camera with a tube and bellows between the objective and the camera provides higher magnifications. Three or four lamps or a neon ring provides adequate light.

TRANSMITTED LIGHT

Thin sections of rocks or of isolated gyrogonites may be examined with transmitted light at powers 50× to 500×.

SCANNING ELECTRON MICROSCOPY (SEM)

For charophytes, the SEM allows observations and photography of general views at magnifications from 30× to 200× and details of structure at up to about 20,000×. Specimens are glued to a stub, preferably with a metallic glue that holds the specimen in position and provides good electronic imaging. The metallic coating, whatever medium is used (gold, gold-palladium, platinum), must be thick enough (150 to 200 Å) to avoid the electronic charge inherent in the spherical shape of gyrogonites.

MEASUREMENTS

An ocular micrometer can be used with magnifications of 25×, 40×, or 50× to determine gyrogonite dimensions (see Fig. 12.1) as well as the number of spiral cells and the coefficient of spirality (Demin, 1967). Tests of the reliability of the methods of measurements (Soulié-Märsche, 1989) show that variation due to observer error is lower than those due to the capabilities of the equipment. For example, a difference of five to six percent was observed in a test of four methods for a population of 100 specimens that were 530 μm in mean diameter. Dimensions are important but not of prime significance for distinguishing species, and a margin of error should be considered when comparing numerical data from different charophyte populations.