# CHAPTER 9

# *Lycophyta*

THE Lycophyta is a well-defined group of vascular plants consisting of fossil and living representatives. The known history of this group extends from the Paleozoic Era to the present. There are five living genera with more than 1,000 living species which occur in various parts of the world under varied climatic conditions. The living genera consist of the "ground pine" or club moss *Lycopodium* (Figs. 9-2, 9-3, 9-4); the spikemoss *Selaginella* (Fig. 9-19); the small, tuberous plant *Phylloglossum* (Fig. 9-1), which is greatly restricted in its distribution; the quillwort *Isoetes* (Fig. 9-49); and *Stylites* (Amstutz, 1957) found growing high in the mountains of Peru (Fig. 9-59, A, B).

All of these genera are small plants: some are erect, some live as epiphytes, others grow as creepers on the ground, and yet others produce underground rhizomes. In contrast with these plants of modest stature, many of the ancient lycopods *(Lepidodendron)* were good-sized trees, and their vegetative structures and spores constitute an important part of coal (see Figs. 9-40; 9-41). The importance of this assemblage of vascular plants cannot be measured in terms of the present economic value of living members, but rather by the morphological unity of the entire group and its value in the interpretation of phylogenetic trends in vascular plants.

The vegetative sporophyte is differentiated into a shoot system, consisting of stems and leaves, and a root system. Reminiscent of the Psilophyta, the shoot system of many forms is isotomously branched or modified by anisotomous branching (Chapter 3). Occasionally the axis may be unbranched (as in *Phylloglossum*). The arrangement of leaves is fundamentally helical with modifications (opposite, whorled) characteristic of certain species. Leaves of most living genera are relatively small, whereas those of certain extinct forms were considerably larger. Whatever the arrangement or form of the leaves, each one is generally traversed by a single unbranched vascular bundle. Such a leaf is designated a *microphyll* (Chapter 3). Each leaf of certain genera, such as *Selaginella, Lepidodendron, Isoetes,* and *Stylites* has a curious tonguelike appendage on its adaxial side termed the *ligule* (Figs. 9-25, A; 27; 28 C, D; 50; 54).

The vascular cylinder of the stem in most living species is protostelic. The primary xylem is generally exarch in development and consists primarily of tracheids with scalariform pitting. Whether the vascular cylinder is a protostele or a siphonostele, there are no breaks in the vascular tissue at the point of departure of leaf traces. No leaf gaps exist (Chapter 3). In a stem with a siphonostele a branch gap is present in the vascular cylinder of the main axis

only at the level of divergence of the branch. In most living genera the roots, arising from rhizomes (e.g., in *Lycopodium*), branch dichotomously. In *Isoetes* there is a definite, perennial, root-producing meristem. Although the formation of secondary tissues was very common in ancient arborescent lycopods, this feature is characteristic of only two living genera—*Isoetes* and *Stylites*. A feature that unifies the entire group is the position of the eusporangium. Each sporophyll has a single sporangium which is either attached to the adaxial basal region of the sporophyll or is located in its axil. The conditions of homospory and heterospory are coexistent in the group; heterosporous forms always produce endosporic gametophytes, whereas homosporous forms produce only exosporic gametophytes.

## Classification

LYCOPHYTA: Sporophyte differentiated into leaf, stem, root and eusporangium; microphylls ligulate or eligulate; typically one sporangium attached to or associated with each sporophyll; no leaf gaps; exarch xylem predominates; protostelic or siphonostelic; some have secondary growth.

LYCOPODIALES: Living and extinct plants; sporophytes with primary growth only, no vascular cambium; leaves eligulate; majority have definite strobili; homosporous.

LYCOPODIACEAE: Living and extinct plants; herbaceous; many with definite strobili; exosporic gametophytes; biflagellate sperms in the living genus *Lycopodium*.

*Lycopodium, Phylloglossum, Lycopodites* (extinct).

EXTINCT HERBACEOUS DEVONIAN LYCOPODS: Of Devonian age; low, herbaceous plants; dichotomously branched upright shoots from rhizomes; vascular cylinder cylindrical to lobed; some leaves with one to four sporangia, located near axil or on adaxial side of leaf; no definite, compact strobili; homosporous.

*Baragwanathia, Leclercqia, Drepanophycus, Protolepidodendron, Asteroxylon.*

SELAGINELLALES: Living and extinct plants; with primary growth only; no vascular cambium; microphyllous with ligule; definite strobili formed; heterosporous; gametophytes endosporic; sperms biflagellate in living members.

SELAGINELLACEAE: Characteristics as in Selaginellales.

*Selaginella, Selaginellites* (extinct).

LEPIDODENDRALES: Extinct plants; treelike and most, if not all, with secondary growth; microphyllous with ligule; large root stocks (rhizophores) formed; heterosporous; sporophylls grouped into strobili; some forming seedlike structures.

Selected genera: *Lepidodendron, Stigmaria* (form genus for rhizophores), *Sigillaria, Lepidostrobus* (a form genus for strobili), *Lepidocarpon* (form genus for seedlike structures).

ISOETALES: Living and extinct plants; sporophytes with cormlike stems; secondary growth; perennial root-producing meristem; ligulate microphylls; heterosporous; endosporic gametophytes; sperms multiflagellate in living members.

ISOETACEAE: Characteristics as in Isoetales.

*Isoetes, Isoetites* (extinct), *Stylites*.

PLEUROMEIALES: Extinct plants; upright unbranched stem with ligulate microphylls grouped at its upper end; upper end of axis terminates in a strobilus; rhizophore; heterosporous.

PLEUROMEIACEAE: Characteristics as in Pleuromeiales.

*Pleuromeia, Nathorstiana.*

## Homosporous Forms in the Lycophyta

### Lycopodiales—Lycopodiaceae

The family Lycopodiaceae includes two living genera, *Lycopodium* and *Phylloglossum*. The former, commonly termed club moss, is worldwide in distribution. Most of the species (about 400) of

*Lycopodium* are tropical, but others occur in temperate and arctic regions of the world. *Phylloglossum,* a highly reduced and specialized monotypic plant, is restricted to Australasia (Fig. 9-1). *Lycopodites,* a fossil lycopod from the Carboniferous Period to the Recent Epoch, resembled the modern club moss in many respects.

**LYCOPODIUM.** Although species of *Lycopodium* do not usually form a conspicuous part of the flora of temperate regions, this genus is very diversified in growth habit and abundantly represented in the American tropics (Haught, 1960).

Some species are erect shrubby plants (Figs. 9-2; 9-3, B), others have a trailing or creeping habit (Fig. 9-4, A, C), and still others grow as epiphytes. Some of the terrestrial prostrate types form "fairy rings" in open, undisturbed areas. Active rhizomatous growth takes place at the margins of such a circle, while that part of the colony produced in previous years decays. The ring may be in the shape of a circle, and increase in diameter as a function of time, resulting in an exponential curve of surprising exactness. One ring, measuring 11.25 meters in diameter in 1964, was estimated to have originated in 1839 (Van Soest, 1964).

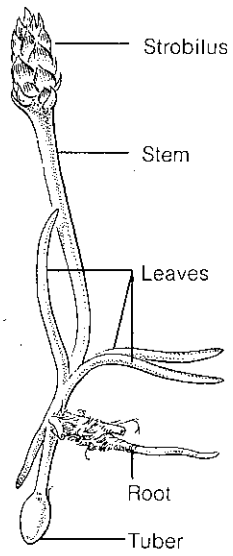



FIGURE 9-1 Habit sketch, *Phylloglossum drummondii.* The "tuber" is a vegetative reproductive body and is capable of developing into a typical plant under favorable environmental conditions.

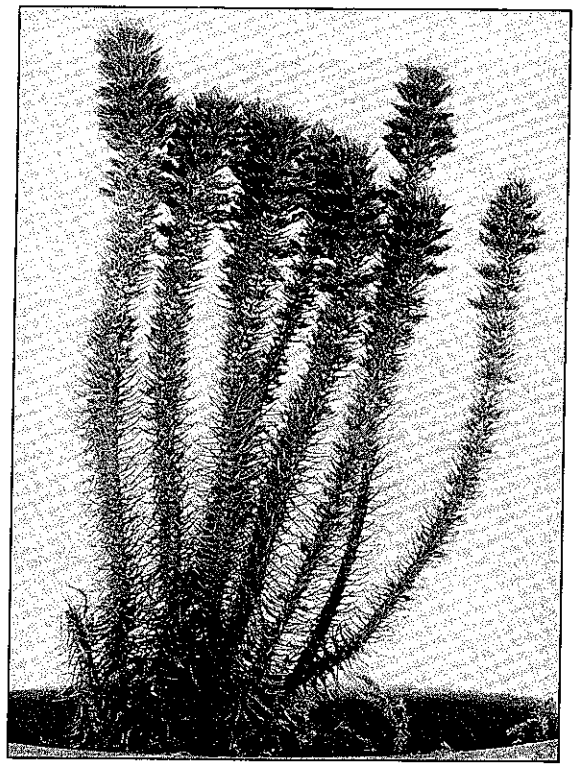


FIGURE 9-2 *Lycopodium* sp. Note the sporangia (white structures) in the axils of certain leaves along the upper half of each branch and the clusters of gemmae on the uppermost portion of each shoot.

It has been a convention to consider all club mosses as a single genus, *Lycopodium.* Some pteridologists (those who study lower vascular plants) believe that the variation in sporophytic and gametophytic features in the genus supports the establishment of at least subgenera within *Lycopodium,* or even for the elevation of subgenera to generic status. These concepts will be discussed later in the chapter after the reader becomes aware of the morphological variation in club mosses.

**ORGANOGRAPHY.** Whether a given species is erect or prostrate, branching is fundamentally dichotomous. The branches of a dichotomy may be equal (Fig. 9-3, A), or the branches of a dichotomy may be unequal, with one branch overtopping the other. The weaker branch system generally becomes determinate, often ending in one or more strobili (Fig. 9-4, C). This mode of branching is termed anisotomous, and it reaches its greatest development in

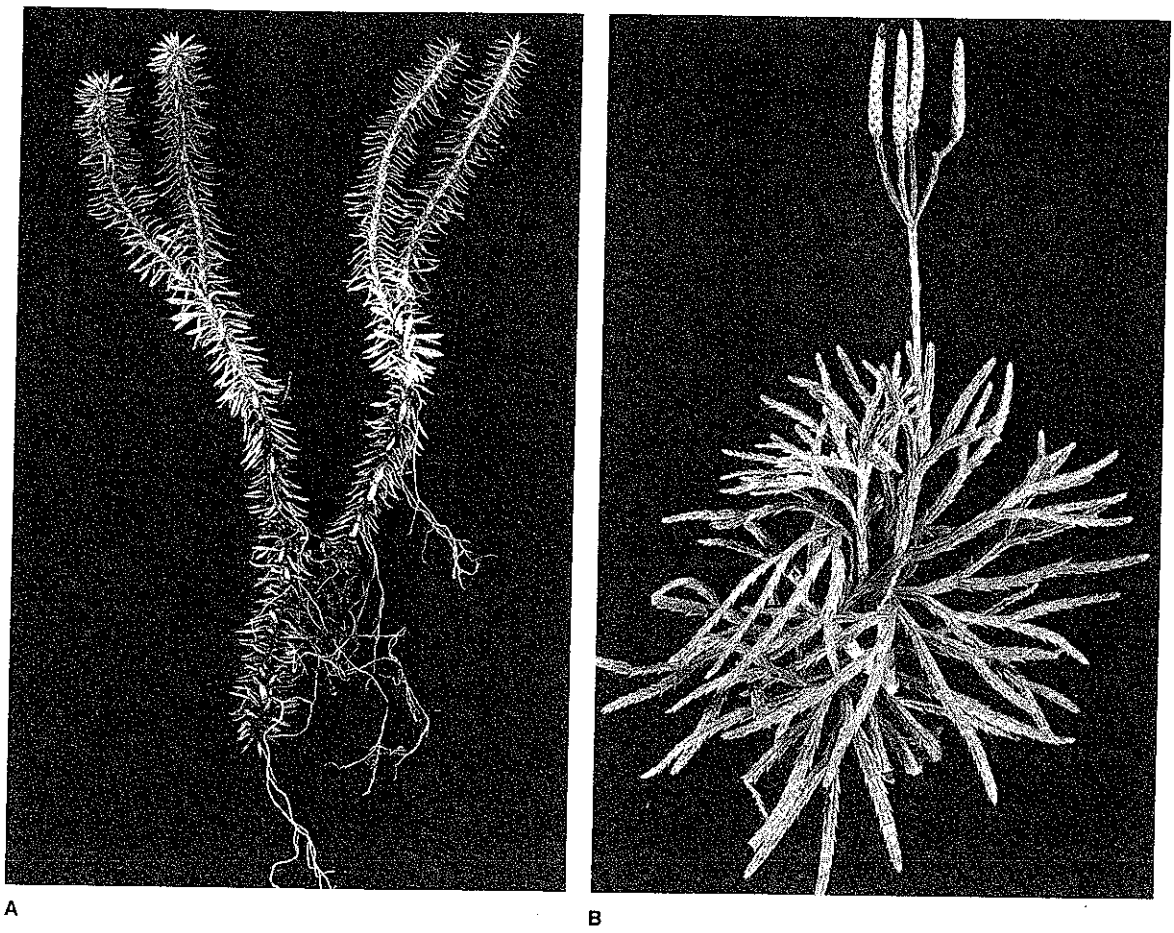



FIGURE 9-3 *Lycopodium.* **A,** *Lycopodium lucidulum;* note dichotomous branching of shoot, and roots. **B,** *Lycopodium digitatum;* note small, scalelike leaves and branch terminating in strobili.

forms with a prostrate rhizomelike main axis (Fig. 9-4, C). The leaves are microphylls which range in length from 2 to 20 millimeters or even up to 25 to 35 millimeters in a few species. Phyllotaxy is basically helical, but the arrangement may appear to be opposite or whorled, or even variable in different regions of the same plant (Bhambie, 1965). In some forms the leaf bases are decurrent (the leaf base is fused with and extends down the stem to varying degrees). In some species the leaves may be of two sizes (anisophylly), especially on lateral determinate branches.

Some species form vegetative reproductive structures — termed gemmae or bulbils — which become detached from the plant and grow into new sporophytes (Fig. 9-2). These structures arise in the positions of leaves and consist of a bud and preformed roots (Takeuchi, 1962). Gemmae have been interpreted as short, specialized branches resulting from anisotomous branching of the main stem axis (Stevenson, 1976). The factors that favor their formation are not well understood (Cutter, 1966).

Roots arise endogenously along the lower side of the stem in prostrate forms (Chapter 3; Fig. 9-4, C). In the upright forms roots may be initiated near the shoot tip and subsequently grow downward through the cortex, emerging at the base of the plant (Figs. 9-3, A; 9-5, A). After the root emerges from the stem it may branch freely in a dichotomous fashion.

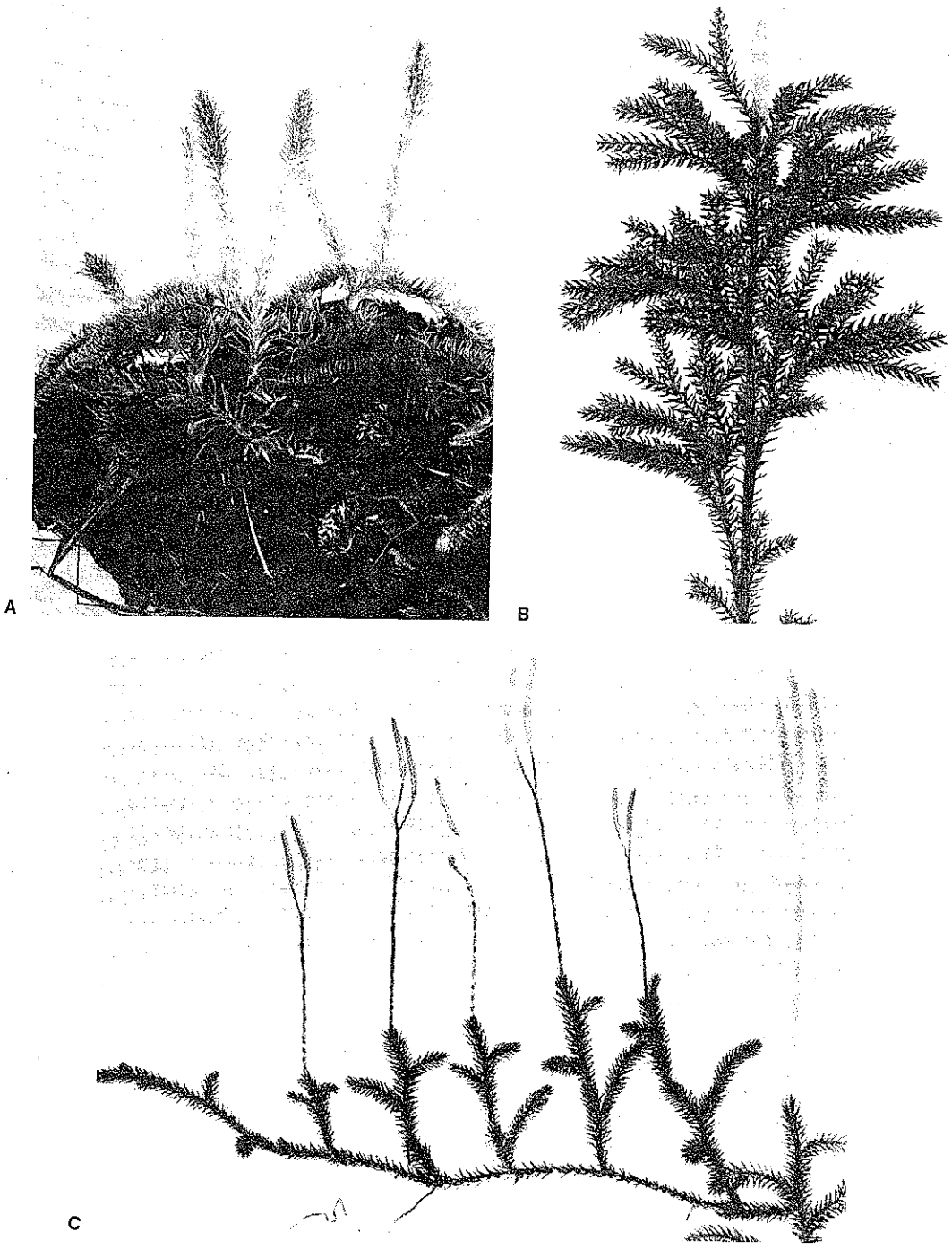



FIGURE 9-4 *Lycopodium.* **A,** *Lycopodium inundatum,* showing prostrate rhizomes and upright fertile shoots terminated by strobili. **B,** *Lycopodium obscurum,* portion of upright branched shoot and terminal strobilus. **C,** *Lycopodium clavatum,* strongly rhizomatous species with determinate, fertile side branches; a root is evident along lower edge of the main axis.

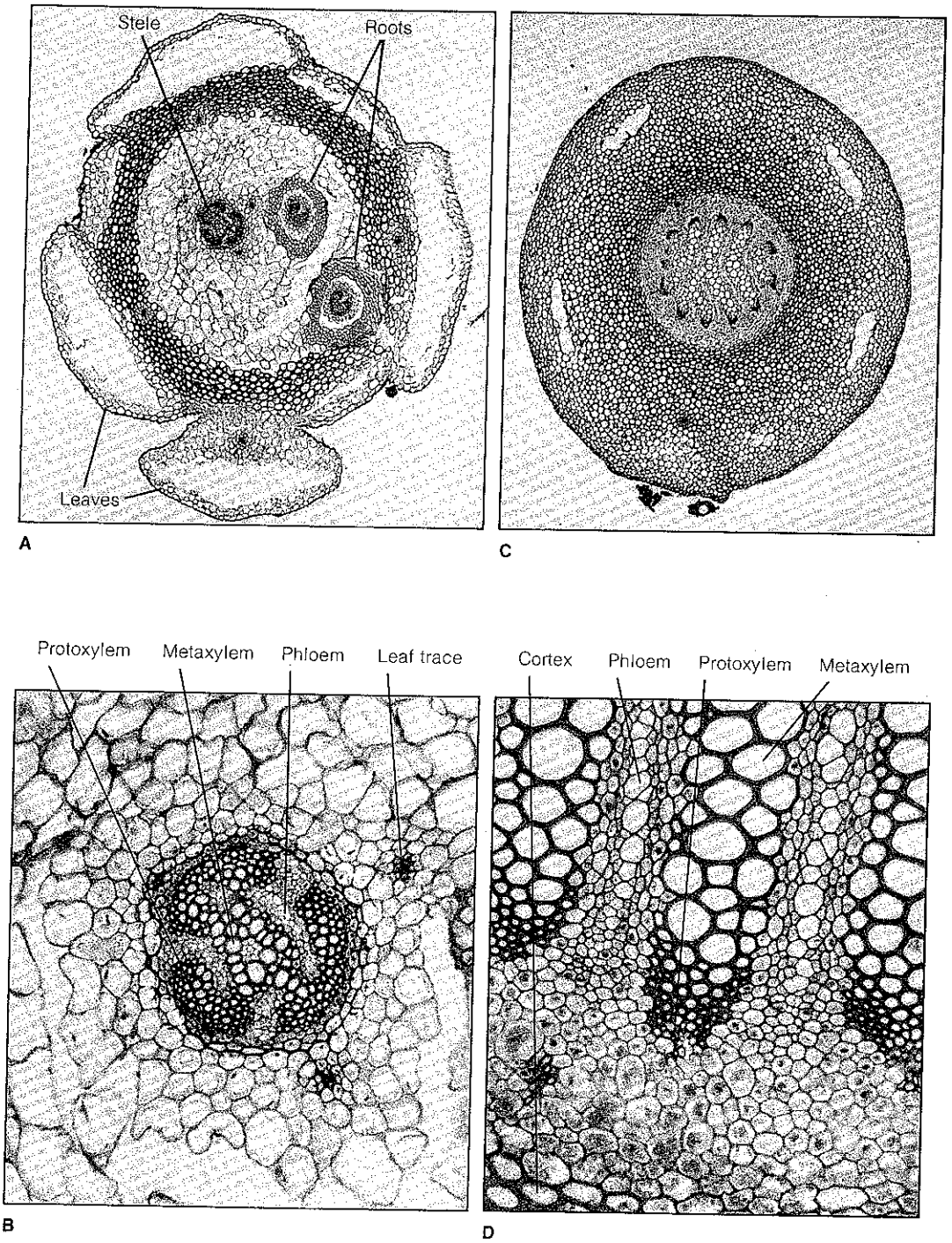



FIGURE 9-5 Stem anatomy in *Lycopodium.* **A,** transection, stem of *Lycopodium selago;* **B,** details of actinostele in *Lycopodium selago;* **C,** transection, stem of *Lycopodium* sp. showing plectostele; **D,** details, portion of stele in C.

Sporangia always occur singly on the adaxial surface of the sporophylls or in their axils. The sporophylls may be aggregated into definite strobili and may be quite different from vegetative leaves (Figs. 9-3, B; 9-4, B, C). In other species, however, "fertile" areas alternate with "sterile" regions along the stems, the sporophylls resembling ordinary foliage leaves (Figs. 9-2; 9-6, A).

**STEM ANATOMY.** The outermost layer of the stem is a uniseriate epidermis. The cortex is highly variable in thickness and structure (Fig. 9-5, A, C). In some species it remains parenchymatous, and in others the cells of specific regions undergo sclerification. Large air space systems may be present that extend into the leaves. Surrounding the vascular cylinder is the pericycle that may be two or three cells wide, or constitute a broad zone of parenchyma. An endodermis with casparian strips is not evident. The transition from pericycle to cortex in some species is abrupt because the walls of the cortical cells are thick (Fig. 9-5, D).

With the exception of the ferns, in no lower vascular plants is there such variation in the pattern of primary xylem and phloem in stems as is found in *Lycopodium*. The same species and even the same individual may show great variation during ontogeny (Wardlaw, 1924). In the mature plant body the vascular cylinder may be *actinostelic* with the primary phloem occupying the regions between the flanges of primary xylem (Fig. 9-5, B). In other species the primary xylem and phloem form strands of tissue which in transverse section appear as alternating bands of xylem and phloem; this type of vascular cylinder is designated a *plectostele* (Fig. 9-5, C, D). In still other species the central mass of xylem may be so modified as to form numerous strands of xylem and phloem (Ogura, 1938). It should be remembered that the seemingly isolated strands of xylem or phloem actually are interconnected. This can be demonstrated if their course if followed throughout the stem.

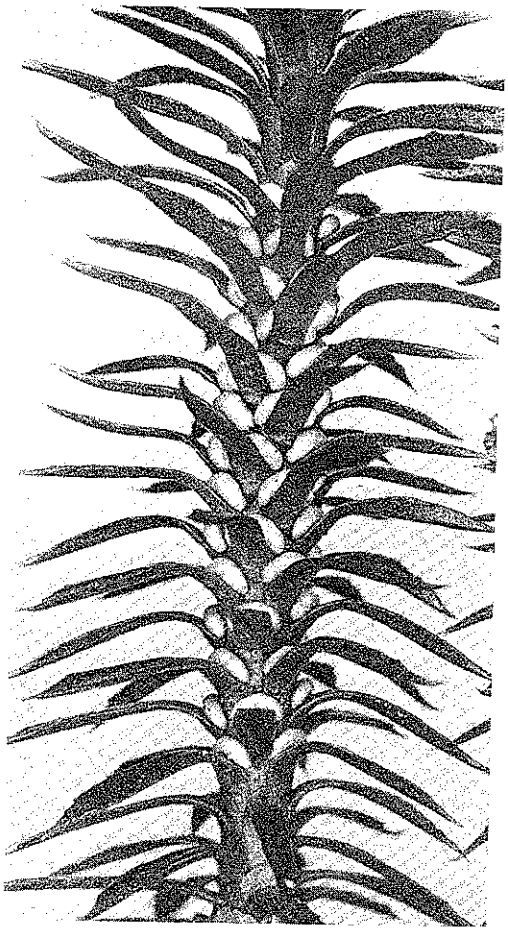
Ontogenetic studies have shown that the young sporophyte in most species is actinostelic. As growth of the sporophyte continues and the stem increases in size there is generally a change in the pattern of xylem and phloem. The actinostelic condition may persist with the formation of more protoxylem poles, or any of the configurations described above may result. In the smaller branches there may be a return to an actinostelic arrangement with only a few protoxylem poles.

Xylem maturation generally has been accepted to be strictly exarch in *Lycopodium*, but the results of a reinvestigation of three species would suggest that xylem development is at least sometimes and possibly always mesarch in indeterminate branches (Wilder, 1970). Mesarchy is, however, inconspicuous in that there are only a few tracheids formed in the centrifugal direction from a protoxylem pole. The bulk of the xylem cylinder, of course, consists of tracheids of the metaxylem, the larger of which have scalariform or circular bordered pits.

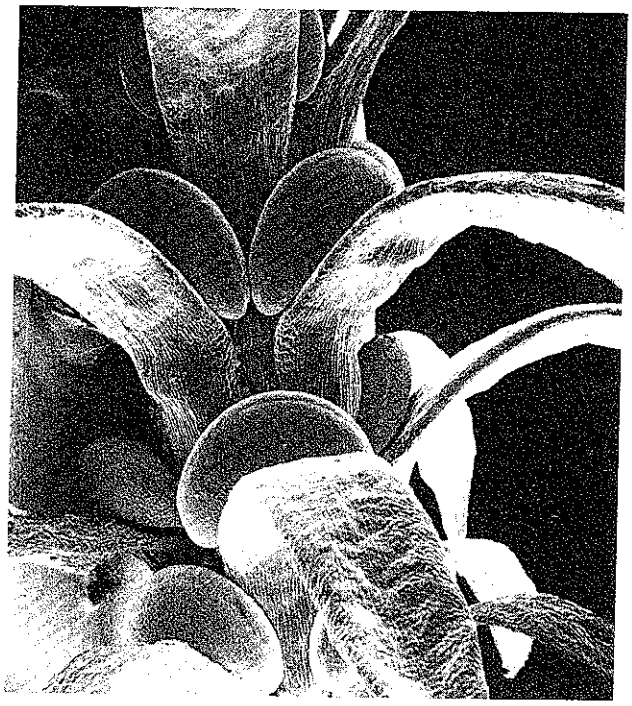
The phloem consists of sieve cells and parenchyma. The sieve cells are elongate with sieve areas distributed over the lateral walls as well as on the long, oblique end walls. The pores of the sieve areas of one investigated species are not lined with the carbohydrate callose, the presence of which is so characteristic of vascular plants in general (Warmbrodt and Evert, 1974). The sieve cells of *Psilotum* also lack callose (Chapter 8, p. 92).

The apical meristem of the shoot tip is reported to consist of a group of apical cells (Turner, 1924; Härtel, 1938; Freeberg and Wetmore, 1967; Nougarède and Loiseau, 1963) which by periclinal and anticlinal divisions contribute to the three primary meristematic tissues: protoderm, ground meristem, and procambium. The derivatives of these three primary tissues differentiate into epidermis, cortex, and vascular tissue, respectively. The centrally located procambium extends very close to the shoot apex, a feature characteristic of many lower vascular plants (Wetmore, 1943; Freeberg and Wetmore, 1967). The cells of the procambium are elongate (Fig. 9-7, A), and divide longitudinally, and frequently in the transverse plane.

To understand vascular differentiation, an examination of transverse stem sections taken at successive levels from the apex is essential. Near the tip the future vascular cylinder is represented by a compact core of procambial cells. Very early, however, within the procambial cylinder of a plectostele, for example, there is the centrifugal blocking-out of the future stele. The first procambial cells to differentiate cytologically are the future



A



B

FIGURE 9-6 *Lycopodium lucidulum.* **A,** fertile region of branch showing sporangia in axils of leaves (sporophylls). **B,** enlargement, as seen with the scanning electron microscope; note line of dehiscence across top of each sporangium. [B courtesy of Dr. R. H. Falk.]

cells of the metaxylem and metaphloem, followed by protoxylem and protophloem (Fig. 9-7, B, C). The future pattern may be established as close as 0.5 millimeters from the shoot apex. This initial centrifugal blocking-out is followed, at a lower level, by a stage of centripetal cellular maturation, the first elements to mature being the tracheids of the protoxylem (Fig. 9-7, D), followed by sieve cells of the protophloem. Maturation then proceeds centripetally until all metaxylem and metaphloem elements are mature. Complete maturation of the vascular cylinder may be complete only at a distance of 4 to 6 centimeters below the shoot apex (Freeberg and Wetmore, 1967).

Although the foregoing description emphasizes radial differentiation and maturation, procambial cells also are elongating and maturation occurs both longitudinally and radially in the stem.

**LEAF ANATOMY.** Initiation of the leaf may occur in a single superficial cell on the flank of the apical meristem, or in several superficial cells for certain species (Bhambie (1965). Growth in length, lateral extension of the lamina, and maturation of tissues produce the mature leaf. The growth of a leaf is associated with the development of a procambial strand into its base from the differentiating vascular tissue of the stem (Härtel, 1938). Differentiation of cells within this original procambial tract produces a vascular bundle, termed a leaf trace in its course through the cortex of the stem, and an unbranched vein within the leaf itself. Leaf traces are attached to lateral flanges or edges of the protostele of the stem axis (Fig. 9-7, C). Mature leaves range from minute scales to larger types that are lanceolate to ovate in outline, and generally lack definable petioles. Stomata may occur on both leaf surfaces or be re-

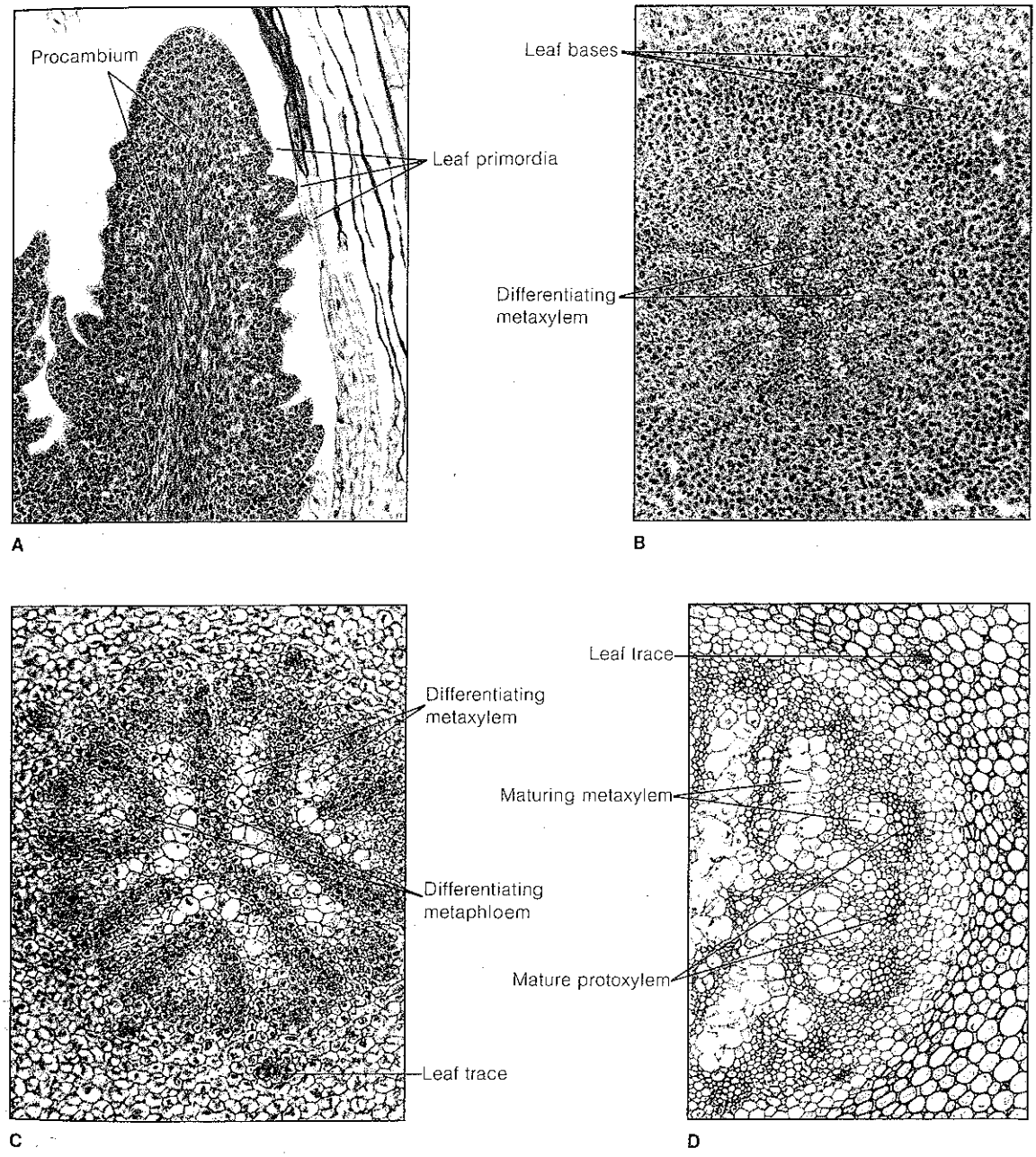



FIGURE 9-7 Development of the vascular cylinder (stele) in *Lycopodium*. A, longisection of shoot tip; B-D, transections of stem near shoot tip (B) and at increasing distances from the shoot apex (C, D). See text for details.

stricted to one (Chu, 1974). The mesophyll in many species is composed of more or less isodiametric cells with a conspicuous intercellular air space system.

The guard cells are formed directly from the original protodermal initial (Bhambie, 1965; Pant and Mehra, 1964).

ROOT. Except for the ephemeral primary root of the young sporophyte, the roots of actively growing plants arise from the stem very near the growing tip. These roots, which arise endogenously from the stem pericycle (Roberts and Herty, 1934), do not break through the cortex and epidermis immediately but often traverse the cortex for some distance before emerging. Since roots arise acropetally along the stem, many roots may be found in the stem cortex of the aerial portion of erect and epiphytic species (Fig. 9-5, A). In certain species (e.g., *Lycopodium pithyoides*) as many as 52 roots may be counted in the cortex at one level (Stokey, 1907). Only near the base of the stem do these roots emerge. In prostrate forms the roots take a more direct course from the stem axis to the exterior. After emerging from the stem, a root branches dichotomously, often with great regularity.

In propagating species of *Lycopodium* it is important to (1) obtain a portion of the plant with intact roots, or (2) use the upper portion of a shoot (since roots are initiated near the tip), or (3) secure a portion of the stem with arrested roots which emerge from the stem cortex on contact with a moist surface. Arrested roots may be identified as mounds on the under side of the stem of a prostrate form (Roberts and Herty, 1934).

Stokey (1907) has reported that four distinct groups of initials are present in the root apical meristem: a calyptrogen giving rise to the root cap; a tier of initials contributing to the developing protoderm; a group of initials giving rise to the cortex; and a set of initials for the vascular cylinder. These observations, however, need verification. Procambial differentiation and maturation result in a xylem strand that is crescent shaped (as seen in transverse section) and that partially surrounds a strand of phloem. Near the point of attachment to the rhizome a root may be polyarch (having several protoxylem poles) and maturation is exarch (Pixley, 1968). At this level the vascular cylinder (Fig. 9-8) may resemble that of the stem, and, except for size, it is sometimes difficult to distinguish between the two organs on the basis of stelar anatomy.

SPORANGIUM. One of the definitive characteristics of the Lycophyta is the association of one sporangium with each sporophyll; each sporangium is located on the adaxial side of a sporophyll or in its axil. In certain species of *Lycopodium* (for example, *L. lucidulum and L. selago*) the sporophylls are similar to vegetative leaves (Figs. 9-2; 9-6, A). No definite strobili are formed, but rather there are "fertile" areas on the stem alternating with vegetative or "sterile" regions. In species considered to be more specialized, the sporophylls are aggregated into definite conelike structures or strobili; the sporophylls of such cones may be unlike vegetative leaves in size, shape, and color and exhibit other specializations related to sporangial protection and spore dispersal. These strobili may occur on leafy stems or may be elevated on lateral branches with very small, scalelike leaves unlike those of the vegetative shoot (Figs. 9-3, B; 9-4, B, C).

Developmentally the sporangium is of the eusporangiate type originating from a group of superficial cells which divide periclinally (Fig. 9-9). The outer cells of such divisions form the multilayered wall, and the inner derivatives the sporogenous cells. The innermost layer of the sporangial wall functions as the tapetal layer.

Mature sporangia of most species are reniform (kidney shaped), their color ranges from yellow to orange, and they have a short stalk. There are some interesting relationships among position of mature sporangia, line of dehiscence, and specialization of the sporophyll. In certain species (e.g., *L. lucidulum*) the mature sporangium is axillary to a relatively unmodified sporophyll. Dehiscence is longitudinal or transverse to the long axis of the sporophyll (Fig. 9-6, B). In other species with definite strobili, the mature sporangia are foliar in position, and the sporophylls are imbricated and have abaxial extensions (Fig. 9-10, B). Dehiscence is modified, the opening being between the sporophyll and the abaxial extension of the sporophyll directly above. In still other species the sporangia are axillary, protected by sporophyll modifications, and open in a similar manner. Whether the sporangium is protected or not, the line of dehiscence

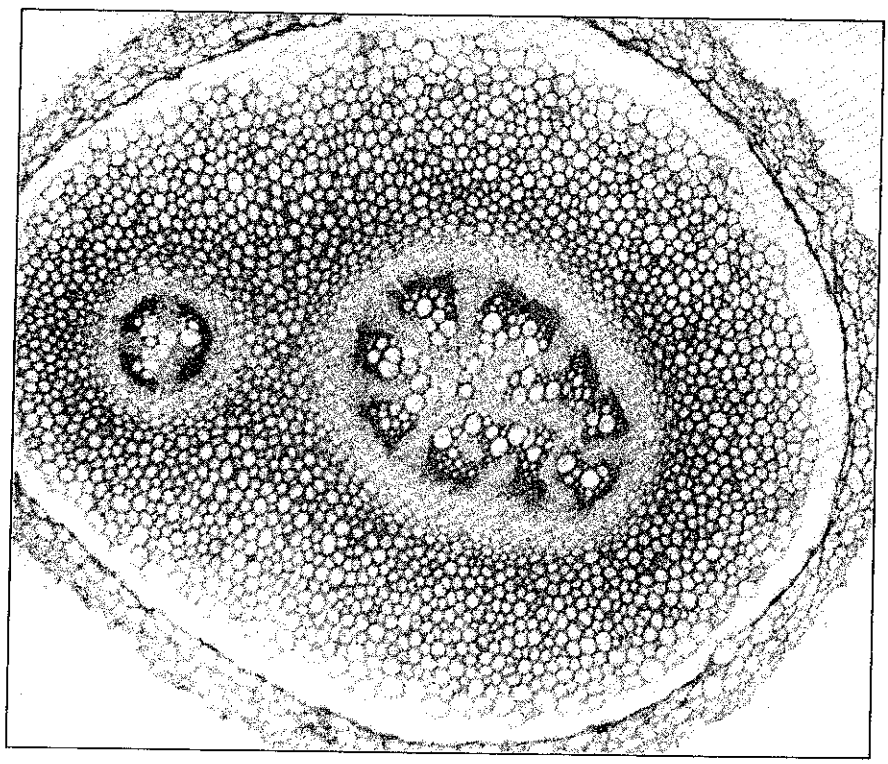


FIGURE 9-8 Transection of large root of *Lycopodium* sp. showing general similarity in organization of stele to that of a *Lycopodium* stem.

occurs in such a position as to insure efficient dispersal of spores (Sykes, 1908).

Meiosis occurs in the sporocytes, resulting in spore tetrads. The mature spores are yellow. The spore wall consists of an inner layer, called the intine, and an outer layer, the exine that displays an ornamentation that varies with the species. A triradiate ridge, present on the inner (proximal) face of each spore, is indicative of the mutual contact between members of a spore tetrad (Fig. 9-11, A, B). Spore morphology is useful in delimiting subgroups within the genus. (See Wilce, 1972, for illustrations of differences in spore wall ornamentation.)

The spores of certain species of *Lycopodium* are collected and sold as "lycopodium powder." This powder has been used in the manufacture of fireworks, but its use as a dusting powder on surgical gloves and pills has been discouraged; apparently the spores of *L. clavatum* cause inflammations in operative and other wounds (Whitebread, 1941).

THE GAMETOPHYTE. Depending on the species of *Lycopodium*, the spores may germinate immediately or after a delay of several years. A gametophyte plant of the first type *(L. cernuum, L. inundatum),* generally found on the surface of the substrate, is ovoid to axial-dorsiventral, with short green aerial branches; the entire plant may not be over 3 millimeters long (Fig. 9-12, B). Rhizoids occur on the colorless basal portion. An endophytic fungus, entering the gametophyte plant early in development, is present in most species, occupying a definite region within the gametophyte. The sex organs generally occur near the bases of the aerial lobes. The time interval between spore germination and appearance of sex organs may vary from eight months to one year (Treub, 1884; Chamberlain, 1917; Eames, 1942).

After spore germination and when 6 to 8 cells have been formed, gametophytes of the second type may enter into a rest period of a year or more.

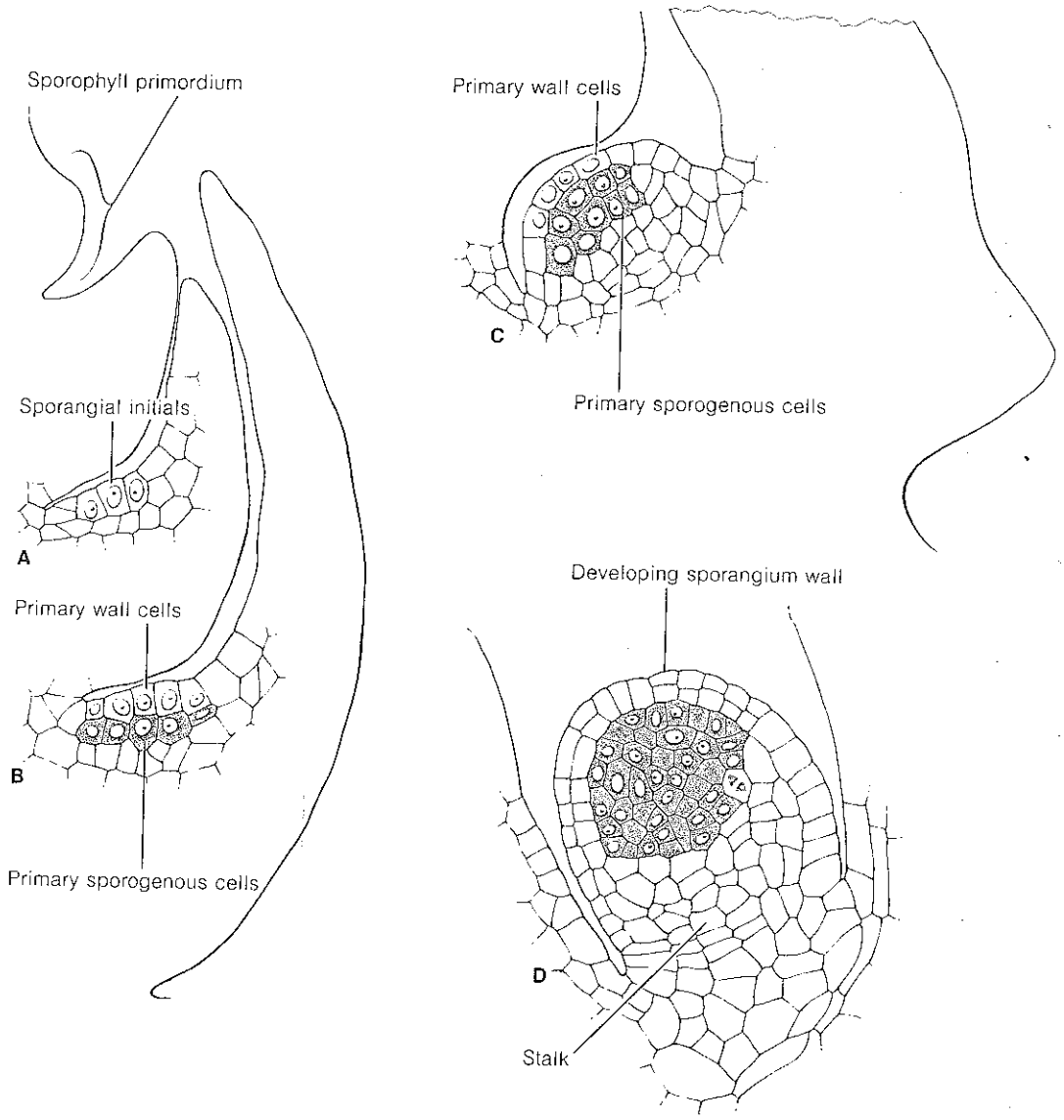
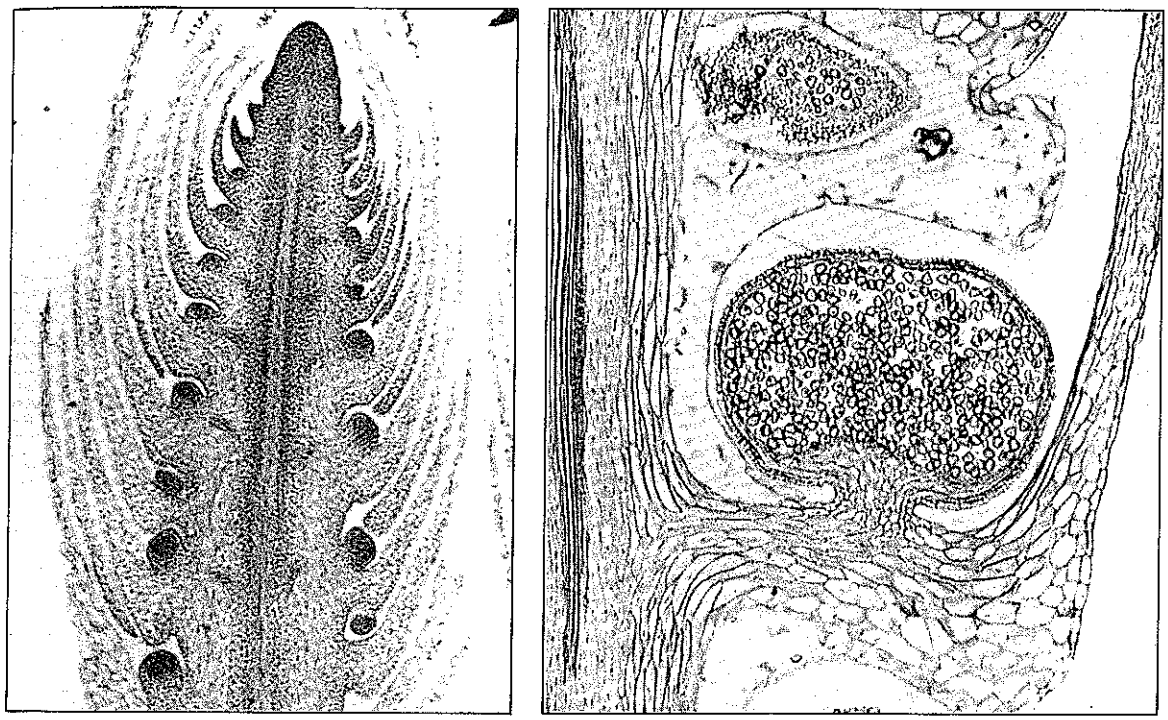



**FIGURE 9-9** Ontogeny of the sporangium in *Lycopodium clavatum*. Note that initiation of the sporangium takes place in superficial cells by periclinal divisions, setting aside primary wall and primary sporogenous cells (**A, B**). The tapetum ultimately arises from inner cells of the sporangium wall.

Apparently, further development is dependent on the entrance of a fungus. If this infection does not occur, all further growth ceases (Bruchmann, 1910). Physiologically, the fungus must supply certain substances vital for proper growth of the gametophyte plant. Subsequent development to a stage in which mature sex organs are present may require ten years or more (Eames, 1942). Development takes place beneath the surface of the ground or within a layer of humus. The gametophyte (e.g., *L. clavatum*) becomes disc shaped, with a convolute margin resembling a "walnut meat" (Fig. 9-12, A).



A B

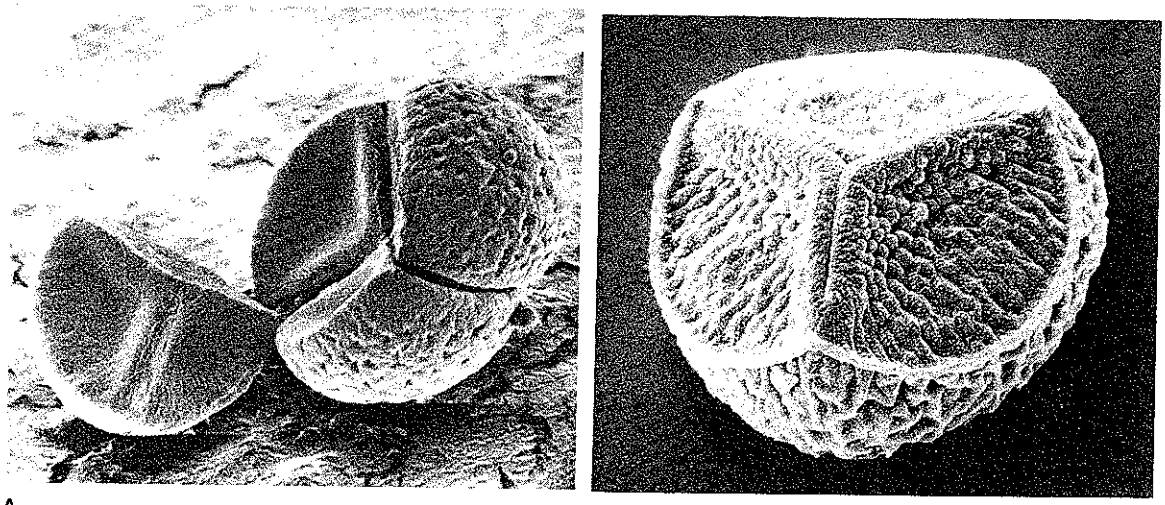
**FIGURE 9-10** **A,** longisection of entire young strobilus of *Lycopodium clavatum;* developing sporangia can be seen near the bases of sporophylls. **B,** a mature sporangium of *Lycopodium* sp. attached to sporophyll; note numerous spores and the overarching abaxial extension of the sporophyll above; the cone axis is to the left.

But in other species the gametophyte may be cylindrical and branched, or assume the shape of a tiny carrot (Fig. 9-12, C). All of the subterranean gametophytes are colorless or yellowish to brown, developing chlorophyll only in those portions that become exposed near the surface (Spessard, 1922).

The subterranean forms are long lived, increasing in size by a marginal ring of meristematic tissue. Old gametophytes may be up to 2 centimeters in length or width.

In species whose gametophytes are of the green, annual type, antheridia and archegonia are generally intermingled near the bases of the upright lobes, whereas in the subterranean forms the sex organs are segregated into definite groups (Fig. 9-13, A) except in certain species (Spessard, 1922). In the course of development, antheridia generally appear first near the middle of the crown of the gametophyte. Initiation of archegonia and more antheridia then occurs in the immediate derivatives of the meristematic ring (Fig. 9-13, A).

The dependence of *Lycopodium* species on the infection of the gametophyte by a fungus presents an interesting physiological problem. It has been possible to culture gametophytes, particularly the annual type, to maturity by sowing the spores on soil taken from the original habitat (see Koster, 1941). Wetmore and Morel (1951a) were able to culture to maturity, in the laboratory under sterile conditions, the gametophyte of *L. cernuum* (a green annual type that in nature is associated with a fungus). After the spore coat had been sterilized with calcium hypochlorite, the spores were sown on a culture solution containing minerals and glucose. In some cultures the upright green branches became club shaped, while in others a filamentous "pin-cushion" type resulted (Figs. 9-14; 9-15, C). After six months of continued growth, under regu-



A B

FIGURE 9-11 Scanning electron micrographs of *Lycopodium* spores. A, spore tetrad of *Lycopodium reflexum* (×1650). B, *Lycopodium inundatum;* note prominent triradiate ridge and contact faces with the other three spores (×1250). [Courtesy of Dr. G. Breckon.]

lated conditions, antheridia and archegonia were formed and many sporophytes developed (Fig. 9-14).

In 1957 Freeberg and Wetmore reported that they were able to germinate the spores of *Lycopodium selago* and *L. complanatum var. flabelliforme (L. digitatum).* Under natural conditions the gametophytes of both species are subterranean and long lived. However, under artificial cultural conditions the gametophytes were green and similar to those of *L. cernuum* discussed previously. On the basis of these results, some pteridologists expressed the belief that the gametophyte would be of little systematic value if the form of the gametophyte could be changed by simply altering the conditions for growth. Approximately 20 years later Bruce (1976a) made a detailed study of the sporophytes that developed on the gametophytes of all three species and concluded that all of the cultures were of *L. cernuum.* Apparently some spores of *L. cernuum* had been inadvertently introduced into the culture tubes, and they were the only spores that germinated. Pteridologists have now returned to the conviction that the form and physiology of the gametophyte can be of importance in the taxonomy and systematics of *Lycopodium.* In support of this belief Whittier (1977, 1981) was able to germinate spores *in vitro* and obtain mature gametophytes of *L. obscurum* and *L. digitatum.* Spores will germinate only if the culture tubes are placed in the dark for six or more months. The resulting gametophytes of *L. digitatum,* devoid of the endophytic fungus, are similar in form to those from nature. They are carrot shaped with a tapering base, and have a constricted neck below a cap like portion (Fig. 9-15, D). Although the endophytic fungus is not present, a layer of radially elongate cells is present that matches the region occupied by the endophytic fungus in nature. Gametophytes do turn green upon exposure to light after the required dark period, but the form remains the same.

The ontogeny of gametangia in *Lycopodium* has been described in detail in Chapter 5. A remarkable similarity in development exists in the early stages of ontogeny of the sex organs — namely initiation in a single superficial cell by a periclinal division, which sets aside the sterile jacket cell and the primary spermatogenous cell of the antheridium, and a division which forms the primary cover cell and the central cell of the young archegonium. The latter cell is the progenitor of the axial row (Fig. 9-13, B, C–E). At maturity an antheridium consists of a sterile jacket, one cell thick, enclosing many spermatids. Each one matures into a biflagellate sperm

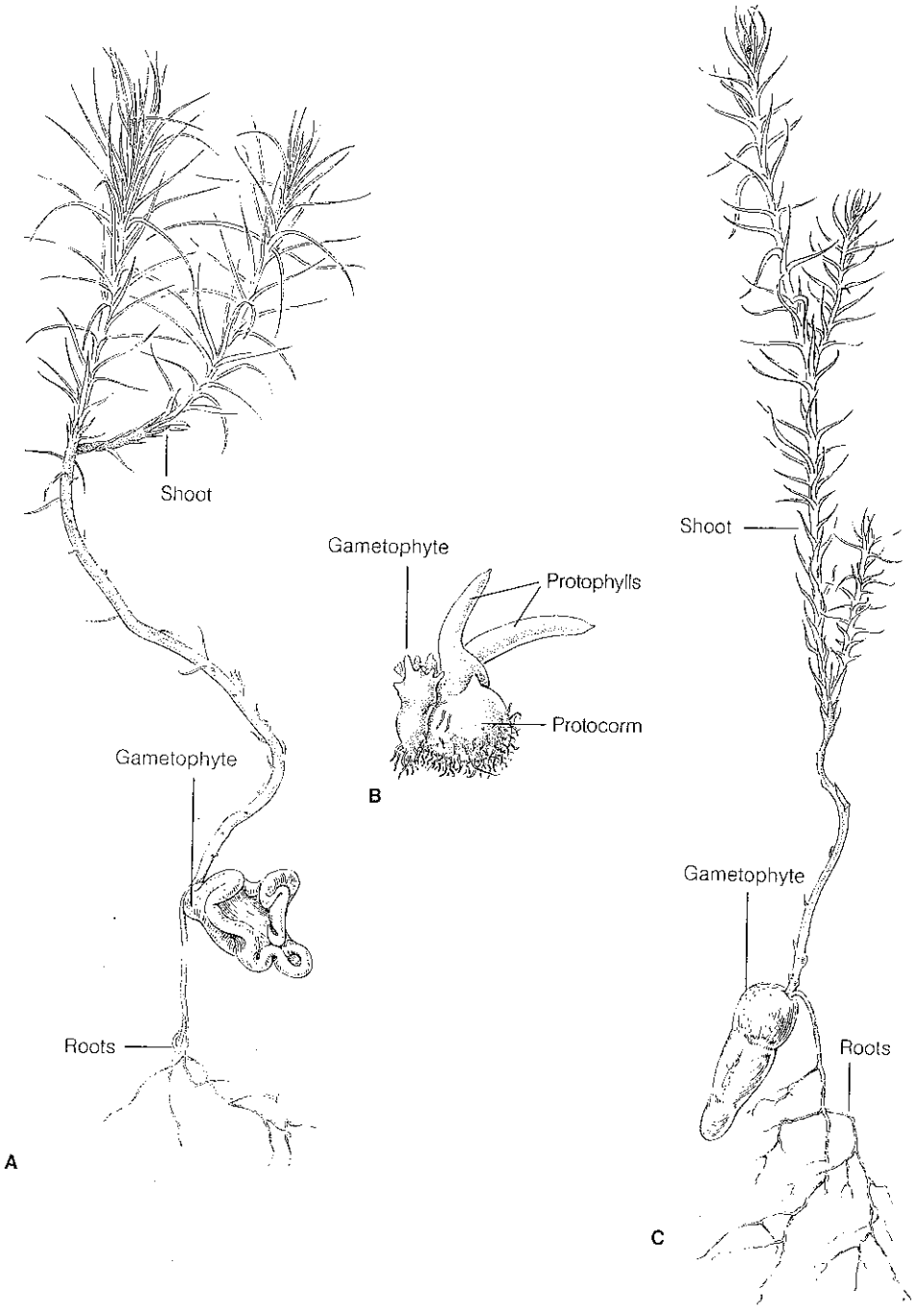



FIGURE 9-12 Gametophytes of *Lycopodium*. **A**, subterranean gametophyte of *Lycopodium clavatum*, with attached sporophyte; **B**, the subaerial or terrestrial type, *Lycopodium laterale;* **C**, subterranean type, *Lycopodium complanatum*. [**A** and **C** drawn from specimens supplied by Dr. A. J. Eames; **B** redrawn from Chamberlain, *Bot. Gaz.* 63:51, 1917.]

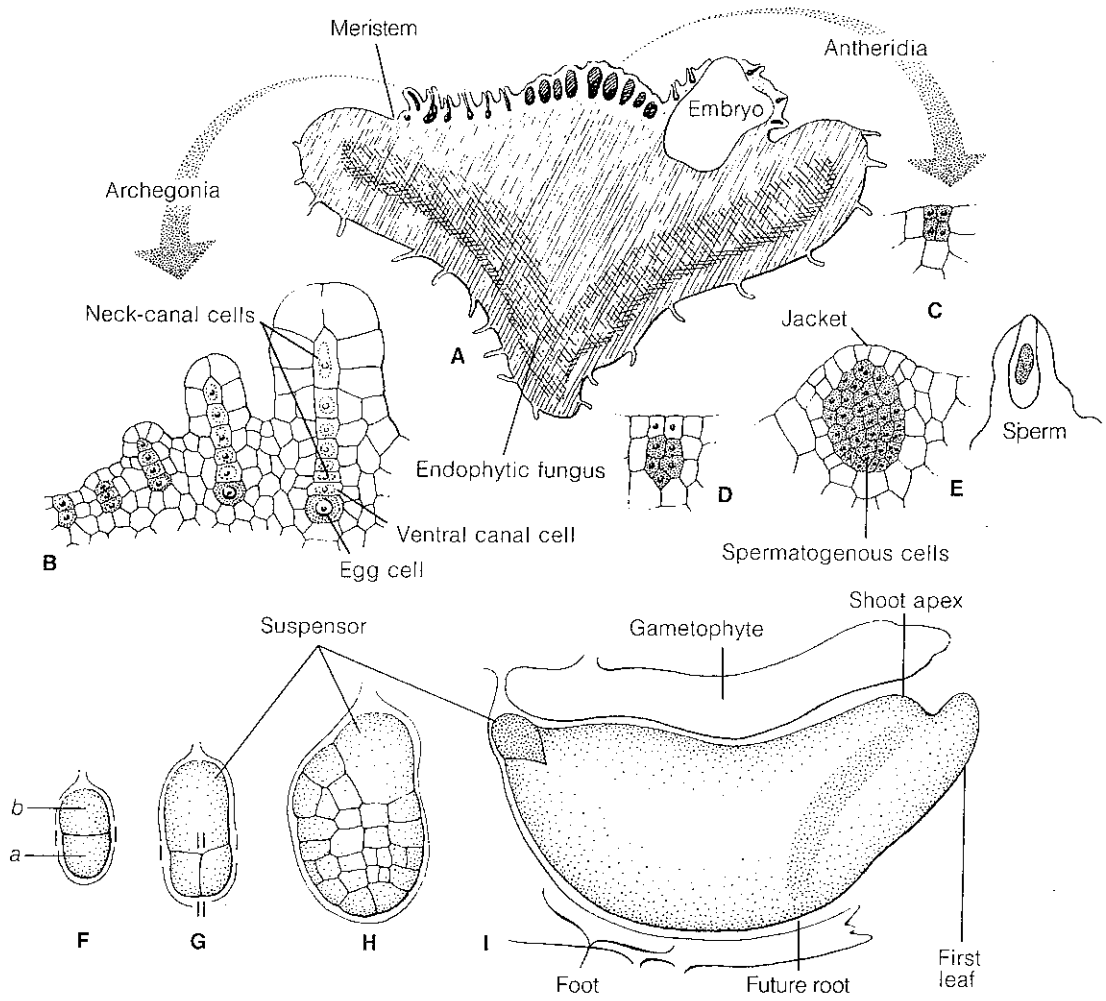



**FIGURE 9-13** **A,** longisection of the gametophyte of *Lycopodium clavatum* showing the position of antheridia and archegonia, and one embryo; **B,** stages in development of an archegonium, *Lycopodium selago;* **C-E,** stages in ontogeny of an antheridium, *Lycopodium clavatum;* **F-I,** development of the embryo, *Lycopodium selago* (mouth of the archegonium is directed toward the top of the page). (Consult text for details.) [**A** redrawn from *Syllabus der Pflanzenfamilien* by Engler and Gilg, Berlin: Gebrüder Borntraeger, 1924; **B, G, H, I** redrawn from Bruchmann, *Flora* 101:220, 1910; **C-E** adapted from *Morphology of Vascular Plants. Lower Groups* by A. J. Eames. McGraw-Hill, New York, 1936.]

which closely resembles the sperms of certain algae. A sperm is a blunt-ended, fusiform cell, 8 to 10 micrometers long by 4 to 5 micrometers wide. The two flagella, each about 38 micrometers long, trail behind the cell as it swims. In its ultrastructure the sperm has a large coiled mitochondrion closely associated with a multilayered structure with microtubules forming a supportive spline. A large amyloplast, containing starch grains, occurs at the posterior end. The basal body of each flagellum lies close to the multilayered structure (Robbins and Carothers, 1978; Fig, 9-16).

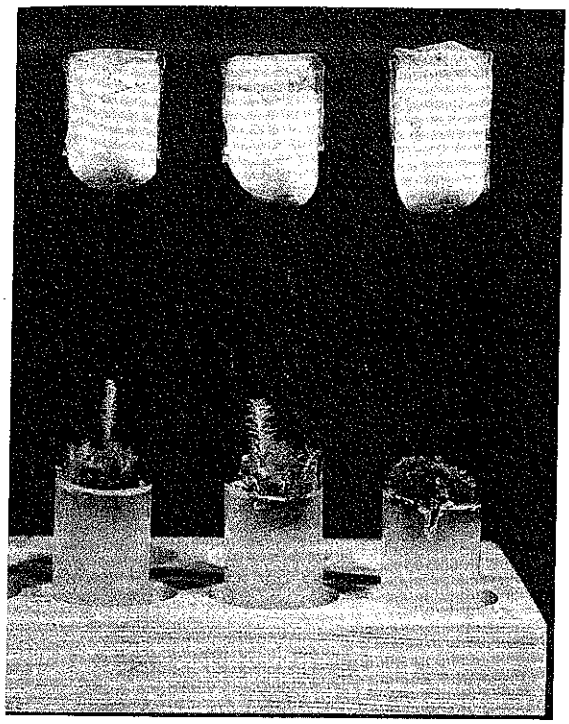


FIGURE 9-14 *Lycopodium cernuum.* In vitro cultures of gametophytes with young sporophytes attached, in two culture tubes at the left. [Courtesy of Dr. Ralph H. Wetmore.]

The archegonia of surface-living, green, short-lived gametophytes have only three or four tiers of neck cells (Treub, 1884) and usually one neck-canal cell, whereas, according to Spessard (1922), archegonia of the subterranean forms have long necks with six or more neck-canal cells (Fig. 9-13, B). In either type the venter is embedded in the gametophyte tissue. In certain forms a doubling of the axial row may occur (Spessard, 1922), and archegonia may be formed with exceedingly long necks.

With the degeneration of the neck-canal cells and the ventral canal cell a passageway is created for the entrance of the motile biflagellate sperm, which reach the archegonium by swimming through a film of water on the surface of the gametophyte. Free citric acid or salts of citric acid may play a role in the attraction of sperm to the archegonia (Bruchmann, 1909; Doyle, 1970).

Despite the fact that the gametophytes of *Lycopodium* are bisexual, the results of an electrophoretic study of enzymes from the sporophytes of several to many colonies of three species indicate that the rates of intragametophyte selfing (self-fertilization) are very low. The gametophytes of these three species predominantly cross fertilize. The mechanism(s) promoting cross fertilization are unknown (Soltis and Soltis, 1988).

**THE EMBRYO.** Embryogeny is correlated, to some degree, with the type of gametophyte, but closer examination reveals a common basic plan. To gain an understanding of embryogeny, we will begin with a species possessing an underground gametophyte (Fig. 9-13, F-I). The embryo in *Lycopodium* is endoscopic, that is, the future shoot apex is directed away from the mouth of the archegonium. The first division of the zygote is transverse to the long axis of the archegonium, setting aside an apex, cell *a*, and a base, cell *b* (Fig. 9-13, F). Cell *b* undergoes no further divisions and becomes a suspensor. In our example, cell *a* then divides at a right angle to the original wall (Fig. 9-13, G, wall II–II). Additional divisions produce a multicellular embryo (Fig. 9-13, H). At about this developmental stage the future shoot apex grows laterally and upward, and a foot develops along the lower side of the embryo. The root is variable in position, but commonly arises between the first leaf and foot. With continued growth, the shoot tip emerges from the gametophytic tissue Fig. 9-13, I). The foot enlarges and maintains close connection with the gametophyte, acting as an haustorial structure until the sporophyte becomes physiologically independent. Sexually mature gametophytes may continue to live for some time, supporting one or more young sporophytes in various stages of development.

In certain species that have green, surface-living gametophytes, a foot is formed as well as a spherical parenchymatous body, termed a protocorm. No roots are produced on the protocorm, but leaflike structures—protophylls—arise on the upper surface, and rhizoids occur on the lower surface (Fig. 9-12, B). Only later does a shoot apical meristem become organized in cells of the protocorm, and a "normal" type of shoot is produced. In *L. carolinianum* a foot is formed but the protocorm stage is

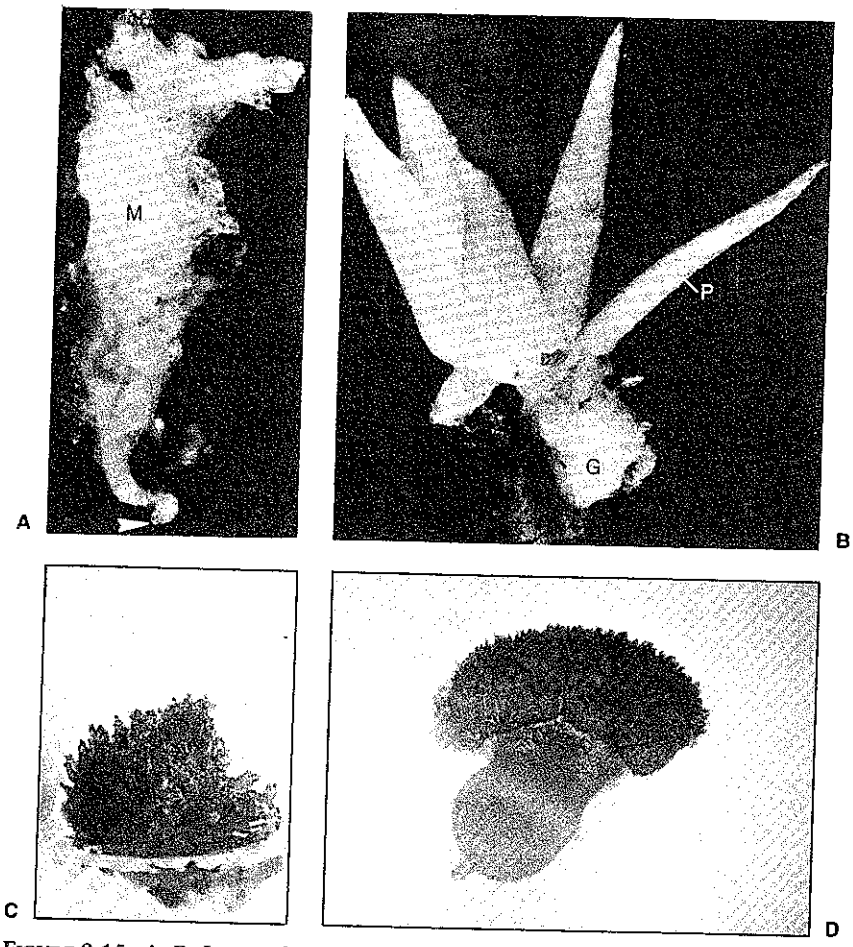



**FIGURE 9-15** **A, B,** *Lycopodium carolinianum.* **A,** gametophyte showing position of meristem (M) and spore (white arrow; ×40). **B,** gametophyte (G) with attached sporophyte showing protophyll (P) and four leaves (×20). **C, D,** gametophytes grown in sterile culture. **C,** *Lycopodium cernuum;* gametophyte is green under natural conditions and in artificial culture (×1.2). **D,** *Lycopodium digitatum;* gametophyte is subterranean in nature, but becomes green in culture when exposed to light (×7.8). [**A, B** from Bruce, *Amer. Jour. Bot.* 66:1156–1163, 1979; C courtesy of Dr. J. A. Freeberg; D courtesy of Dr. Dean P. Whittier.]

absent. The emerging sporophyte forms a single protophyll (Fig. 9-15, A, B) and a rhizome apex (Bruce, 1979).

## Generic and Subgeneric Concepts

As mentioned previously there have been recommendations over the years to establish subgenera or genera of *Lycopodium.* One early suggestion was to recognize two subgenera—one, *Urostachya,* in which well defined strobili are not formed (e.g., *L. lucidulum, L. selago*) and branching is essentially isotomous. The second subgenus, *Rhopalostachya,* includes species that have definite cones and branching is anisotomous (e.g., *L. digitatum, L. clavatum*). In another system two families are recognized—the Urostachyaceae with one genus and the Lycopodiaceae with three genera. All four genera are said to have different basic chromosome numbers (Löve and Löve, 1958), and the chemistry of their flavones (Voirin and Jay, 1978), phenolics, and lignins differ (Towers and Maass, 1965).

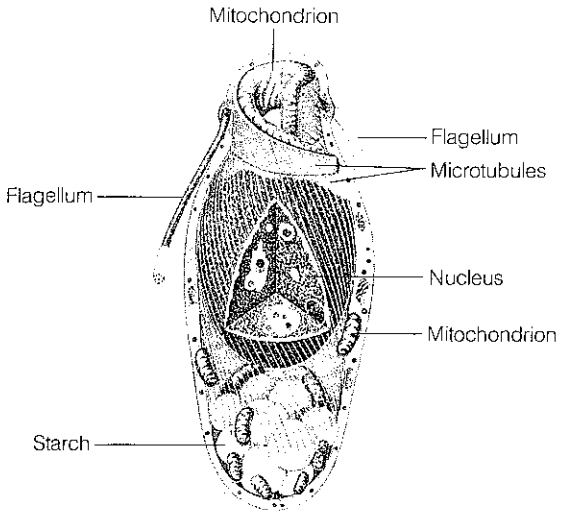



**FIGURE 9-16** Perspective diagram of a mature sperm of *Lycopodium cernuum*, with wedge of nucleus removed to show internal organization (approx. ×7,000). [Courtesy of Dr. Robert R. Robbins.]

Other pteridologists believe that the one genus *Lycopodium* (the species of which are easily recognized) should be retained until more information is available on aspects such as gametophytes, life histories, and chromosome numbers (Hauke, 1969). In recent years more information has become available on the gametophyte generation (see p. 115). Chromosome counts of a limited number of species on a worldwide basis range from $n = 23$ to $n = 264$ (Löve et al., 1977). These counts indicate a history of hybridity and possibly polyploidy, although few multiple series (replications of entire chromosome sets) are evident.

Space does not permit a review of all proposed subgenera, but one system of classification, applied more specifically to North American species, will be presented. The classification is based upon the totality of morphological characteristics and chromosome numbers (Beitel, 1979; Wagner and Wagner, 1980; Beitel and Wagner, 1982).

## Subgenera

*Huperzia* (e.g., *H. selago, H. lucidulum;* (Fig. 9-3, A). Little distinction between rhizome and upright branch system as a result of isotomous branching; leaves spirally arranged; sporophylls unmodified or slightly modified, arranged in zones on the stem or in pendant tassels; gametophytes rod shaped, branched or unbranched, subterranean or buried in moss or humus on trees. A known chromosome number of $n = 67$ may be indicative of an aneuploid series based upon multiples of the theoretical base number $x = 11$ plus one chromosome $[(6 \times 11) + 1]$.

*Lycopodium* [(in limited sense) e.g., *L. clavatum, L. obscurum;* Fig. 9-4, C]. Anisotomous branching; prostrate rhizome and upright terminal branches; leaves spirally arranged; sporophylls aggregated into definite strobili; gametophytes disc shaped and subterranean; chromosome base number of $n = (3 \times 11) + 1 = 34$.

*Diphasiastrum* (e.g., *L. complanatum, L. digitatum;* Fig. 9-3, B). Anisotomous branching; creeping rhizome and upright evergreen branches; majority have stems with four rows of leaves, fused with stem most of their length, forming flattened branches; definite strobili; gametophytes subterranean, cone shaped like tiny carrots; chromosome base number $n = (2 \times 11) + 1 = 23$.

*Lycopodiella* (e.g., *L. inundatum, L. carolinianum, L. cernuum;* Fig. 9-4, A). Considerable variation in growth habit from an erect, much-branched plant to creeping rhizomes with upright fertile branches that bear spirally arranged leaves; only the swollen rhizome tips in some species remain alive at the end of the growing season; gametophytes, where known, resemble tiny, green pincushions or are axial-dorsiventral with upright green lobes; chromosome base number $n = (7 \times 11) + 1 = 78$ appears to be representative, although there is considerable variation in the group, especially for *L. cernuum* and *L. carolinianum* (Löve et al., 1977; Bruce, 1976b).

One note of caution should be cited with respect to high chromosome numbers in *Lycopodium* (sensu lato) and the role that possible repeated episodes of polyploidy played in evolution. Soltis and Soltis (1988) pointed out that if lycopod species with high chromosome numbers are truly highly polyploid, they should possess many isozymes (different forms of an enzyme encoded by different gene loci). They found in their study of eight species (in three subgenera) that the number of isozymes present was typical of diploid seed plants for all but one enzyme. They conclude that there is no genetic

evidence for widespread polyploidy in lycopods and no evidence to support the low, theoretical base numbers suggested for these plants, e.g., $x = 11, 12$. They favor the concept that the ancient ancestors of lycopods and other homosporous pteridophytes initially had high chromosome numbers.

## Some Herbaceous Devonian Lycopods

A plant with definite lycopod characteristics is *Baragwanathia* from the Lower Devonian of Australia (Jaeger, 1962). It has been reported also from the Silurian, however this record needs verification. The plant branched dichotomously, and the stem was covered with helically arranged leaves, 0.5 to 1 millimeter wide and as long as 4 centimeters. Sporangia are associated with some leaves, but it is unknown (because of poor preservation) whether they were on the adaxial side of leaves or near the axils of leaves.

The genus *Protolepidodendron* from the Lower to Middle Devonian had leaves that forked near the tip. The plant was rhizomatous with upright dichotomous branches bearing helically arranged leaves, some of which had solitary sporangia located on the adaxial side of leaves (Fig. 9-17, A). Similar types of sporophylls have been reported for the new genus *Estinnophyton;* this genus, however, had one pair or two pairs of short, stalked sporangia on the adaxial side of the forked sporophylls (see Gensel and Andrews, 1984).

Another lycopod from throughout the Devonian is *Drepanophycus* (Fig. 9-17, C). As reconstructed, it had a rhizome with upright dichotomous branches with stiff, curved leaves. Stalked sporangia in some forms occurred singly on some leaves midway between the enlarged leaf base and tip (Grierson and Hueber, 1968).

The Lower Devonian genus *Asteroxylon* was formerly classified with the "psilophytes" because it was assumed to have had terminal sporangia. It was transferred to the Lycophyta because vascularized, stalked sporangia were borne laterally on stems, interspersed among leaves (Lyon, 1964). *Asteroxylon mackiei* grew to a height of 0.5 meters. The plant had a naked, dichotomously branched subterranean rhizome. Small branch systems probably functioned in anchorage and absorption. The tips of some dichotomies grew upright, bearing numerous, closely appressed, small leaves. The main upright axes exhibited irregular or anisotomous branching, whereas smaller side-branch systems displayed more regular dichotomous growth (Fig. 9-17, B).

The stem of *Asteroxylon* had an epidermis with thick outer walls, which was interrupted in places by stomata. The cortex was differentiated into outer, middle, and inner portions (Fig. 9-18). Occupying the central region of the stem was the vascular cylinder, which may be designated as an actinostele (Chapter 3). Primary xylem in the form of a fluted cylinder occupied the center of the stem. Protoxylem occurred near the extremities of the lobes but was surrounded on all sides by metaxylem, making the xylem mesarch in development. "Leaf" traces, departing from the vicinity of the lobes, passed obliquely through the cortex and ended abruptly near the base of each leaflike appendage. These traces were concentric; that is, each trace consisted of primary xylem surrounded by primary phloem. In one species, *Asteroxylon elberfeldense,* the vascular cylinder was siphonostelic in lower portions of the aerial stem.

*Leclercqia,* a recently described lycopod from Middle Devonian rocks, is the best preserved and most completely known herbaceous lycopod (Banks et al., 1972). Its leaves were 4.0 to 6.5 millimeters long and were five partite: an elongate tip and two lateral portions each of which was forked. Tracheids have been identified in the midvein. A branch vein extended to the base of each lateral division but did not enter the two segments. The stem had an exarch protostele with 14 to 18 protoxylem poles. There was one adaxial sporangium on each sporophyll and the plant was probably homosporous. In 1979, Grierson and Bonamo discovered the presence of a ligule on vegetative leaves and sporophylls. This discovery constitutes the first record of the ligule in a pre-Carboniferous, herbaceous, homosporous lycopod. It has long been thought there is a strict correlation between the ligulate condition and heterospory as characterized by the Carboniferous Lepidodendrales and the present day genera *Selaginella* and *Isoetes.* No doubt *Leclercqia* will be brought into future discussions on the phylogenetic interrelationships of Devonian and Carboniferous lycopods (see Stewart, 1983).

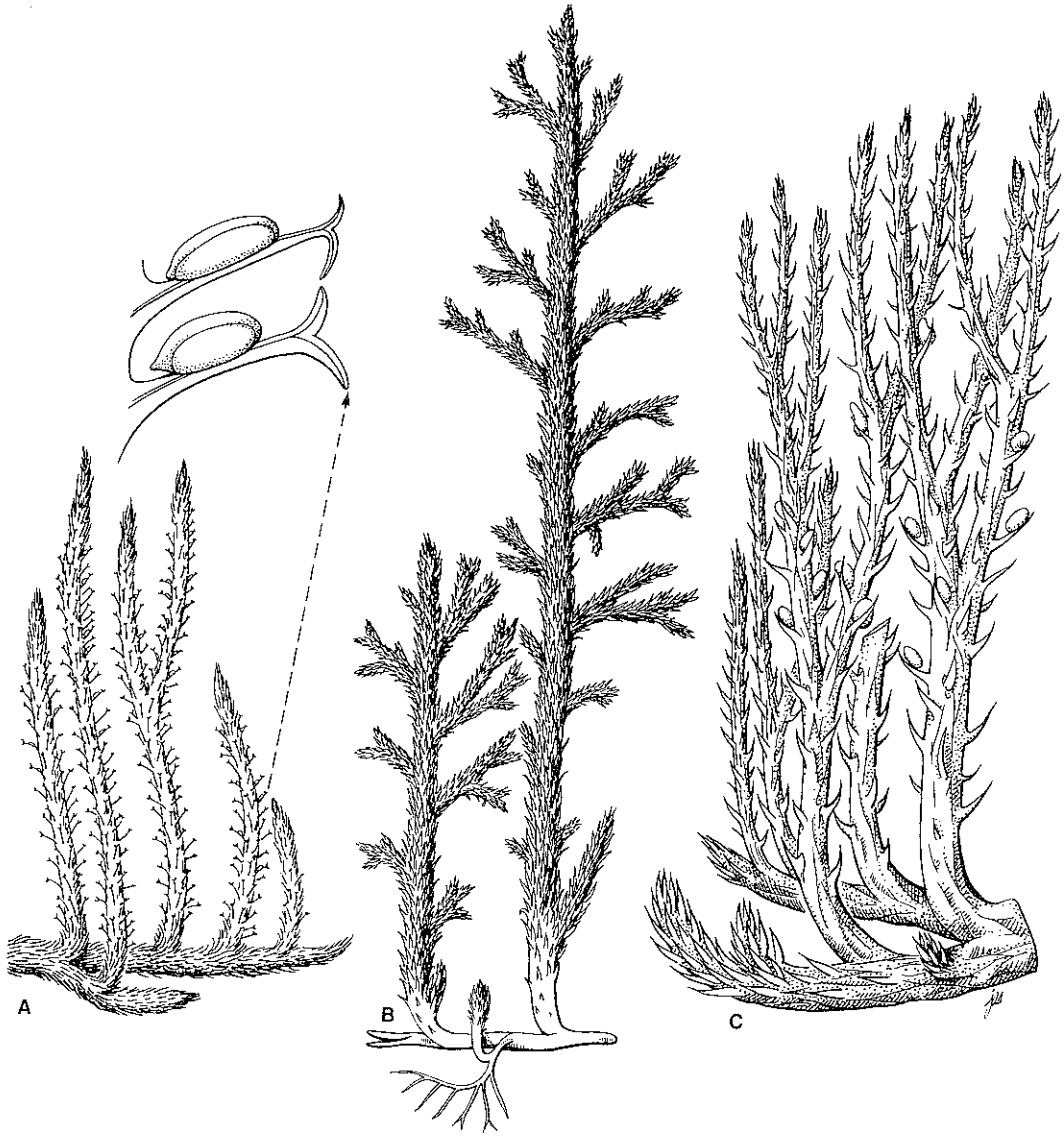



FIGURE 9-17 Diagrammatic reconstructions of extinct lycopods from the Devonian Period. A, *Protolepidodendron scharyanum;* sporophylls and sporangia enlarged above; B, *Asteroxylon mackiei;* C, *Drepanophycus spinaeformis.* [A redrawn from Kräusel and Weyland, *Senckenbergiana* 14:391–403, 1935; B redrawn from Kidston and Lang, *Trans. Roy Soc. Edinb.* 52, Part IV, 1921; C redrawn from Kräusel and Weyland, *Palaeontographica* 80(B):171–190, 1935.]

Some of the Devonian lycopods just described were probably ancestral forms in the line leading to *Lycopodites* (Middle Devonian-Carboniferous species) and then to the extant genus *Lycopodium.* The Devonian types were, in turn, undoubtedly derived from members of the Zosterophyllophyta — one of the divisions of early vascular plants (Chapter 7).

## Heterosporous Groups in the Lycophyta

### Selaginellales — Selaginellaceae: *Selaginella*

GENERAL CHARACTERISTICS. The genus *Selaginella,* the small club moss or spike moss, is widely

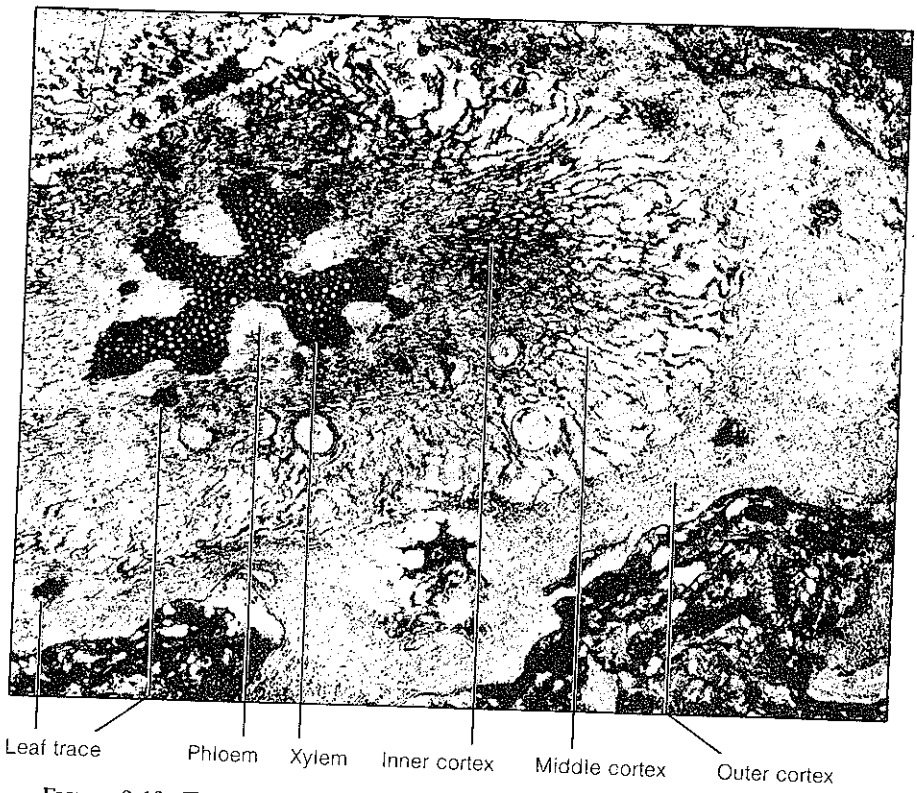



FIGURE 9-18 Transection, stem of *Asteroxylon mackiei*. [From Kidston and Lang, *Trans. Roy. Soc. Edinb.*, Vol. 52, 1920–21.]

distributed over the earth. But even though the genus includes about 700 species, it does not form a conspicuous part of the world's vegetation. Whereas many species of *Lycopodium* may be relatively large and coarse, most species of *Selaginella* are small and delicate. It is in the tropics that *Selaginella* is most abundantly represented, often being the dominant element of the forest floor in mesophytic tropical woodlands (Haught, 1960). Some species grow where climates are cold, and many others inhabit temperate regions, growing in damp areas or even occupying exposed rocky ledges. The genus is especially well represented on the eastern slopes of the Andes from Colombia to Bolivia (Tryon and Tryon, 1982). One species, *Selaginella lepidophylla*, caespitose in habit, has adapted to existence on a Mexican desert and in the arid regions of southwestern United States. The entire plant forms a tight ball during periods of drought; in the presence of moisture the branches expand and lie flat on the ground. This species is commonly known as the "resurrection plant." *Selaginella* is a greenhouse favorite, and is often used as a border plant. Species of this genus growing together in a greenhouse present an array of color shades — dark to light green, bluish — and some are iridescent.

**GROWTH FORM.** In growth habit there is considerable variation, although most species can be referred to two or perhaps three growth types. Some species are erect or often form tufts or mounds (Fig. 9-19, A). The leaves are helically arranged and are of the same size and shape (isophylly). In other species the plant may be flat, creeping along the surface of the ground or scrambling over shrubs (Fig. 9-19, B). Still others have a strongly developed rhizomatous stem with large, frondlike side branches which stand erect (Fig. 9-20, C). In the last two types anisophylly (the production of small and large leaves) is a prominent feature and the stems have dorsiventral symmetry (Fig. 9-21). Branching generally is considered to occur by a more or less equal bifurcation of the shoot apex in the establishment of two shoot axes, one of which overtops the other, resulting in

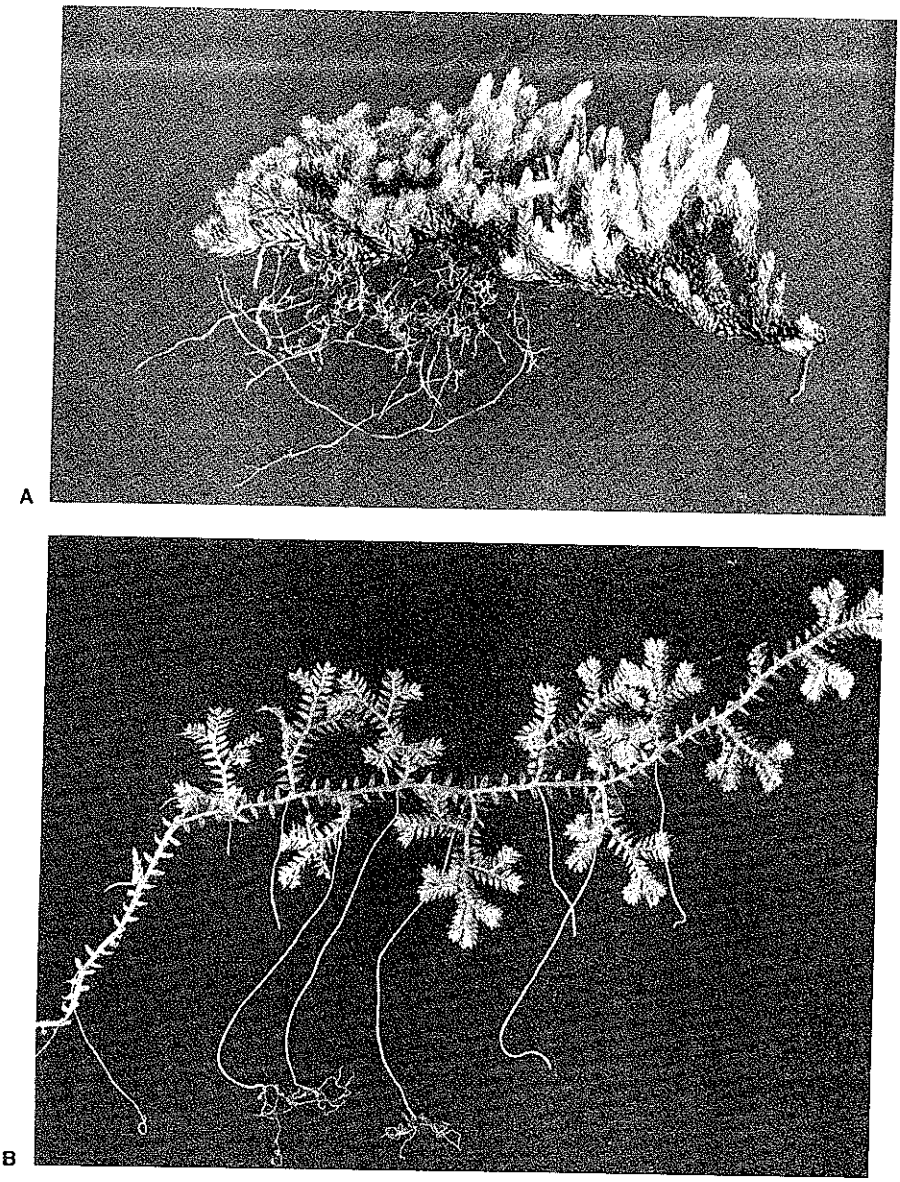
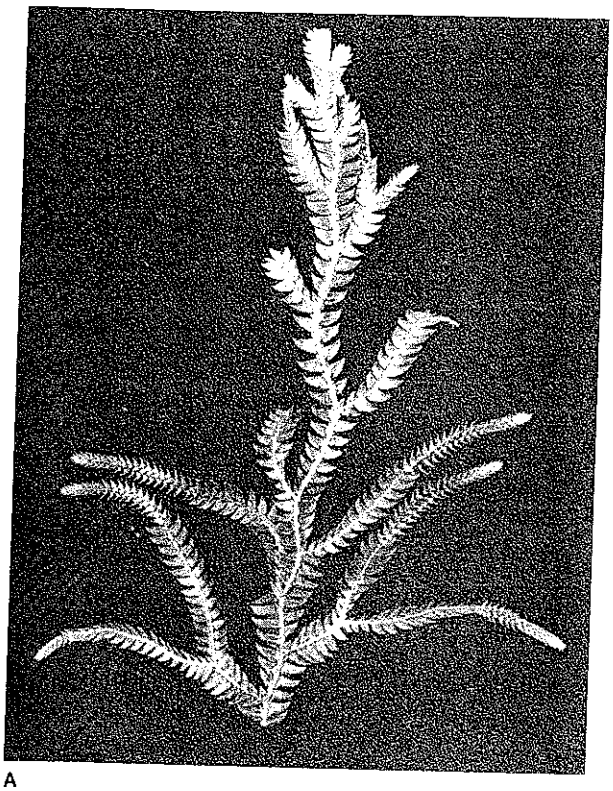



FIGURE 9-19 Two species of *Selaginella* showing contrasting growth forms. **A,** *Selaginella watsoni,* ascending branches with leaves of uniform size (isophylly). **B,** *Selaginella kraussiana,* a creeping or scrambling type; note that roots arise at points of branching. [B courtesy of Susan Larson.]

anisotomous branching. However, Hagemann (1980) has shown that a new branch in *Selaginella speciosa* arises in a lateral position on the dome-shaped apical meristem. Both the original axis as well as the new branch continue to grow. The branching pattern resembles the anisotomous type.

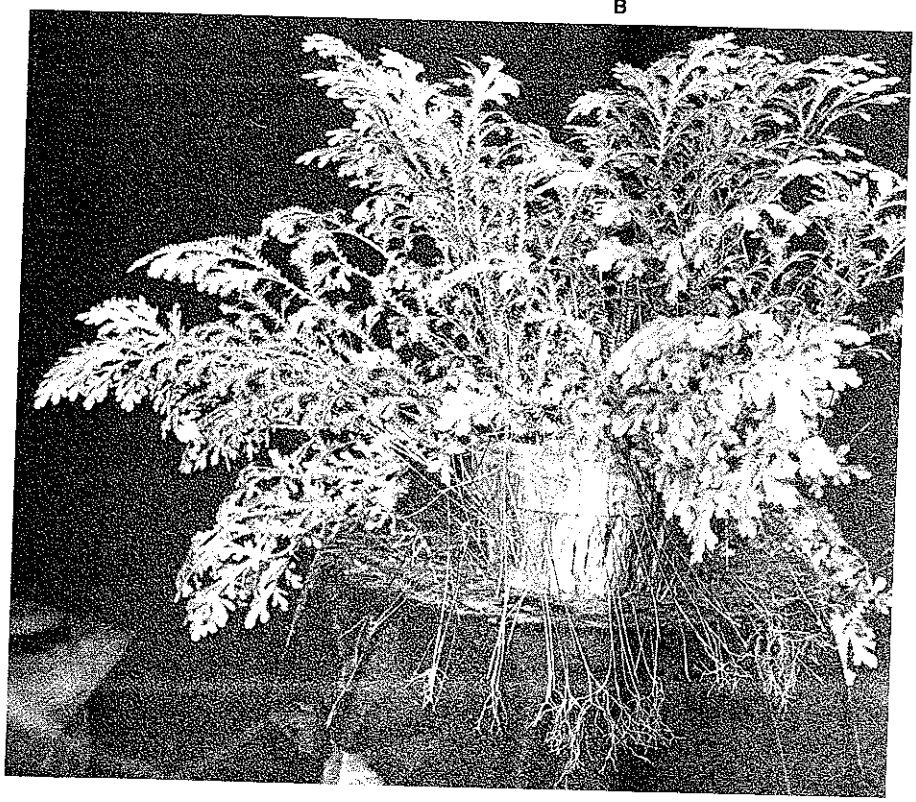
Peculiar leafless, proplike structures, originating from the stem at points of branching, have been termed "rhizophores," (see Figs. 9-19, B; 9-20, C; also Fig. 9-22, B); more will be said of their morphological interpretation in a later section of this chapter.

STEM ANATOMY. The outer cell walls of the epidermis are cutinized. Stomata are said to be lacking. In many species there are several layers of thick-



A

B



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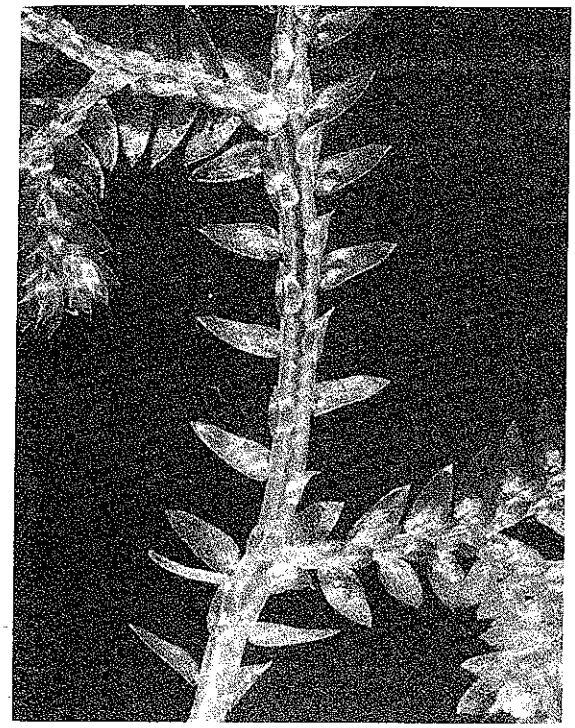


FIGURE 9-21 *Selaginella kraussiana,* showing two rows of small dorsal leaves and two rows of larger ventral leaves (anisophylly). [Courtesy of Susan Larson.]

walled cells beneath the epidermis, which merge gradually with thin-walled chlorophyllous cells of the inner cortex. In most species the trailing stem or prostrate rhizome is protostelic. Plants with a radial symmetry may have a simple, cylindrical protostele in the stem, whereas dorsiventral species may have two or more vascular strands that are either circular or ribbon-shaped as seen in transverse section (Fig. 9-22, A). The ribbon-shaped protostele in the rhizome (Fig. 9-22, D) may be replaced in the upright branches by a number of vascular bundles (meristeles). It should be emphasized that the vascular system of a shoot is interconnected and the term meristele is used only for convenience in describing a portion of the vascular system as seen in transverse section. An even more complex stelar pattern has been described for the tropical species *Selaginella exalta*. In this species the stele of the very large erect stem is a three-lobed plectostele, termed an "actinoplectostele" (Mickel and Hellwig, 1969).

In still other species the rhizome may be solenostelic, and the upright branches may have as many as 10 to 15 separate meristeles. Experimentally it has been shown that if such an upright branch is placed in a horizontal position, the newly developed portion of the shoot will become solenostelic (Wardlaw, 1924). Whatever the stelar configuration, the one central vascular cylinder or each meristele is supported in a large air-space system by radially elongated endodermal cells designated *trabeculae* (Figs. 9-22, A; 9-23; 9-24). These cells have the characteristic casparian strips. If the air-space system is large, each support or trabecula may consist of several cortical cells as well as the endodermal cell.

Regardless of stelar organization, the primary xylem is exarch in development, and the metaxylem consists primarily of tracheids with scalariform pitting. Ribbon-shaped steles may have more than one protoxylem pole (Zamora, 1958). Many years ago (Duerden, 1934) certain species were shown to possess vessels. The phloem of *Selaginella* consists of sieve cells and parenchyma. The end walls of the sieve cells are usually slightly oblique and scattered single "sieve pores" occur on the lateral and end walls rather than having typical sieve areas, i.e., groups of pores clustered in thin-walled areas (Lamoureux, 1961). Each mature sieve cell contains a degenerated nucleus and "refractive spherules" which are probably plastids (Burr and Evert, 1973).

**LEAF ANATOMY.** Leaves of all species are small, attaining a length of a few millimeters. In form the leaves may be ovate, lanceolate, or orbicular with one vein running nearly the length of the leaf. Exceptions to this condition have been reported for two species that have a branched venation system similar to megaphylls (Wagner, et al., 1982). Although helical arrangement is a common feature in *Lycopodium,* most species of *Selaginella* have leaves that are arranged in four rows along the stem (Fig. 9-21). There are two rows of small leaves on the

FIGURE 9-20 **A, B,** branches of two species of *Selaginella* showing strobili at tips of determinate branches. **C,** *Selaginella martensii,* showing large ascending or erect branches and numerous proplike roots.

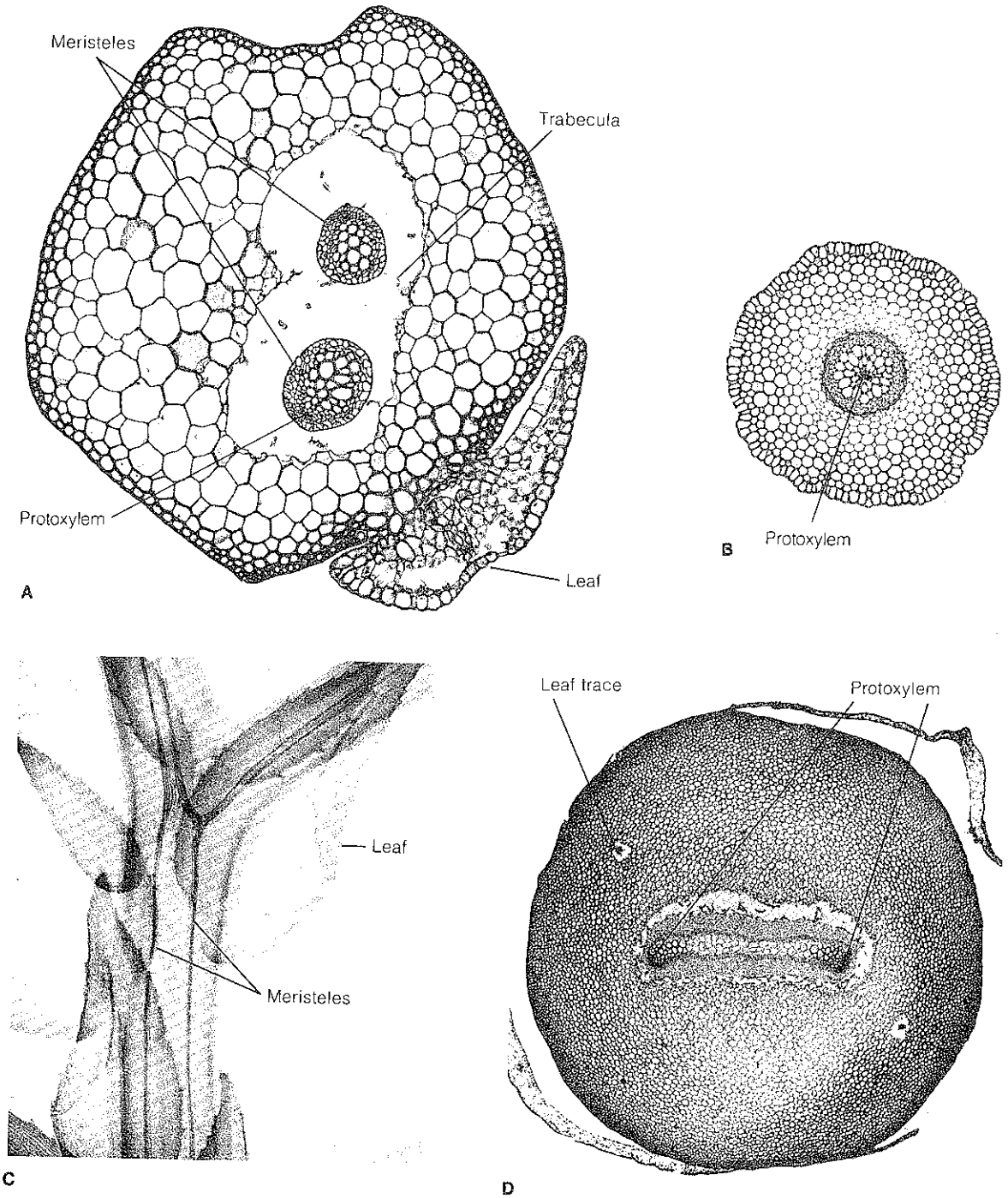



FIGURE 9-22 A, transection of stem of *Selaginella kraussiana;* only a few trabeculae appear in a transection of the stem; B, transection, rhizophore of *Selaginella* sp.; C, "cleared" and stained shoot of *Selaginella kraussiana* showing course of the vascular strands (meristeles) at a dichotomy of the stem; D, transection, stem of *Selaginella pallescens* near the base of a large branch.

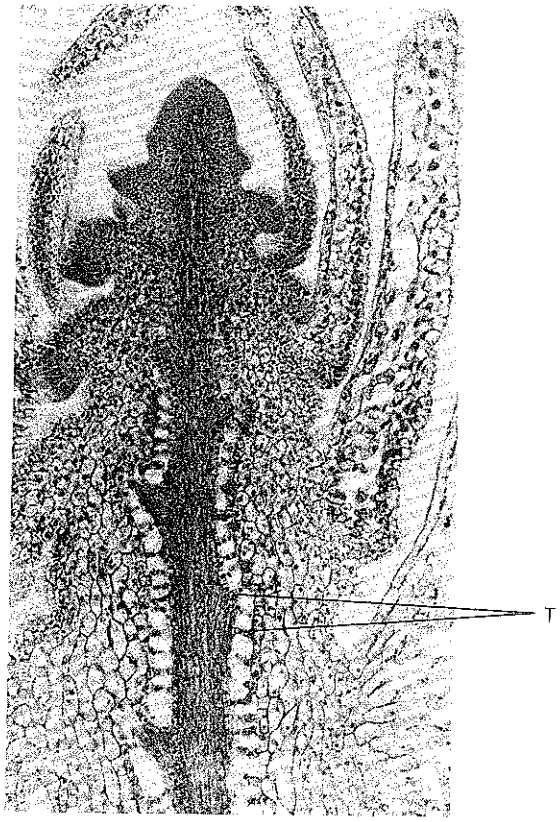



FIGURE 9-23 Longitudinal section of *Selaginella* sp. stem showing development of numerous trabeculae supporting the vascular cylinder in a large air-space system. T, trabeculae.

dorsal side of the stem, and two rows of larger leaves on the ventral side or in a lateral position. A small tonguelike structure, the ligule, is on the adaxial side of each leaf near the base. Anatomically the mature leaf may vary considerably. The cells of the two epidermal layers may be similar, or in some species they may be somewhat different (Hsü, 1937). Some species have bristles or short hairs extending out from the epidermis. The mesophyll may consist of a distinct palisade layer and spongy parenchyma, or the entire mesophyll may be a reticulum of lacunate parenchyma. Generally stomata are on the abaxial surface, although in certain species they are present on both surfaces.

In certain investigated species the shoot is terminated by an apical cell (Barclay, 1931; Hsü, 1937). The apical cell is tetrahedral and appears as an inverted pyramid in longitudinal sections of the shoot apex (Fig. 9-24). Through divisions of this apical cell, derivative cells are produced on the three "cutting" surfaces. Each of these cells (segments) undergoes a periclinal division, forming an outer and an inner cell. The outer cell, by further divisions, will produce the epidermis and cortex. Endodermis, pericycle, and vascular tissues are derived ultimately from derivatives of the inner cell (Fig. 9-24). Certain other species are reported to have one three-sided cell (Hagemann, 1980), or two adjoining apical initial cells at the shoot tip (Williams, 1931), or a group of apical initials (Bhambi and Puri, 1963).

Leaves have their origin in superficial cells located along the flanks of the apical meristem. A developmental study was made of anisophylly in *S. martensii* to determine if the smaller dorsal leaf is only an arrested form of the larger ventral leaf. Each pair of leaves—dorsal and ventral—originate at about the same time from the apical meristem, but the ventral leaf primordium is larger at the time of inception. The two can be distinguished when the leaves are only 0.1 millimeter in length. The subsequent pattern of histogenesis of both types of leaf is similar, but the smaller dorsal leaf is distinguished primarily by precocious maturation of tissues (Dengler, 1983a, b).

Early in its development a leaf is traversed by a procambial strand which is continuous with the vascular cylinder of the stem. Procambial cells eventually differentiate into primary xylem and primary phloem of the leaf vascular bundle.

A ligule (from Latin, *ligula,* "a small tongue"), located on the adaxial side of each vegetative leaf and sporophyll, makes its appearance through periclinal divisions in two or more short rows of surficial cells (Fig. 9-28). At maturity, a ligule is a surprisingly complex structure, considering its small size and short life. The ligule may be to some extent sunken in the leaf and has a basal sheath of cells with casparian strips and an adjacent group of large, vacuolated cells termed a *glossopodium* (from the Greek words *glossa,* meaning "tongue", and *podion,* meaning "foot"). The sheath cells resemble endodermal cells and may well perform a regulatory role in the movement of water and dissolved substances. The frequent development of tracheidlike cells (transfusion tissue) between the ligular sheath

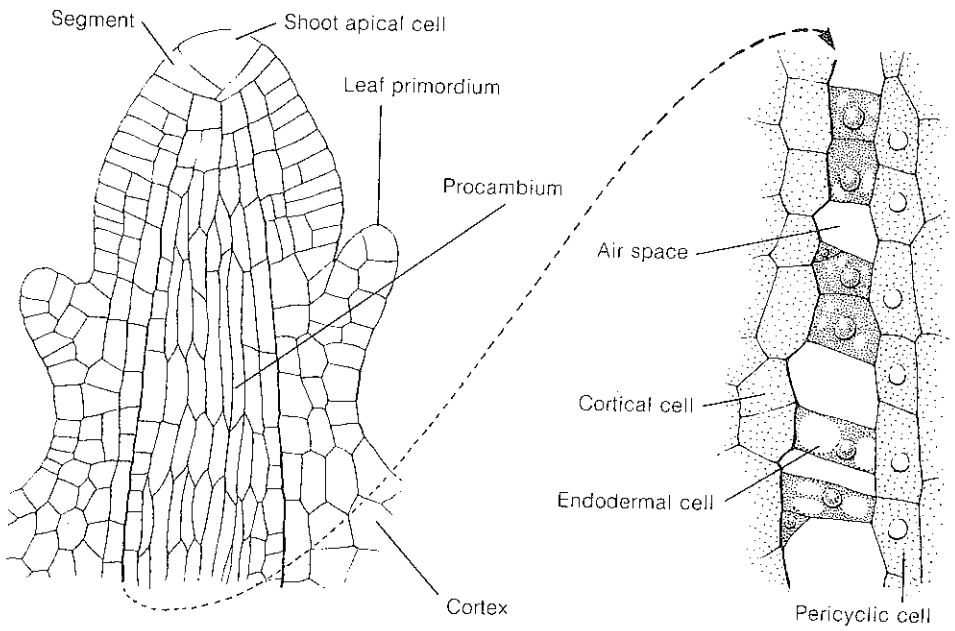



**FIGURE 9-24** Stem development in *Selaginella sinensis*. Longisection of the shoot tip (left), and early development of trabeculae (endodermal cells) and of the air-space system surrounding the vascular cylinder (right); endodermal cells become separated from one another and undergo radial extension. [Redrawn from Hsü, *Bull. Chinese Bot. Soc.* 3:75, 1937.]

and adjacent vein of the microphyll strengthens the concept of direct conduction of substances to the base of the ligule.

A ligule develops precociously soon after initiation of the microphyll, and it has been assumed that it might function as an excretory structure in keeping the young leaf primordium (and sporangium of a sporophyll) moist during early development. There is now convincing evidence that the ligule in certain species, during early development, secretes a mucilage consisting of carbohydrates and proteins which also coats the apical meristem (Bilderback, 1987; Fig. 9-25, A, B).

Ligules also occur in *Isoetes* and *Stylites*. Phylogenetically, the origin and homology of the ligule are obscure problems. But the antiquity of ligules is shown by their presence in extinct arborescent lycopods of the Carboniferous. More recently, the ligule was shown to have been present in the herbaceous, homosporous Devonian lycopod, *Leclercqia* (see p. 124).

**THE ROOT.** Except for the primary root of the young sporophyte, the roots of most species arise at points of branching of the stem. Each root takes its origin from a meristem, termed an angle meristem, but the root generally remains visibly unbranched until it contacts the substratum. Traditionally, the leafless axis has been interpreted as stemlike (rhizophore), giving rise to roots at its distal end upon reaching the soil or humus. The young, initially unbranched root has no definite root cap, and occasionally develops into a leafy shoot under natural conditions or can be induced to do so under experimental conditions (Williams, 1937; Cusick, 1954).

Detailed histogenetic studies of root development have been made on three species of *Selaginella*. In *S. wallacei* (an isophyllous species) the root initially lacks a root cap, but very early the apical cell gives rise to root cap cells. Soon after root cap formation, the root apical meristem divides in preparation for branching. The apical meristem may continue to divide in an isotomous manner, but actual branching is not evident externally until the axis comes into contact with the soil (Webster and Steeves, 1964). Root development is essentially the same for the anisophyllous species, *S. kraussiana*. A root may become 4 to 5 centimeters in length be-

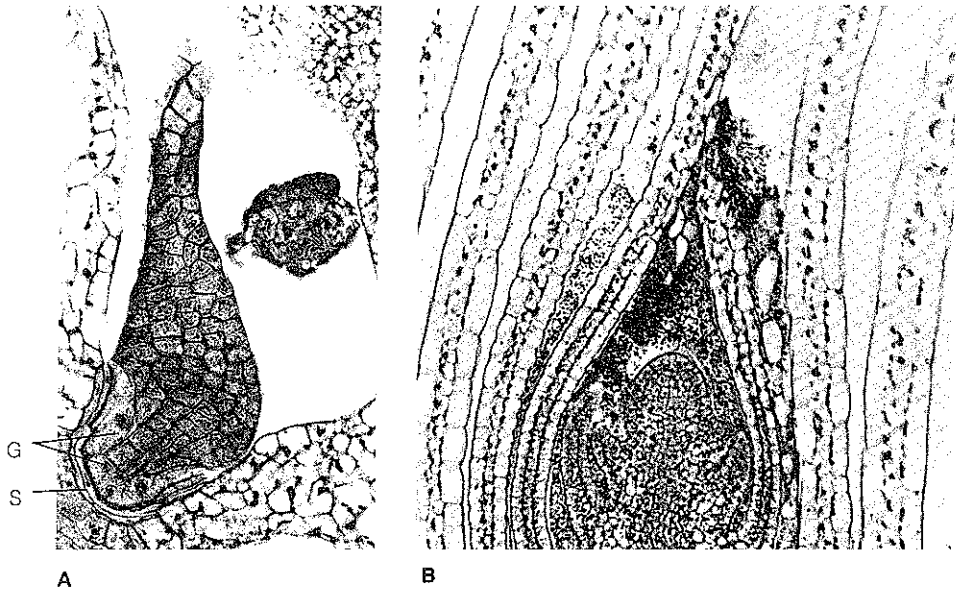



FIGURE 9-25 A, ligule of *Selaginella wallacei* with a mucilage body next to ligule. B, stem tip of *Selaginella kraussiana* grown in sterile culture with sucrose; mucilage surrounds the shoot apex and young leaves. G, glossopodium; S, sheath. [Courtesy of Dr. D. E. Bilderback.]

fore branching is evident externally. A root cap is lacking in *S. martensii* (an anisophyllous species) even when the root is several to many centimeters in length. Although not always evident to the eye, branching of the apical meristem has already occurred upon reaching the soil. Root cap formation then takes place and the dichotomously branching roots become evident (Webster and Steeves, 1967).

Physiological support for the conclusion that the leafless axis ("rhizophore") is in fact a root, rather than a stem, has come from experiments on auxin transport in *S. willdenovii*. In this species there are two angle meristems: one on the ventral side (lower side) of the flabellate shoots and one on the dorsal side. A root is formed from the ventral meristem and a leafy shoot may develop from the dorsal meristem. Experimental results have shown that auxin transport in the root is acropetal (toward the root tip) rather than basipetal (Wochok and Sussex, 1974). This acropetal transport is similar to that in the roots of angiosperms. Basipetal transport is the prevailing condition in shoots. When grown on a basic medium supplemented with 0.5 *M* naphthaleneacetic acid (NAA), 1-millimeter tips of roots less than 20 millimeters long continued to grow as roots (Wochok and Sussex, 1976). Twenty percent of the roots grown only on the basic medium or in the presence of triiodobenzoic acid (TIBA), an antagonist of auxin, developed into leafy shoots.

In summary, recent evidence supports the conclusion that the rhizophore is, in fact, a root. The occasional formation of a leafy shoot from an angle meristem that otherwise normally develops into a root perhaps might occur because in a primitive plant such as *Selaginella* the fate of a meristem may not be so precisely determined as in seed plants (Webster and Steeves, 1967; Mickel and Hellwig, 1969).

However, the seemingly unending debate on the homology of the rhizophore may not be over. An electrophoretic analysis of polypeptides from stem, leaf, root, and rhizophore of *S. kraussiana* revealed that the polypeptides of the rhizophore more closely resemble those of the stem rather than subterranean roots (Jernstedt and Mansfield, 1985).

**THE STROBILUS.** Unlike *Lycopodium*, all species of *Selaginella* form strobili or cones. Strobili occur terminally on side branches, although in some forms the apical meristem of the cone may continue meri-

stematic activity, producing vegetative leaves. All sporophylls of a strobilus are generally alike (although not differing from vegetative leaves as much as in certain species of *Lycopodium*) and are arranged in four distinct rows. The sporophylls may fit tightly together, or the entire strobilus may be lax or an open type of cone (Figs. 9-20, A, B; 9-26, A).

Because *Selaginella* is heterosporous, sporangia are of two types: microsporangia and megasporangia (Figs. 9-26, B; 9-27). The sporophylls associated with these two types of sporangia are termed, respectively, microsporophylls and megasporophylls. The one mature sporangium associated with each sporophyll is generally axillary in position, although its origin may be from cells of the axis or the base of the sporophyll. There is variation in distribution of sporangia within the strobili of different species. Strobili may consist entirely of microsporangia or of megasporangia. However, the mixed condition is more common. The lower portion of a strobilus may consist of megasporangia and the upper portion of microsporangia, or the two types of sporangia may be mixed indiscriminately. A common arrangement is two vertical rows of each type (Fig. 9-26, B). In certain species (e.g., *Selaginella kraussiana*) only one megasporangium is present at the base of each strobilus.


FIGURE 9-26 Strobili of *Selaginella*. A, two enlarged, compact strobili comprising four rows of sporophylls; B, one-half of a strobilus that has been "cleared" and stained; microsporangia to the left and megasporangia to the right; note the four megaspores in each megasporangium, also the vascular bundle in each sporophyll that passes beneath the sporangium.

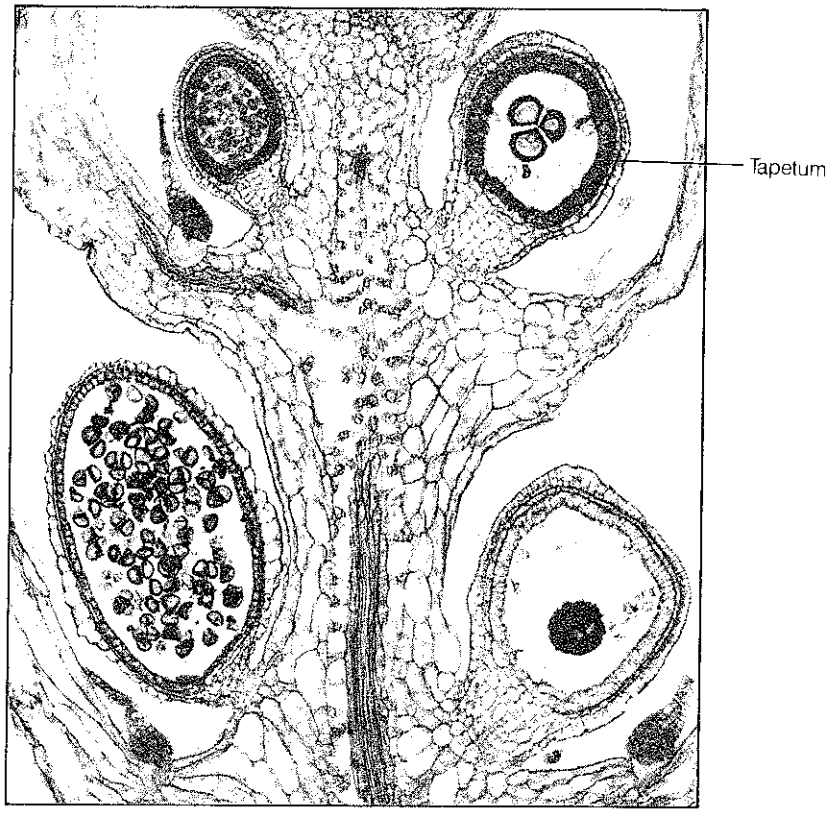



FIGURE 9-27 Portion of longisection of strobilus of *Selaginella* sp. showing late stages in development of sporangia. At upper left, a microsporangium with microsporocytes; note median sectional view of ligule. At lower left, a mature microsporangium with numerous microspores. Three megaspores, surrounded by degenerating sporocytes, are evident in megasporangium at upper right. The megasporangium at lower right is nearly mature, and at this level of section only a single megaspore is seen. (Consult Fig. 9-28 for details of early ontogeny of sporangium in *Selaginella*.)

The type and distribution of sporangia within the strobili of *Selaginella* have never been submitted to a detailed analysis by using mass collections. However, Horner and Arnott (1963) examined the pattern of distribution of megasporangia and microsporangia within strobili of species related taxonomically and geographically. They recognized three major patterns of sporangia distribution in 30 North American species of *Selaginella:* Pattern I—strobili having a basal megasporangiate zone with an upper zone of microsporangia; Pattern II—strobili having two rows of microsporangia and two rows of megasporangia; Pattern III—strobili that are wholly megasporangiate. It was concluded that sporangial arrangement is a useful taxonomic tool in *Selaginella*. In general, a natural group or series of species is all of one type. Furthermore, Horner and Arnott (1963) concluded that Pattern I is more primitive since this arrangement was exhibited by Carboniferous tree lycopods and fossil species of *Selaginellites*. From this type other patterns may have evolved.

Mature microsporangia are generally obovoid or reniform and reddish to bright orange. Megasporangia are larger than microsporangia and frequently are lobed, conforming in outline to the

large spores within them. The megasporangia are characterized by lighter colors: whitish-yellow or light orange.

The site of sporangial initiation, of microsporangia or megasporangia, is in superficial cells of the axis, directly above the sporophyll, or in cells near the base of the sporophyll on the adaxial side. Whether two, three, or more superficial initials are involved, periclinal divisions in these initials separate an outer tier of cells — the primary wall cells — and an inner tier — the primary sporogenous cells (Fig. 9-28, A, B). By repeated anticlinal and periclinal divisions of the primary wall cells, a two-layered sporangial wall is formed. The primary sporogenous cells divide periclinally, the outer layer of cells eventually becoming the tapetum (Figs. 9-27; 28,C, D); the inner cells, by dividing in various planes, produce the sporogenous tissue (Fig. 9-28, C). Undoubtedly cells located near the base of the sporogenous tissue, not identified with the original periclinal divisions, serve to complete the continuity of the tapetum (Figs. 9-27; 9-28, D).

A sporangium at this stage consists of an immature sporangial wall of two layers, a short stalk, and a conspicuous tapetal layer enclosing sporocytes which normally round off and separate from each other prior to the meiotic divisions. Up to this stage, microsporangia and megasporangia are indistinguishable, although one study showed that a pair of sporangia (microsporangium and megasporangium) at the same node in the cone exhibit different growth rates up to the premeiotic stage (French, 1972). As development continues, the two types become clearly defined. If a sporangium is to become a microsporangium, a large percentage of the sporocytes undergo meiosis to form tetrads of microspores (Fig. 9-27).

In a potential megasporangium the functional megasporocyte becomes distinct from the nonfunctional ones prior to meiosis. Nonfunctional megasporocytes develop large vacuoles and accumulate starch while the functional sporocyte retains a dense cytoplasm (is rich in RNA) and lacks starch. The functional megasporocyte, encased in a thick coat of callose (Horner and Beltz, 1970), is generally in the central region of the sporocyte mass and undergoes meiosis, forming four megaspores; the nonfunctional sporocytes ultimately degenerate.

What determines why one sporangium will become microsporangiate and another megasporangiate? The earliest histological feature that distinguishes a megasporangium from a microsporangium is the number of potential sporocytes produced. In a megasporangium there are 100 to 150 fewer sporocytes (French, 1972) which means that the sporogenous cells failed to undergo a final *mitotic* division. In some interesting experiments, Brooks (1973) has shown that repeated spraying of *Selaginella wallacei* with Ethephon markedly modifies the determination of sporangia. After Ethephon is absorbed it decomposes, releasing ethylene. After 18 months, 98 percent of the strobili were entirely megasporangiate. Ethylene may inhibit the last mitotic division of sporogenous cells, thus resulting in the production of megasporangia.

Frequently one or more megaspores do not mature, or in certain species more than one megasporocyte is functional, resulting in the production of eight, twelve, and even more megaspores.

The difference in size between microspores and megaspores in *Selaginella* is dramatic (Fig. 9-29, A). Spore size and morphology are useful in the taxonomy of the genus.

CHROMOSOME NUMBERS. Chromosome numbers are known for only about 10 percent of the species of *Selaginella*. The small size of chromosomes (1 micrometer or less long in some species) and difficulties in obtaining suitable cytological preparations may have contributed to the lack of information on the genus. On the basis of a limited amount of information, some investigators recognize at least four basic or primary chromosome numbers ($x = 7, 8, 9, 10$; Löve et al., 1977), with actual chromosome counts of $2n = 14, 16, 18, 20$, with a few being $2n = 50$ to 60 (Jermy et al., 1967). Another investigator concluded that perhaps there are only two basic numbers, $x = 9$ and 10, although 10 may be basic and 9 an aneuploid derivative (Kuriachan, 1963). A majority of the actual counts are indeed $n = 9$ or 10. *Selaginella* remains a genus that has not undergone increases in chromosome numbers and polyploidy apparently has played no major role in evolution.

GAMETOPHYTES. In *Selaginella* development of microspores and megaspores generally begins while

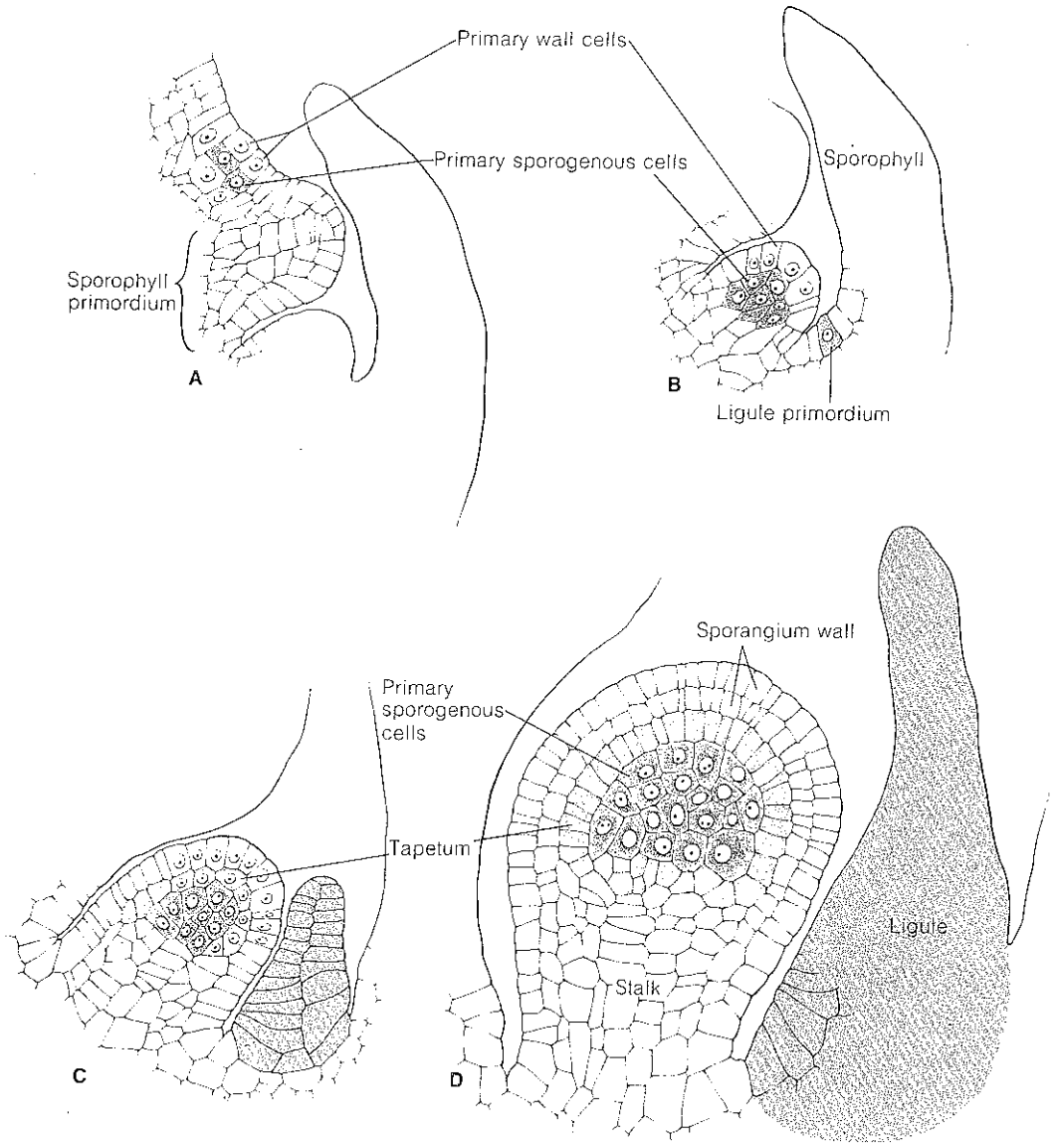
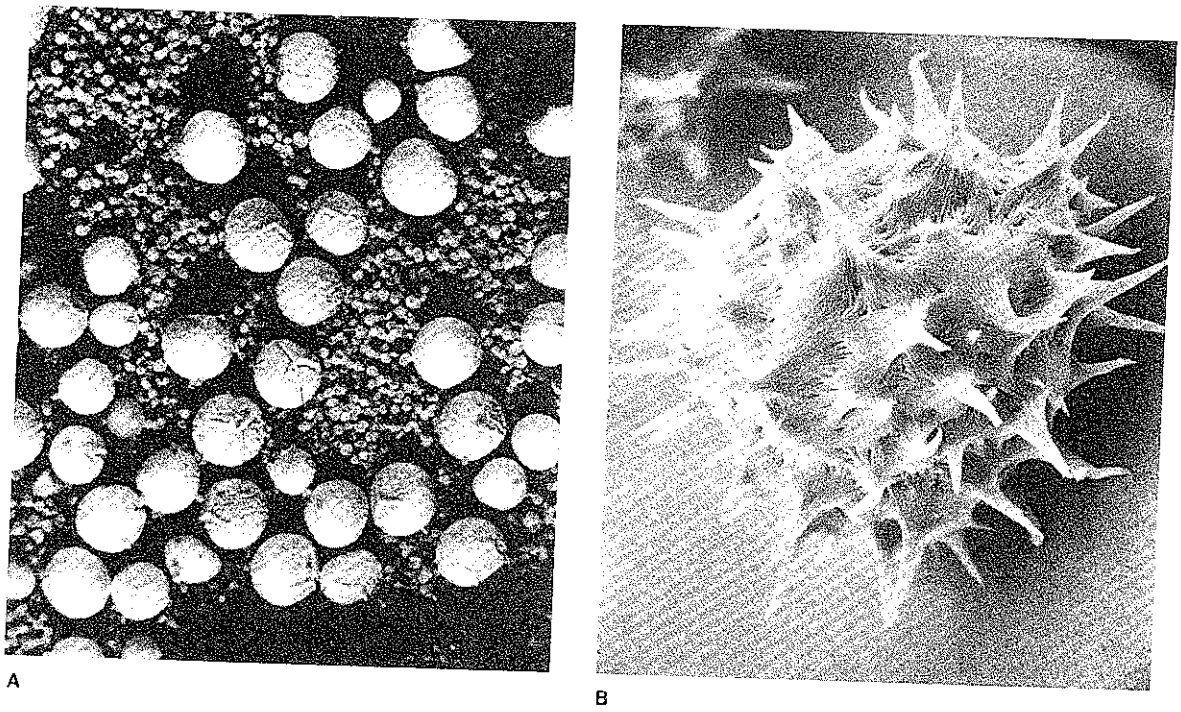



**FIGURE 9-28** Early ontogeny of a sporangium in *Selaginella* sp. **A, B,** periclinal divisions in superficial cells separate primary wall and primary sporogenous cells. **C, D,** the tapetum is formed from outer sporogenous cells. Note precocious development of ligule. Whether the sporangium in **D** would have become a microsporangium or a megasporangium is not evident morphologically at this stage of development. However, physiological specialization may have occurred.



A B

FIGURE 9-29 *Selaginella* spores. **A,** spores of *Selaginella* sp. illustrating dramatic difference in size of large megaspores and numerous small microspores (×35). **B,** scanning electron micrograph of microspore of *Selaginella kraussiana* (×875). [**A,** courtesy of Dr. E. G. Cutter; **B,** courtesy of Dr. R. H. Falk.]

they are still within their respective sporangia. In a microsporangium at a late stage of development the radial and inner tangential walls of the outer layer of the sporangium thicken; the inner wall layer becomes stretched and crushed; the tapetum may still be recognizable. Within the microsporangium the microspores (which may still be held together in tetrads) are thick-walled tetrahedral cells with various types of wall ornamentations such as spines or knobs. The first division in a microspore results in the formation of a small vegetative cell ("prothallial cell") and a large potentially meristematic cell (termed the "antheridial initial"; Fig. 9-30). The antheridial initial divides, and by several additional cell divisions a sterile jacket is established that encloses four primary spermatogenous cells, all within the original microspore wall. Dispersal of the spores (with the enclosed partially developed microgametophytes) may occur at this time or earlier by mechanisms characteristic of species.

In some species a microsporangium dehisces but there is no active mechanism for active spore ejection. In a study of 53 species of *Selaginella* Koller and Scheckler (1986) demonstrated that 21 of them, mainly from xeric environments, have the "passive type" of spore dispersal. The microsporangium dehisces longitudinally and the spores simply fall over the sides of the sporangium and sift down through the strobilus or are carried away on wind currents. The spores in certain species are forcibly discharged. In one type, a sporangium dehisces longitudinally into two valves (halves) and the valves continue to snap back and forth several times (Fig. 9-31, A). This is the "spore-ejector" type. In the third type, at the time of dehiscence, the sporangial wall is ruptured and becomes reflexed (bends back on itself), and the entire sporangium with most of the spores is ejected. Sporangia are ejected up to 16 to 20 centimeters away from the strobilus in certain species. Upon landing, a sporangium continues to undergo snapping motions causing the spores to be dispersed (Fig. 9-31, B, C). When movement stops, a sporangium usually remains in an open position, and may consist of two

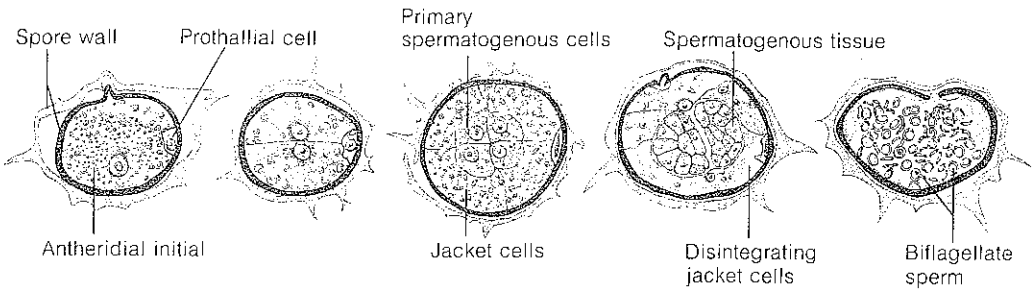



FIGURE 9-30 Development (from left to right) of the microgametophyte in *Selaginella kraussiana*. Early development may occur within the microsporangium prior to sporangial dehiscence. [Redrawn from Slagg, *Amer. Jour. Bot.* 19:106, 1932.]

lobes and a tonguelike portion or variations of this configuration (Fig. 9-31, D). This type of dehiscence is termed the "sporangium-ejector" type. In the two active types of spore dispersal, an annulus of thick-walled cells is involved in dehiscence, functioning much like the annulus of fern sporangia. (See Chapter 13, p. 262 for a discussion of the mechanism.)

The type of spore dispersal may well become useful in the taxonomy and systematics of *Selaginella*. Although only a few species have been studied in detail, it appears that the sporangium-ejector type may prove to be characteristic of the series *Articulatae* in the genus *Selaginella* (Somers, 1982; Koller and Scheckler, 1986).

According to Slagg (1932), the primary spermatogenous cells undergo several divisions, forming 128 or 256 spermatocytes which, on disintegration of the jacket cells and rupture of the spore wall along the triradiate ridge, are liberated as free-swimming biflagellate sperm (Fig. 9-30). Each mature swimming sperm (in *S. kraussiana*) is about 25 micrometers long and very narrow (0.25 micrometers at the anterior end to 0.50 micrometers at the posterior end). One flagellum is attached at the anterior end and the other (posterior flagellum) at about the middle of the sperm. Each flagellum is about 30 micrometers long (Fig. 9-32). In the sperm's ultrastructure a very long mitochondrion (12 micrometers) occupies the anterior half, and the nucleus most of the posterior half. A partial sheath of microtubules extends the entire length of the sperm, functioning perhaps as a support mechanism (Robert, 1974).

Just as early stages in formation of the microgametophyte begin while the microspores are still within the microsporangium, megagametophyte development begins while the megaspores are in the megasporangium. After meiosis in a megasporocyte the resulting megaspores soon develop a thick, layered cell wall. The outer layer, referred to as exospore (or exine) becomes thick, developing spines or ridges in a pattern useful in the identification of species (Figs. 9-33, A; 9-34). Beneath this layer is a thinner layer, the endospore (intine). In some species a third layer (mesospore) develops between the exospore and endospore. The exospore displays a rather unique organization in that there may be packets of aligned granules, each packet forming a polyhedron. In some investigated species there are heavy deposits of silica in the outer parts of the wall (Martens, 1960a, b; Stainier, 1965; Tryon and Lugardon, 1978). Very early a conspicuous vacuole develops within the cytoplasm. In material processed for microscopy the intine may appear to be separate from the exine, however, Pieniązek (1938) reported that the apparent differential growth of exine and intine is an artifact. He states that the spore-wall layers remain in contact throughout development if the spores are not subjected to unusual physical or chemical changes (e.g., use of chemical preservatives). Concomitant with the enlargement of the vacuole the megaspore nucleus divides, followed by additional nuclear divisions without cell-wall formation (referred to as a "free" nuclear phase). This results in a thin layer of multinucleate cytoplasm surrounding a large vacuole. The cytoplasm is rich in lipid globules and protein bodies. The formation of cell walls around nuclei begins initially at the apical end beneath the triradiate ridge (Fig. 9-34). In some species cell wall formation is a continuous process proceeding basi-

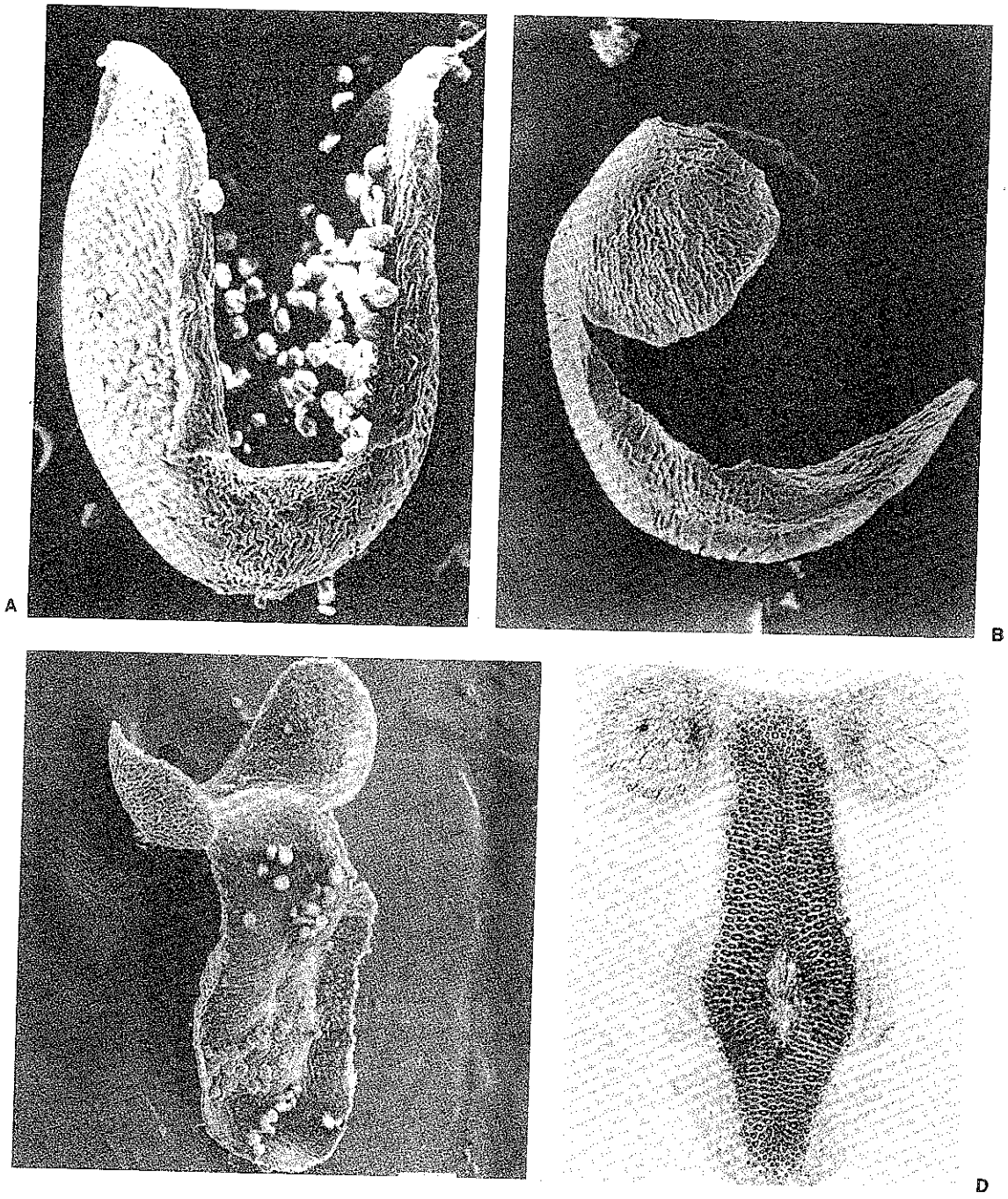



FIGURE 9-31 Microsporangia of *Selaginella* showing active spore dispersal types. **A**, *S. hoffmanii:* spore-ejector type (×160). **B, C,** sporangium-ejector type; **B**, *S. diffusa:* partially reflexed sporangium (×120); **C**, *S. galeottii:* reflexed sporangium; some spores still present (×86). **D**, *S. diffusa:* after dehiscence and completion of snapping motions; whole stained mount; annulus consists of thick-walled cells; region of former attachment to the sporophyll indicated by the lighter staining cells toward the lower end of the annulus. ×86 [Courtesy of Dr. A. L. Koller.]

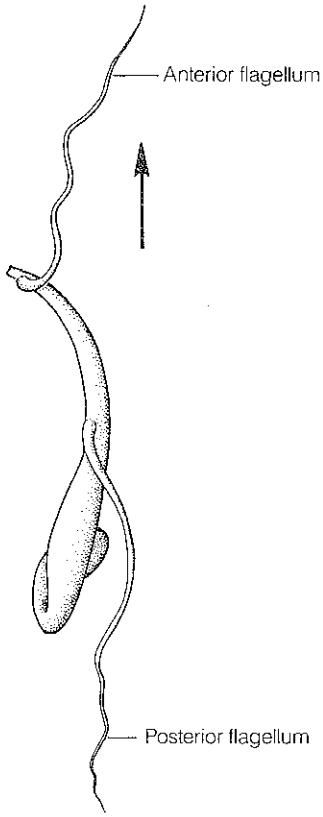



FIGURE 9-32 Biflagellate sperm of *Selaginella kraussiana* (approx. ×1,500). [Based on Robert, *Ann. Sci. Nat. (Bot.)*, Sér. 12, 15:65–118.]

petally from the apical region, until the megagametophyte is entirely cellular or a multinucleate storage vesicle may remain at the basal end. In others, e.g., *S. kraussiana*, soon after cellularization begins, a conspicuous arching diaphragm (wall) connected to the intine is formed beneath the apical patch of tissue (Fig. 9-35). Cell walls do form around some nuclei in the storage vesicle below the diaphragm. The latter is perforated so that there is continuity between the two regions (Robert, 1971a, b).

Apparently there is considerable variation in the genus as to when sporangial dehiscence occurs and the stage of megagametophyte development. Dehiscence may occur at any time before or during the cellular stage. The final stages of megagametophyte development and fertilization take place while the megaspore with its enclosed megagametophyte rests on moist soil or humus. The megagametophyte increases in size resulting in the splitting open of the megaspore wall along the triradiate ridge. The exposed gametophyte may develop tufts of rhizoids which probably play a role in absorption of water, and possibly in anchorage (Fig. 9-38, A).

Archegonia make their appearance in the apical region (Fig. 9-35). Each archegonium develops from a single superficial cell and at maturity consists of eight neck cells, arranged in four rows of two cells each. There is one neck-canal cell, a ventral canal cell, and the egg. Only the terminal neck cells extend beyond the surface of the gametophytic tissue. The microgametophytes complete their development while situated on the exposed megagametophyte or in close proximity to it. After the biflagellate sperm are liberated they swim to the archegonia in a thin film of dew or rain water.

**EXPERIMENTAL STUDY OF THE MEGAGAMETOPHYTE.** Wetmore and Morel (1951b) cultured the female gametophytes of two species of *Selaginella*. On the culture medium the gametophytes remain alive for six months, and if vitamins are added large masses of undifferentiated tissue are produced, which are covered with rhizoids and archegonia.

**THE EMBRYO.** After fertilization, the sporophyte generation is established. The first division of the zygote is transverse, separating a suspensor cell (that cell toward the archegonial neck) and the embryonic cell (labeled "apex" in Fig. 9-36, A) that will form the remainder of the embryo (see Chapter 6).

The embryo is endoscopic. The suspensor may remain undivided or form several cells. A shoot apical cell is established as a result of longitudinal and oblique divisions within the original embryonic cell. At approximately this developmental stage the embryo proper undergoes a 90-degree turn. The first pair of leaves are formed laterally. A foot is produced on the lower side, and the primary root is formed between the suspensor and foot (Fig. 9-36). Variation may exist with respect to the origin and position of foot and root (Chapter 6, Fig. 6-2).

By continued growth of shoot and root, the young sporophyte emerges from the gametophytic tissue (Fig. 9-37). That portion of stem below the first leaves elongates rapidly (Fig. 9-38, B), and in many species the first branching of the shoot takes place immediately above the first pair of leaves (Fig.

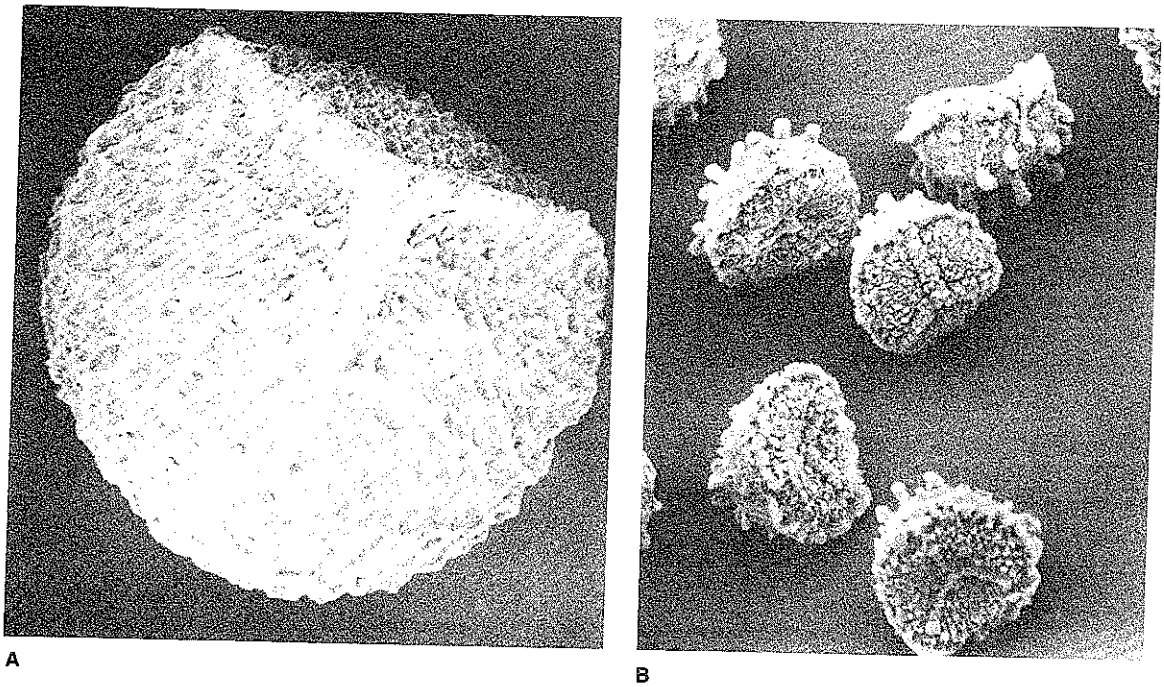



FIGURE 9-33 Scanning electron micrographs of spores of *Selaginella flabellata*. A, megaspore (×200). B, microspores (×650). [Courtesy of Dr. R. H. Falk.]

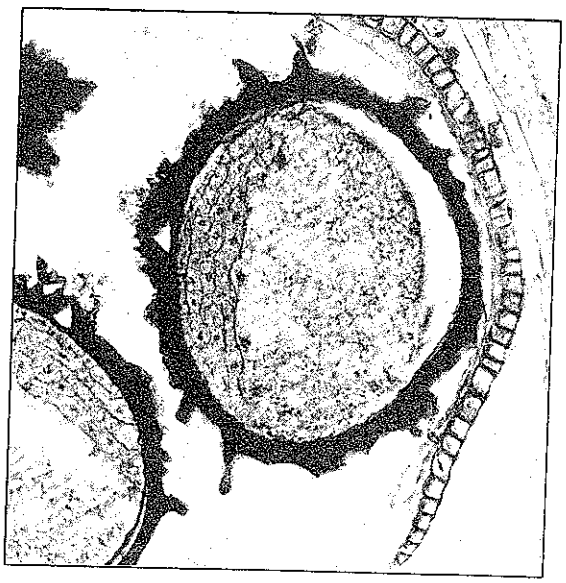


FIGURE 9-34 Section of developing cellular megagametophyte within thick megaspore wall of *Selaginella* sp. A large storage vesicle is present beneath the cellular tissue. The triradiate ridge is indicated by a triangular space (white) at the upper end of the spore (to the left). The developing endosporic gametophyte is still enclosed within the megasporangium.

9-38, C). The primary root grows downward and enters the soil. The foot remains in close contact with the nutritive tissue of the gametophyte contained within the megaspore wall. The complete separation of the sporophyte from the gametophyte may not occur until the sporophyte has undergone considerable growth (Fig. 9-38, C).

Most of our knowledge of embryo ontogeny is based on very old studies (e.g., Bruchmann, 1912). The descriptions may well be accurate, but we need confirmation with modern techniques and equipment.

Webster (1979) has developed a controlled artificial crossing technique for *Selaginella* for inheritance studies. *Selaginella kraussiana*, a common greenhouse plant, is green. *Selaginella kraussiana* var. *aurea* is a pigment-deficient cultivar with yellow-green foliage. When selfed, it produces green, yellow-green, and white (lethal) young sporophytes in a 1 : 2 : 1 ratio. These results together with results of additional crosses have shown that the *aurea* character is controlled by a single nuclear gene with two partially dominant alleles (Webster and Tanno, 1980).

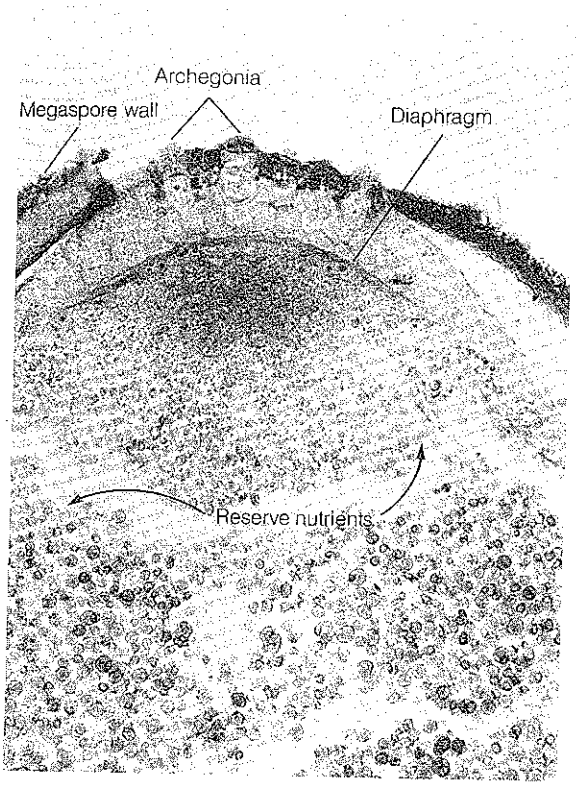



FIGURE 9-35 Section of megagametophyte of *Selaginella kraussiana* surrounded by megaspore wall. Note diaphragm that separates upper (apical) portion of gametophyte from the lower portion.

*Selaginella martensii* f. *albovariegata* is a variegated sport which produces white tissue in an irregular fashion. Reciprocal crosses between the green *S. martensii* and f. *albovariegata* revealed maternal inheritance of variegation. Additional crosses, involving progeny of selected reciprocal crosses, also indicated a lack of direct nuclear influence on variegation. Character expression and inheritance can be accounted for on the basis of random sorting of normal and defective cytoplasmic factors at cell division (Tanno and Webster, 1982a, b).

## Carboniferous Relatives of *Selaginella*

Some herbaceous plants from the Carboniferous Period resembling *Selaginella* have been known for many years. The plants were small and were clearly heterosporous. Some of them were described as *Selaginellites,* others as *Selaginella.* One paleobotanical case history is of interest. Most species thought to be herbaceous and *Selaginella*-like, were described from compressions in a poor state of preservation. But in 1954 a ligulate lycopod was described from the Carboniferous, which had small stems and spirally arranged leaves. This plant was given the generic name *Paurodendron.* The stems were in an excellent state of preservation (Fry, 1954). In 1966 another plant of the same genus was found that had a basal root-producing portion, termed a rhizomorph (Fig. 9-39), that had secondary growth (Phillips and Leisman, 1966). Complete knowledge of the plant was obtained in 1969 when bisporangiate strobili (microsporangia and megasporangia) were found attached to stems. The entire plant was then described as *Selaginella fraiponti* because it resembled to a remarkable degree the modern-day species *Selaginella selaginoides,* which has a centralized basal root system (Schlanker and Leisman, 1969). This is of great morphological interest because it indicates that *Selaginella* existed in the Carboniferous, and provides information on possible ancestral forms of *Selaginella.* However, based upon a study of the meristem of the rhizomorph of *Paurodendron,* Rothwell and Erwin (1985) have aligned the genus with the Isoetales rather than with the Selaginellales.

## Lepidodendrales

In addition to the low-growing lycopods of the Carboniferous Period, there were also large, tall trees with lycopsid features. The first evidence of these forms are found in rocks of the Upper Devonian, although they were not as tall as those in the Upper Carboniferous. These trees must have formed a conspicuous element of the Carboniferous "coal" swamps (Fig. 9-40) and are some of the best known fossils. They were arborescent, heterosporous, and ligulate, and had distinctive persistent leaf bases on the stems.

LEPIDODENDRON. Well-preserved material, especially of the trunks, is common in coal beds in Great Britain and central United States. Many of these dendroid plants attained a height of 30 to 35 meters or more and were at least 1 to 2 meters in diameter at the base. The trunks were unbranched for 10 to

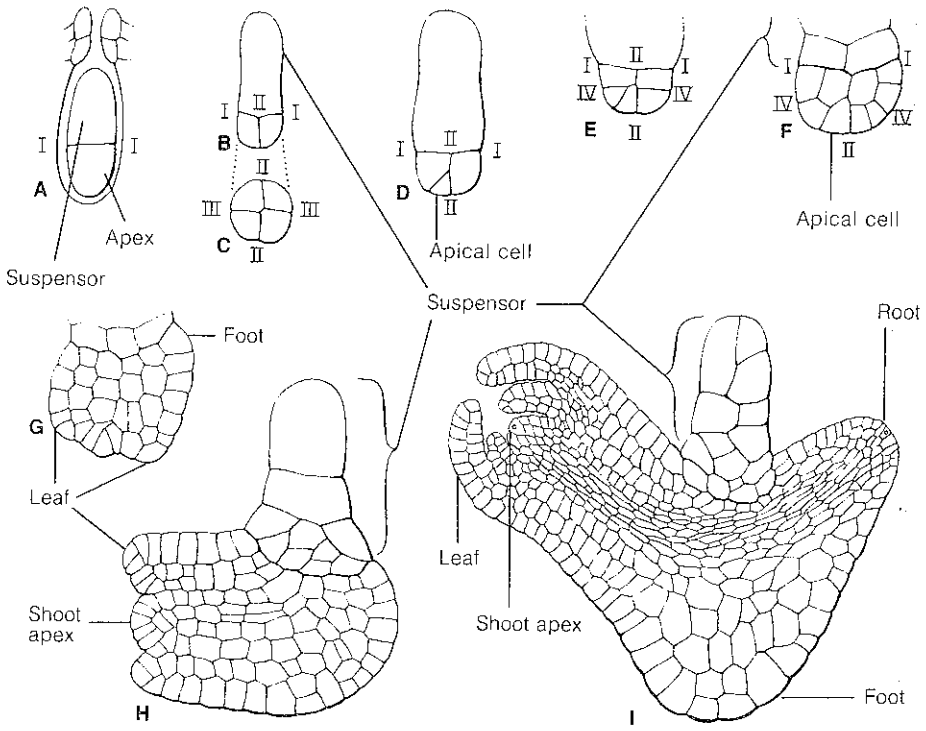



FIGURE 9-36 Embryogeny in *Selaginella martensii*. In all of the sketches the neck of the archegonium is directed toward the top of the page. The walls resulting from early cleavages are indicated as I-I, II-II, etc. An apical polar view of **B** is shown in C. (For details consult text.) [B-I redrawn from Bruchmann, *Flora* 99:12, 1909.]

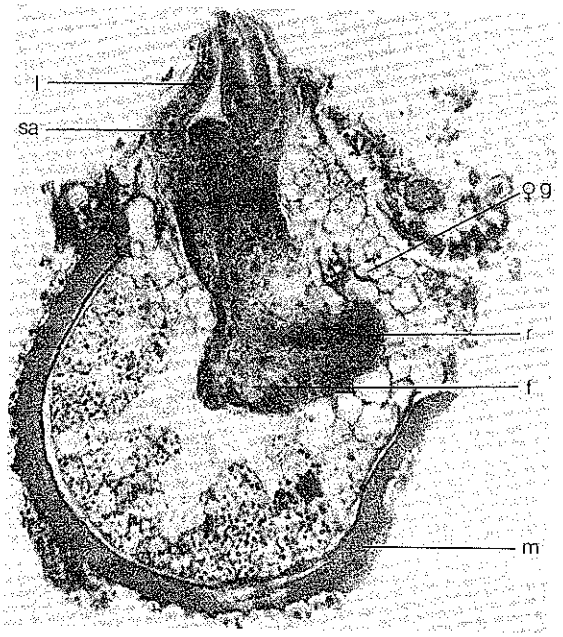



FIGURE 9-37 *Selaginella* sp. Section of megaspore, enclosed female gametophyte, and young sporophyte. f, foot; 1, leaf; ♀g, female gametophyte; m, megaspore wall; r, first root; sa, shoot apex. [From Bold et al., *Morphology of Plants and Fungi*, Harper and Row, New York 1980.]

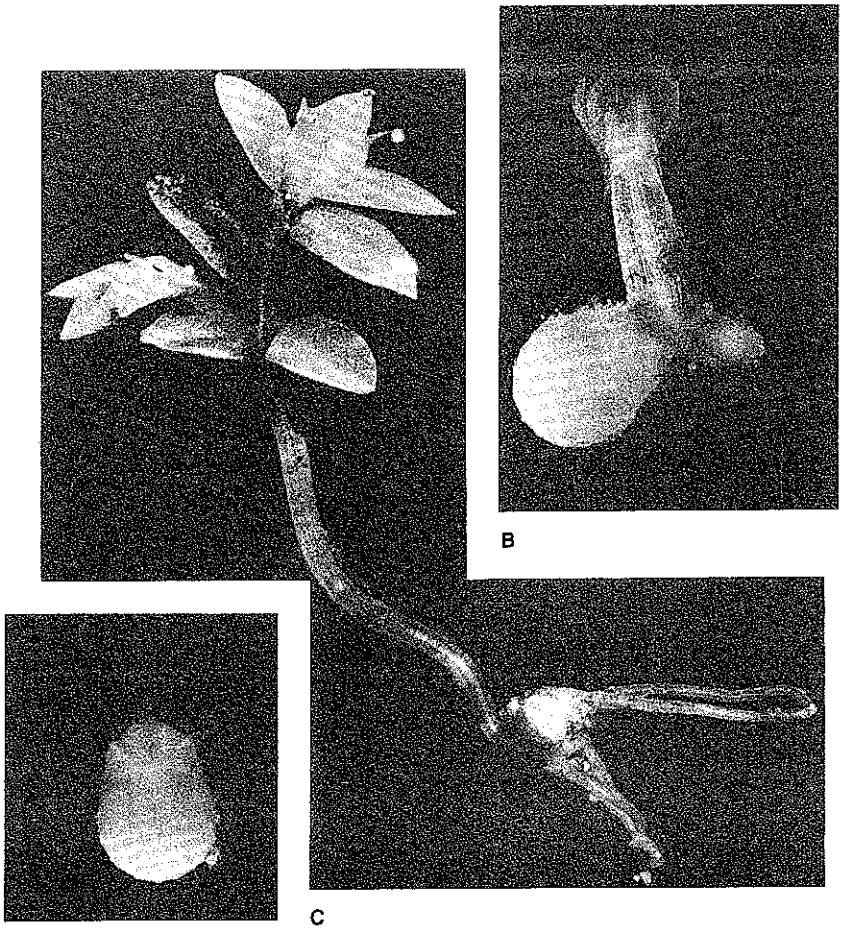



**FIGURE 9-38** Megagametophyte and young sporophyte of *Selaginella kraussiana*. **A,** gametophyte tissue protruding through cracked spore wall; **B,** young sporophyte attached to gametophyte, showing root (to the right), stem, and first pair of leaves (oriented in the plane of the page); **C,** older sporophyte (megaspore wall with enclosed gametophyte is still visible at the juncture of stem and root).

20 meters or more and then terminated in an isotomously or anisotomously branched, umbrellalike shoot system in which the branches were clothed with spirally arranged, linear, or awl-shaped leaves (Fig. 9-41, A). In some species the branched portion consisted of two systems of branches, each of which branched dichotomously. It is believed that the helically arranged leaves on the trunks were grasslike, reaching lengths of several to 78 centimeters. Upon abscission of a leaf blade, a distinctive base (leaf cushion) remained attached to the stem (Fig. 9-42, A). The shorter leaves on the upper crown were persistent. The form of the leaf cushions and the details on the face of the scar left by the abscised leaf blades are useful in separating species in the genus. An examination of a leaf scar reveals a vascular bundle scar flanked by two small scars, each of which is termed a *parichnos* (from the Greek word, meaning "footprint") (Fig. 9-42, A). In the living condition each parichnos consisted of a strand of loosely organized parenchyma that extended from the stem cortex into the leaf blade, functioning presumably as aerating tissue. On some lepidodendrid leaf cushions there are two additional parichnos channels below the leaf scar (Fig. 9-42, B). The base of the trunk was divided into

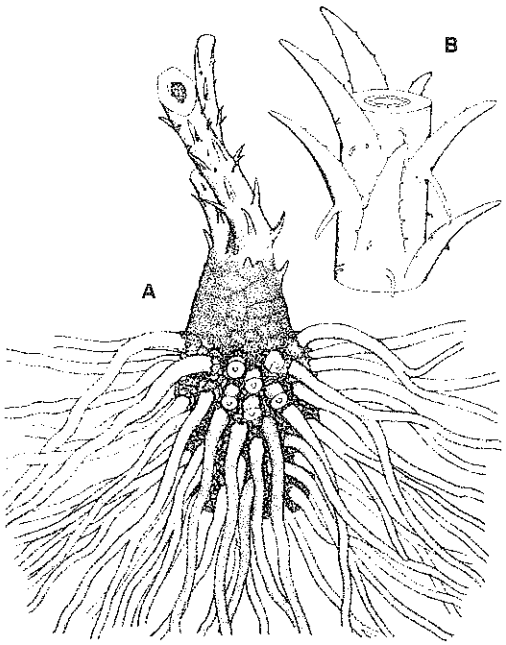



FIGURE 9-39 *Paurodendron (Selaginella) fraiponti.* **A,** reconstruction of lower portion of plant. **B,** enlarged portion of aerial stem. [From Phillips and Leisman, *Amer. Jour. Bot.* 53:1086–1100, 1966.]

four large rootlike structures, termed rhizophores, each of which branched repeatedly in dichotomous fashion (Figs. 9-41, A, B; 9-43).

Even though the trunk of one of these trees was very large, it had very little vascular tissue. Whether the primary vascular cylinder was protostelic or siphonostelic, the primary xylem was exarch in development. A vascular cambium produced a narrow cylinder of uniform secondary xylem, consisting of scalariform-pitted tracheids and uniseriate rays. Apparently the vascular cambium did not produce secondary phloem; only primary phloem has been identified (Eggert and Kanemoto, 1977). The entire vascular cylinder, even in large stems, was never more than several centimeters in diameter.

The bulk of the stem consisted of primary and secondary cortex (periderm). The primary cortex consisted of inner, middle, and outer tissue zones (Fig. 9-44). In some species the secondary cortex was produced centripetally from a ring of meristematic tissue (phellogen) which had its origin through dedifferentiation of outer primary cortical cells (Fig. 9-44). In others there was the production of successive layers of secondary cortex from meristematic cells derived from more deeply lying pri-

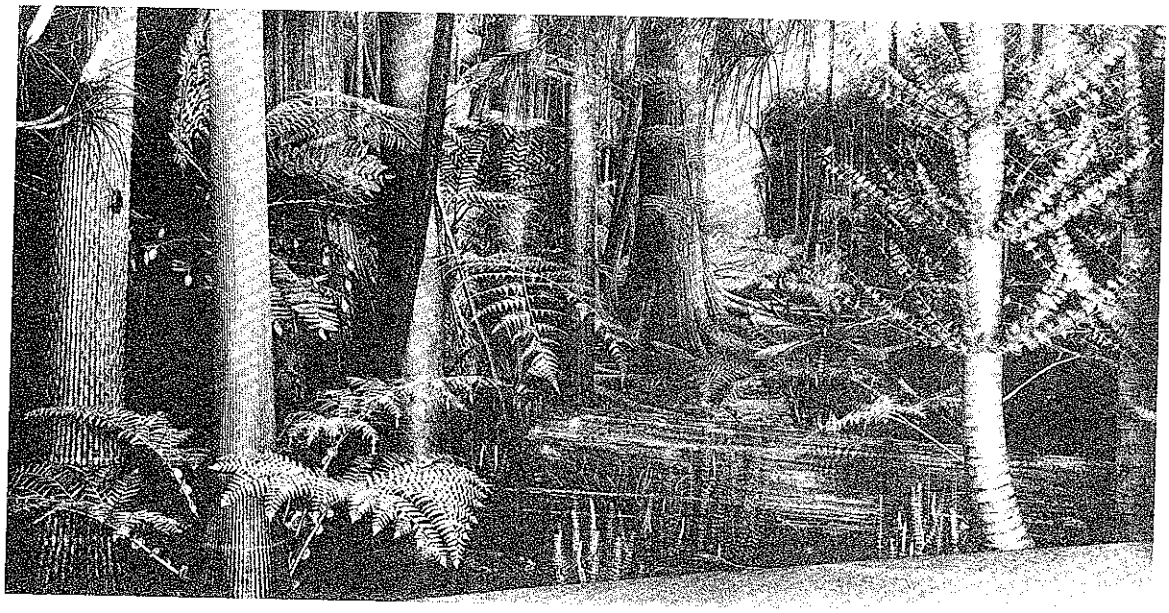


FIGURE 9-40 Reconstructions of plants in a Carboniferous swamp; trunks of lepidodendrids at left foreground with seed ferns behind them; *Equisetum*-like plant (a calamite) at right foreground. [Courtesy of Field Museum of Natural History, Chicago.]

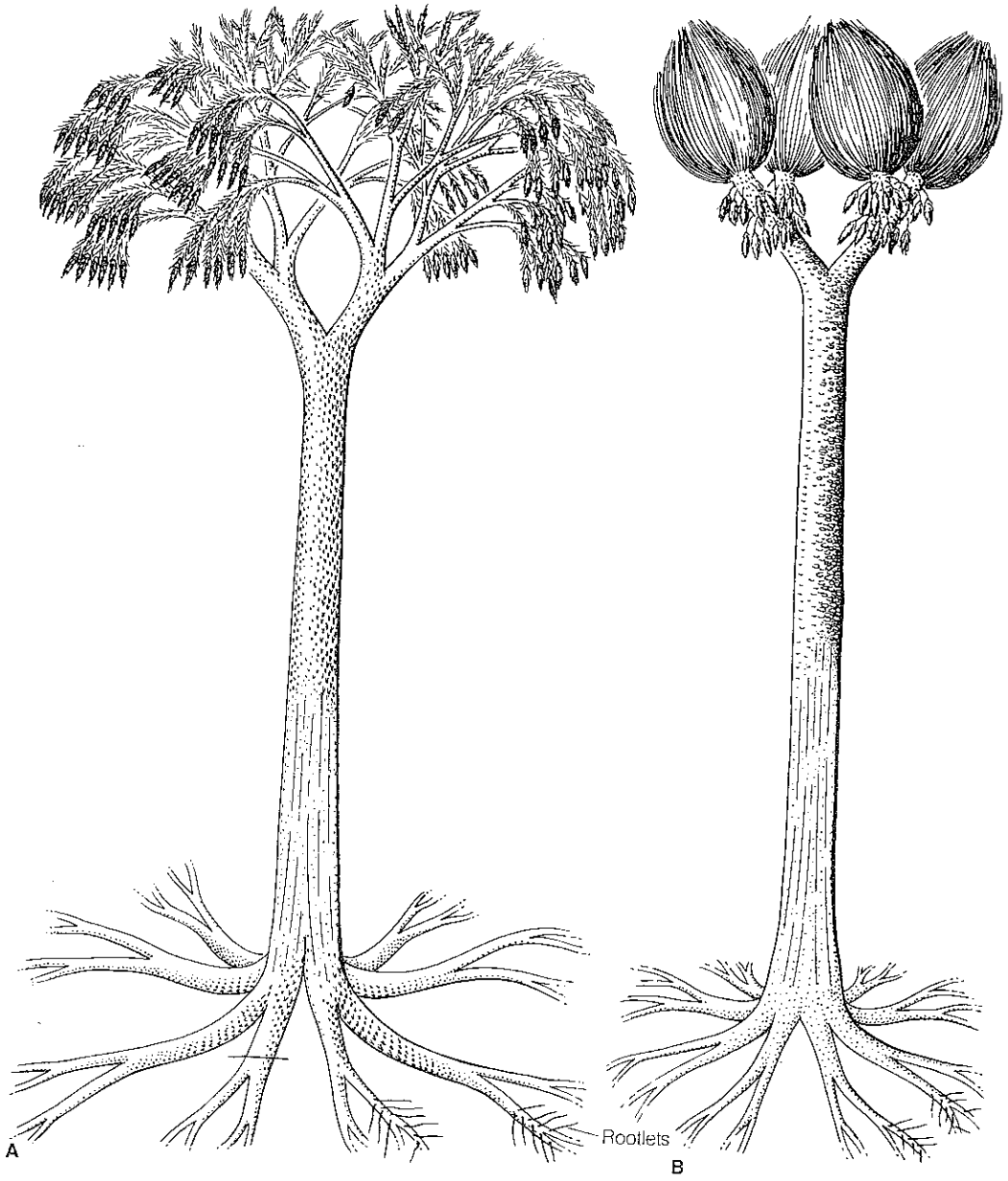



FIGURE 9-41 Suggested reconstructions. **A,** *Lepidodendron* sp.; **B,** *Sigillaria elegans.* Note strobili and the large rhizophores with attached rootlets at base of trunks. *Form* or *organ* genera exist for all basic parts of the plants. (Consult text for pertinent information.) [Modified from *Handbuch der Paläobotanik* by M. Hirmer. R. Oldenbourg, Munich. 1927.]

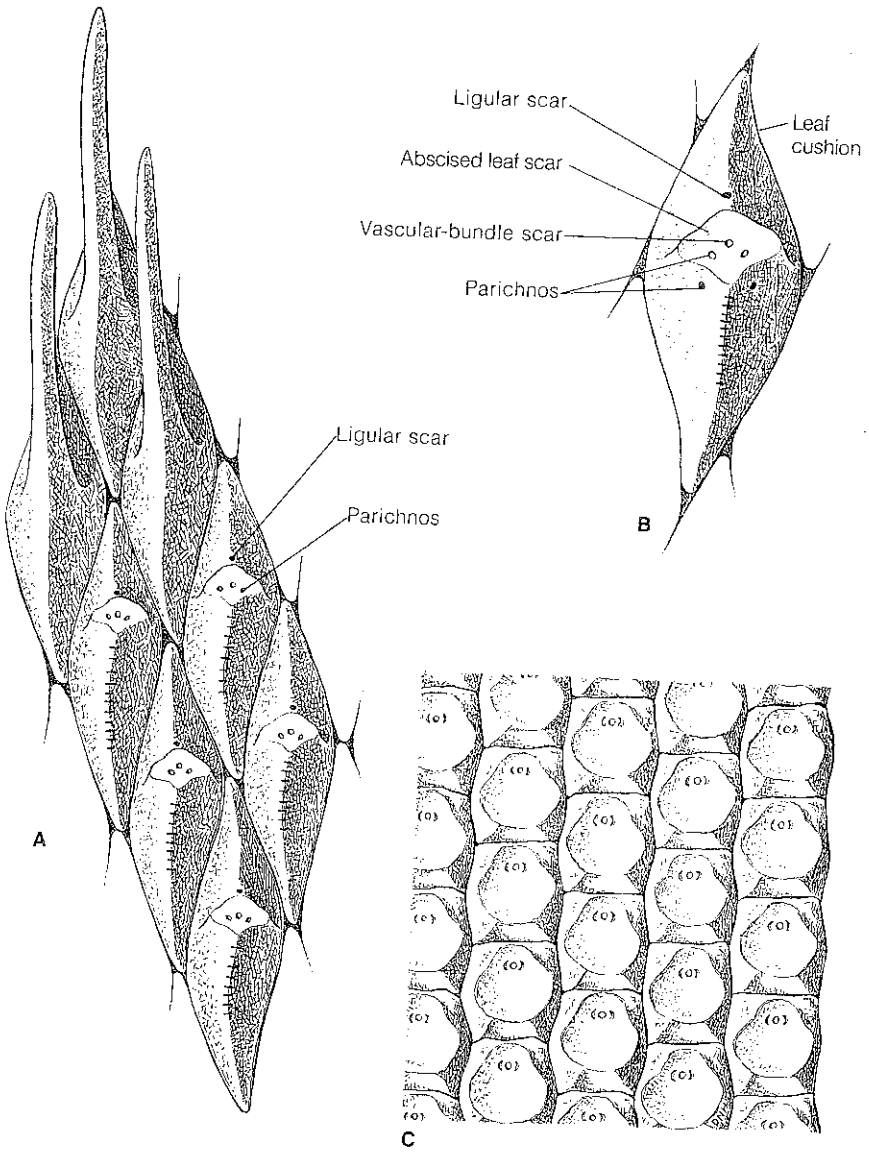



**FIGURE 9-42** **A,** diagrammatic representation, portion of the surface of a branch of an arborescent lycopod (*Lepidodendron* sp.) showing three attached leaves and the scars left by the abscission of five others. **B,** one leaf scar of a lepidodendrid showing two sets of parichnos scars. **C,** surface of the stem of *Sigillaria* sp.

mary cortical cells. The development of secondary cortex brought about the separation of the outer primary cortical cells from that portion nearer the vascular cylinder. Also, the massive production of secondary cortical tissue resulted in the separation of leaf cushions and decortication of the lower half or more of a trunk (Fig. 9-41, A, B).

The small amount of xylem produced in relation to the large size of the trunk is remarkable. The secondary cortex did consist partly of fiber cells in some species, but this would not have resulted in the strength provided by secondary xylem of modern trees. The disparity between tree size and strengthening tissue may have literally led to the

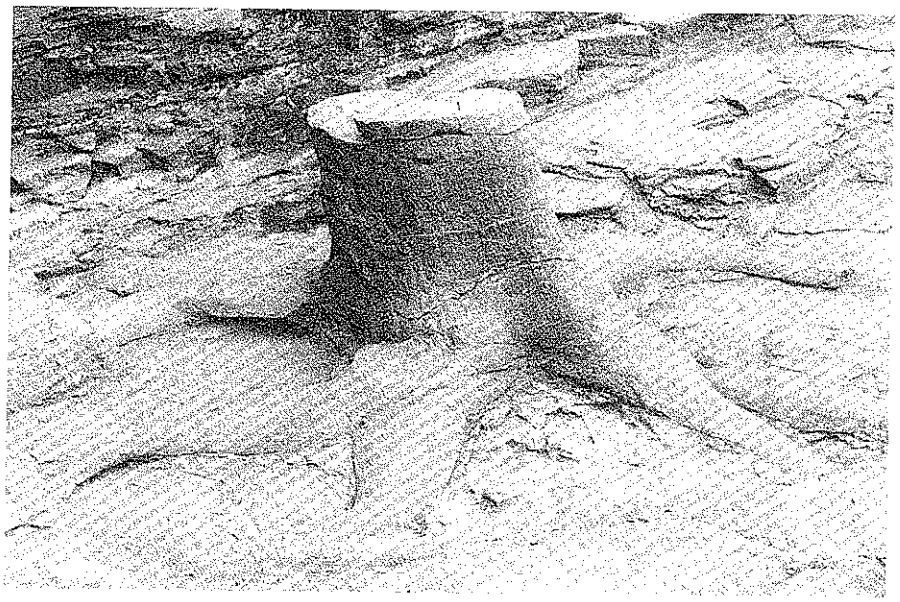


FIGURE 9-43 Tree stump of a lepidodendrid in "Fossil Grove," Victoria Park, Glasgow, Scotland. The basal dichotomously branched lobes, to which rootlets were attached, are designated *Stigmaria* (an organ genus). The fossil is a cast of the original tree. Stumps, which measure 15 to 40 inches at their widest diameter, were exposed by carefully removing the hard rock that encased them. [Photograph courtesy of Dr. E. G. Cutter.]

downfall of the tree-lycopods and their disappearance from the world's flora toward the end of the Carboniferous.

Other genera coexisted with *Lepidodendron* in the Upper Carboniferous. They likewise were tall trees and were somewhat similar to *Lepidodendron*, but enough is known about them to assign them to different genera.

*Lepidophloios* was similar in general growth habit but differed from *Lepidodendron* primarily in the shape and organization of leaf cushions.

*Sigillaria* was unbranched with one or two dichotomies at the distal end (Fig. 9-41, B). The grasslike leaves of the distal branches were generally longer (up to 1 meter) than in the other two genera. The leaves were helically arranged, but the leaf cushions appear to lie in vertical rows. The leaf-blade scars were hexagonal to oval (Fig. 9-42, C). Strobili were borne terminally on short lateral branches at the base of the leafy crowns or intermingled with the leaves (Fig. 9-41, B). Where known, the vascular cylinder was siphonostelic with exarch primary xylem, surrounded by secondary xylem (Fig. 9-45, A, B).

ONTOGENY. Preservation of tree-lycopods is so excellent that the ontogeny of a plant can be deduced from fossil remains. As can be seen in the reconstruction illustrated in Fig. 9-46, the main trunk tapered slightly from the base to the initial dichotomy. Branches resulting from the first dichotomy were reduced in size. With each successive dichotomy there was a progressive decrease in size of branches which led finally to determinate growth, often in the production of strobili. *Lepidodendron* had a definitive life span. Eggert (1961) was able to correlate the successive decrease in size of branches with a decrease in complexity of the vascular cylinder. The xylem was protostelic at the base, siphonostelic with a relatively wide pith near the level of initial branching, and ultimately protostelic in the tiny terminal branches (Fig. 9-46). Associated with this progression, there was a decrease

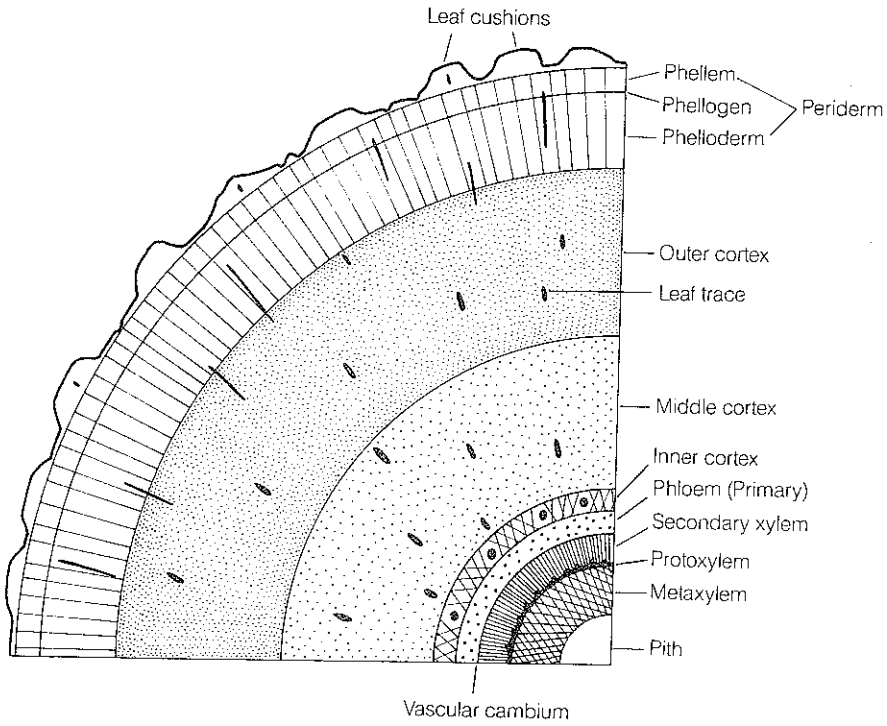



**FIGURE 9-44** Diagrammatic representation of a transverse section of one type of *Lepidodendron* stem.

in size of the secondary xylem cylinder, as well as in the amount of cortical tissue produced. (See also Andrews and Murdy, 1958, and Delevoryas, 1964, for additional discussions.)

**ORGAN GENERA.** From the account in the previous section the reader might conclude that the discovery of intact remains of these arborescent genera is indeed remarkable. However, the story is not that simple. To uncover a fossil plant with all parts intact is the dream of paleobotanists; unfortunately, at best only unconnected portions or separate organs of large plants are generally discovered. This is understandable if we consider the amount of breakage possible during transport by water, for example, to sites of final preservation. The ontogeny of a plant, as discussed previously, has a bearing on the problem because stem structure, for example, may vary at different levels. Also, some structures (strobili, spores, etc.) may have been formed either at regular intervals or only near the end of the life of a plant. This dilemma, appreciated by early paleobotanists, led to the establishment of *organ genera* or *form genera* which has resulted in the accumulation of numerous, but necessary, genera for organs such as leaves, stems, and strobili. For example, the genus *Lepidodendron* originally referred to portions of the stem of lycopods with the distinctive leaf-cushion, leaf-scar pattern. Fossil leaves with one unbranched vein are placed in the organ genus *Lepidophylloides*. *Sigillariophyllum* is the name applied to leaves that are vascularized occasionally by two veins.

The massive underground rootlike organ is known as *Stigmaria,* another organ genus (Fig. 9-43). These dichotomizing axes (rhizophores) bore "stigmarian" rootlets in a helical pattern. Paolillo (1982) has suggested that the rootlets were derived from cell derivatives of an apical meristem rather than from a secondary meristem as in the corm of *Isoetes* (see p. 159). Usually the rootlets are found detached; only the scars are seen on the rhizophores. The internal structure of these rootlets is interesting because it resembles to a remarkable

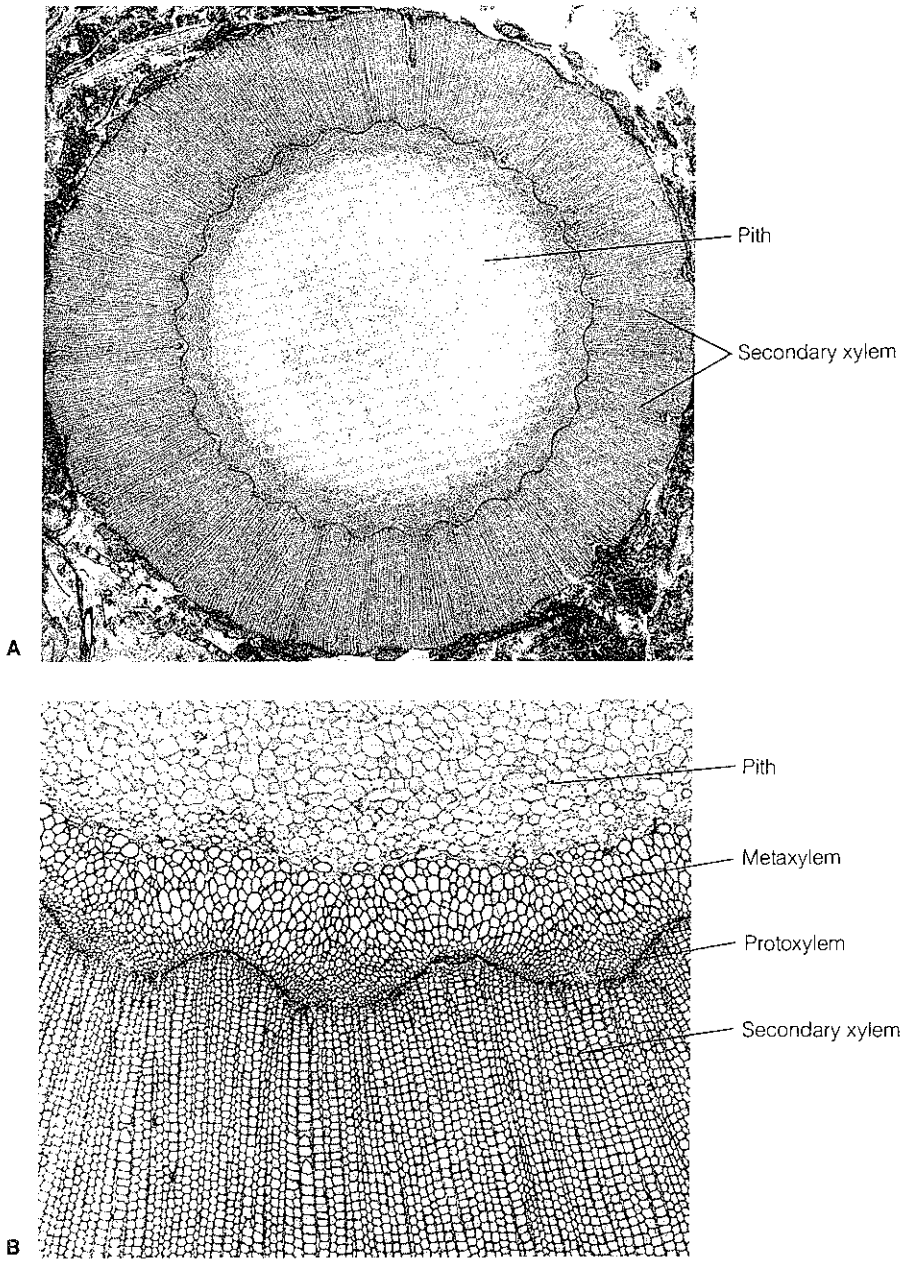
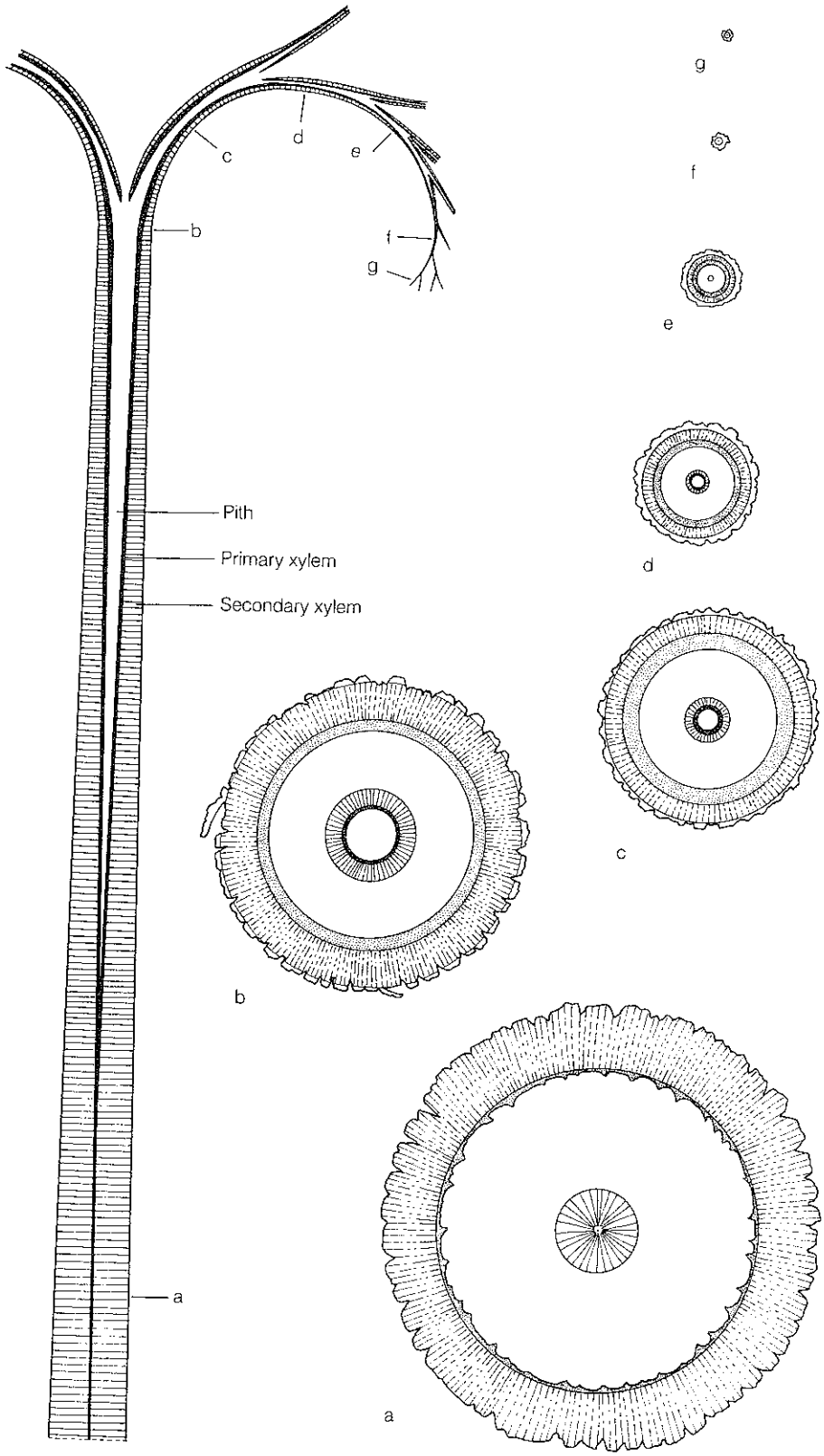



FIGURE 9-45 A, transverse section of a siphonostele of *Sigillaria approximata*. B, details of a section from A. [Courtesy of Dr. T. Delevoryas.]


c
d
e
b
f
g
Pith
Primary xylem
Secondary xylem
a
g
f
e
d
c
b
a

degree that of the roots of *Isoetes;* in both, the root has a monarch vascular strand supported by a flange of the cortex in a large central air cavity (Fig. 9-53).

By continuing comparative studies, and with due consideration given to ontogeny, a better picture is emerging as to what constitute natural or biological species in *Lepidodendron.* For example, DiMichele (1981) has been able to document the occurrence of five distinct species that grew in the coal swamps of the Upper Carboniferous in Europe and America.

The strobili of *Lepidodendron* were either bisporangiate (having microsporangia and megasporangia in the same cone) or monosporangiate. The organ genus *Lepidostrobus* has been applied to both types. The cones were large (8 to 20 centimeters or even 35 centimeters long) with helically arranged overlapping ligulate sporophylls (Fig. 9-47). In bisporangiate cones the microsporangia, containing large numbers of microspores, occurred in the upper portion of the strobilus, and the megasporangia, containing fewer megaspores, were present in the lower portion. One sporangium was present on the adaxial side of each sporophyll, similar to *Lycopodium* and *Selaginella.* The spores were shed from the sporangia and the endosporic gametophytes developed much like those of extant *Selaginella* and *Isoetes.* Well preserved megagametophytes with archegonia, surrounded by the megaspore wall, have been described (Brack-Hanes, 1978) which resemble those of *Isoetes.* Microgametophytes have also been found which resemble those of *Selaginella* in a three-celled developmental stage. These preparations also reveal structures that have been interpreted as chromosomes (Brack-Hanes and Vaughn, 1978).

Some members of the Lepidodendrales progressed to an evolutionary level of producing seed-like structures. *Lepidocarpon* is an organ genus represented by monosporangiate strobili in which typically only one functional megaspore, with its enclosed megagametophyte, was retained within the sporangium (Fig. 9-48, A, B). Conclusive evidence of embryos in *Lepidocarpon* was produced in 1975 by Phillips et al., and further documented by Phillips in 1979. The embryos were vascularized, and post-embryonic stages were obtained showing the establishment of aerial stem and basal rhizophore axes. It was also shown that some of the megagametophytes described by earlier workers were in reality nonvascularized embryos that did not germinate. Another feature of considerable evolutionary interest is the enclosure of the sporangium and enclosed megagametophyte by two lateral extensions (lateral laminae) of the sporophyll, except for a slitlike opening along the top. The entire structure was very much like a seed, the lateral laminae functioning as an integument. The sporophylls were shed from the strobilus, and it is thought that each unit could float on water; hence, there was a mechanism for aquatic dispersal. Fertilization could occur during the flotation period or when the sporophyll unit came to rest on the floor of the muddy swamp. Fertilization probably was effected much like that in *Selaginella.*

## Isoetales – Isoetaceae: *Isoetes*

The genus *Isoetes* is a most interesting and enigmatic vascular plant. The plant body of all species is relatively small with a greatly shortened axis, and has tufts of leaves and roots (Fig. 9-49).

The first popular names recorded for *Isoetes* were "quillwort" and "Merllyn's Grass." The former name is frequently applied to the genus in America; in Europe it is still known as Merlin's Grass. Economically the genus is relatively unimportant today, but there are past records of the plants being eaten occasionally in Europe (Pfeiffer, 1922). Great quantities of starch and oil are present in the plant body. Birds, pigs, muskrats, and ducks may eat the fleshy plant body, and cattle often graze on the leaves.

*Isoetes* includes about 100 described species, although some taxonomists recognize only 50 to 70 as being valid species. The genus is worldwide in distribution, occurring, for example, in America, Europe, Asia, India, and Australia. In America it occurs from coastal Alaska, southward through the United States, Mexico, and most of South America.

FIGURE 9-46 A, diagram of a longitudinal section of the vascular system of an arborescent lycopod showing the distribution of primary and secondary xylem from base to ultimate branches. Levels *a–g* indicate level of transverse sections *a–g.* Note that the axis was protostelic at the base, becoming siphonostelic and then once again protostelic in the small terminal branches. [Redrawn from Eggert, *Palaeontographica* 108B:43, 1961.]

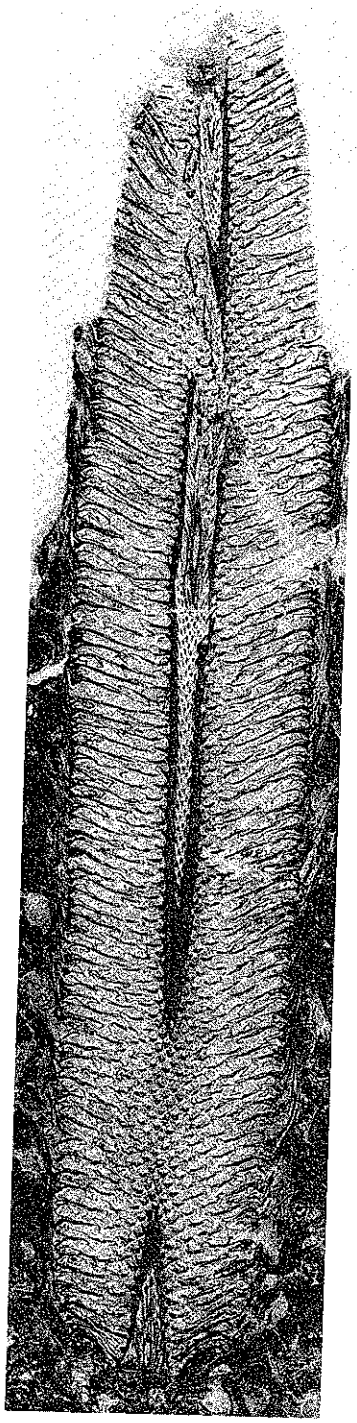


FIGURE 9-47 *Lepidostrobus*, longitudinal section of a large cone of a lepidodendrid. The sporophylls were helically arranged as can be seen in the lower part of the cone where the sporophylls have been cut transversely. The actual specimen is 35 centimeters long.

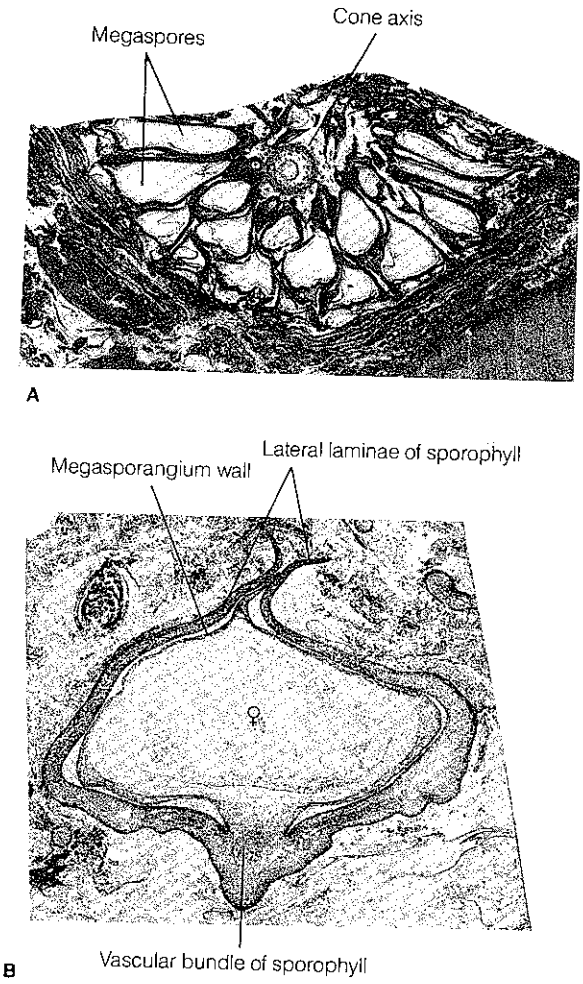



FIGURE 9-48 *Lepidocarpon*. **A**, transverse section of a portion of a fossilized cone showing the cone axis, sporophylls, and large megaspores (one per megasporangium) (×1). **B**, transverse section of one megasporophyll showing structural features and the developing megagametophyte (♀) (×4).

It grows in lakes, ponds, rivers, swampy areas, and in ephemeral pools (e.g., vernal pools) that become dry during part of the year (Tryon and Tryon, 1982). It also grows in lakes that are covered with ice during winter months.

**ORGANOGRAPHY.** The axis is a short, erect structure commonly referred to as a corm. The basal portion of a young plant is usually two-lobed, and may remain so during the life of the plant, but the

corm may become three, or rarely, four-lobed in older plants of certain species by the development of additional furrows (Karrfalt and Eggert, 1977a). Along the sides of the grooves or furrows are numerous roots. The upper part of the axis is covered with a dense cluster of leaves which have broad overlapping bases (Figs. 9-49; 9-50, A-C). The shoot apex is completely hidden in a depression by the tightly overlapping leaves (Fig. 9-51, A). The sides of mature plants become very rough with layers of sloughing tissues. Each increment includes leaf bases and severed roots of previous growing seasons.

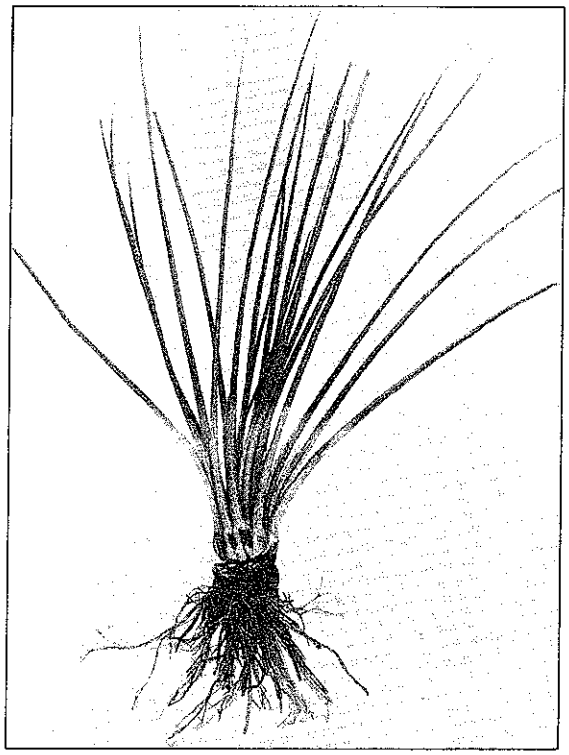


FIGURE 9-49 Entire plant of *Isoetes* sp. showing crown of tightly packed leaves (sporophylls), short "corm" (dark) and roots.

ANATOMY OF THE CORM. Any morphological interpretation of the corm of this peculiar plant must necessarily be based partially on internal structure. The corm has been described variously as an erect rhizome; as a stock; as a stem; as a stem combined with a stigmarian type of rhizophore; and as an upper leaf-bearing part, the stem, and a lower root-bearing part, the rhizomorph. (See Paolillo, 1963, for a review of the subject.) If a mature plant is cut longitudinally in the plane of the basal groove or furrow (considering that the plant is two-lobed), the shoot apex is seen at the bottom of a depression with surrounding leaf bases and leaf traces (Fig. 9-52, A). In a well-established plant the shoot apex is a low dome or a small cone, about twice as broad as high; a group of cells occupy the distal region of the cone (Paolillo, 1963; Michaux, 1966). A single cell may dominate the apical group, but generally there is no conspicuous apical cell. However, Karrfalt (1977) has described apices of older plants in which the arrangement of cells appears to be related developmentally to a tetrahedral apical cell with three initiating faces. The xylem core is in the outline of an anchor—cylindrical in the upper part with the lower part extended horizontally with upturned arms. Roots and root primordia are evident below the xylem core.

Secondary growth is a characteristic feature of *Isoetes* and the specialized cambium comprises two parts: (1) a *lateral meristem* that gives rise to vascular tissue (prismatic layer), centripetally, and to secondary cortical tissues, centrifugally (Figs. 9-51, B; 9-52, B); (2) a *basal meristem,* continuous with the lateral meristem, which adds to the xylem core and produces basally the surrounding ground tissue, in which root primordia become organized (Figs. 9-51, A, B; 9-52, A, B).

Both lobes of the corm are evident in a median longitudinal section cut at right angles to the furrow (Figs. 9-51, B; 9-52, B).

A transverse section through the leaf-bearing portion of a corm reveals the xylem core surrounded by the cylindrical prismatic layer and lateral meristem (Fig. 9-52, C). At a lower level the lateral meristem is evident and the basal meristem appears at two locations, reflecting the curved contour of the xylem core (see Fig. 9-52, C and D).

Of the many debatable features of *Isoetes,* interpretation of the secondary vascular tissue has probably caused the most discussion. The cells of this tissue, which are derived from a definite storied cambium (lateral meristem), are extremely short, being not much taller than they are wide. The form of these cells, combined with the difficulty of interpretation, led to the general acceptance of the noncommittal term "prismatic layer" for this part of the axis. Nevertheless, this tissue has been inter-

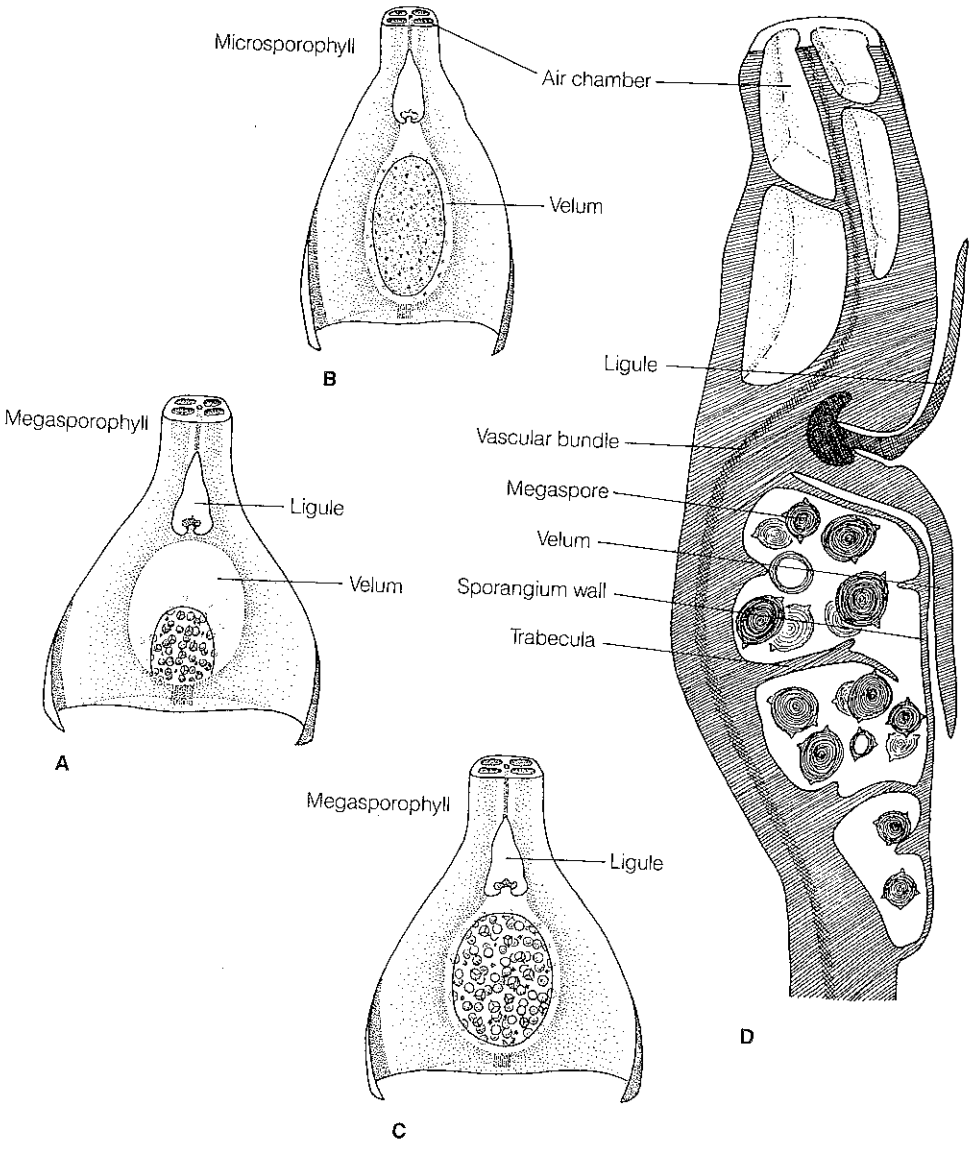
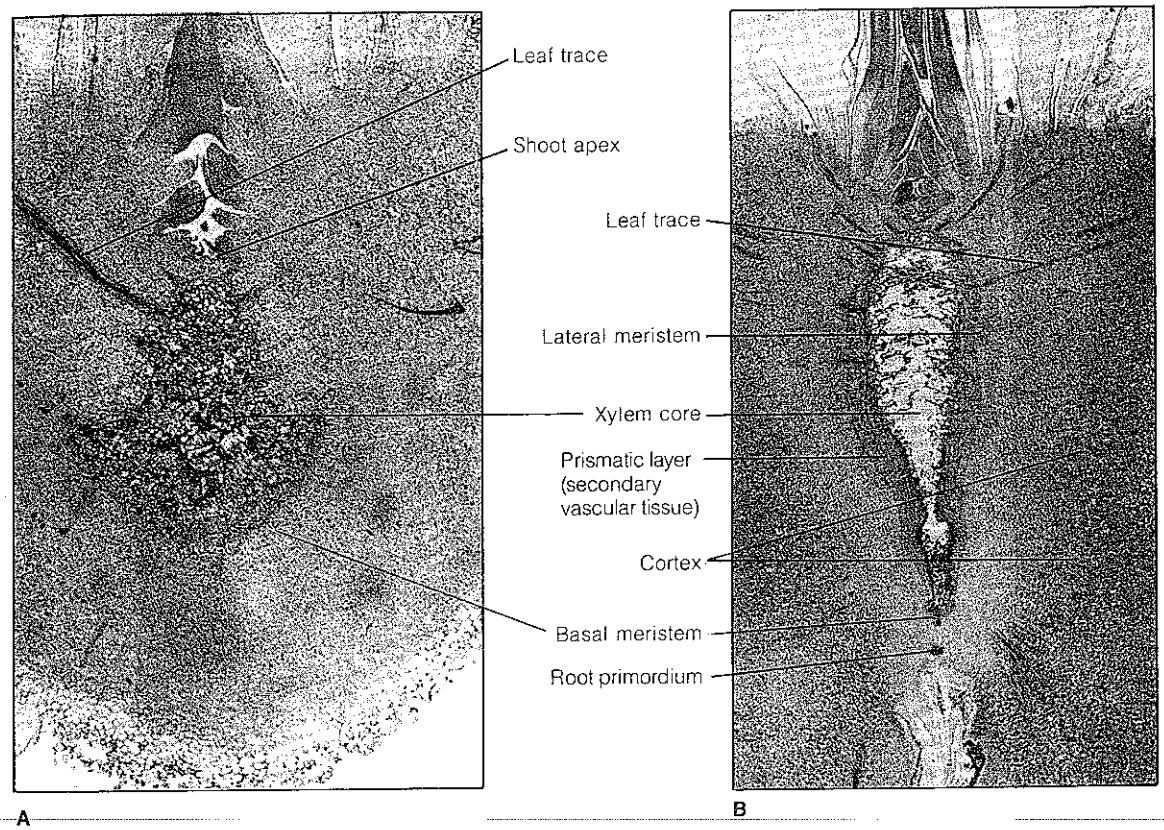



**FIGURE 9-50** A–C, adaxial views of *Isoetes* sporophylls. **A,** *Isoetes* sp.; the velum covers a greater portion of the megasporangium. **B, C,** *Isoetes howellii.* **B,** microsporophyll showing microsporangium (spores are shown as black dots within the sporangium, the ends of trabeculae as larger black asteroids); the velum covers only a small portion of the sporangium. **C,** megasporophyll, showing megasporangium and megaspores. **D,** longisection of a megasporophyll comparable to that in A (markings on spore walls are entirely schematic).

preted as secondary xylem, as secondary phloem, as a secondary tissue containing both tracheids and sieve elements, and as a tissue composed of (1) occasional tracheids, (2) considerable unmodified parenchyma, and (3) specialized parenchyma cells which are concerned with conduction. (See Paolillo, 1963, for a review of older literature.)

Paolillo (1963) provided a detailed study of the secondary vascular tissue (prismatic layer) of *Isoetes howellii.* In young plants of this species the deriva-



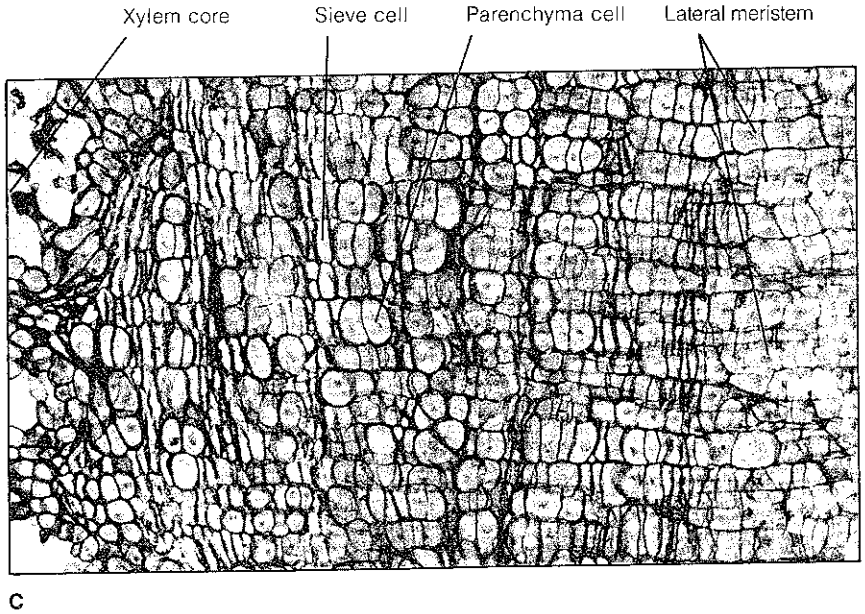




FIGURE 9-51 Anatomy of the corm in *Isoetes howellii*. **A**, longisection, in the plane of the basal groove; **B**, median longisection, at right angles to the basal groove (several root traces can be seen at lower right); **C**, a portion of the secondary vascular tissue and adjacent tissues.

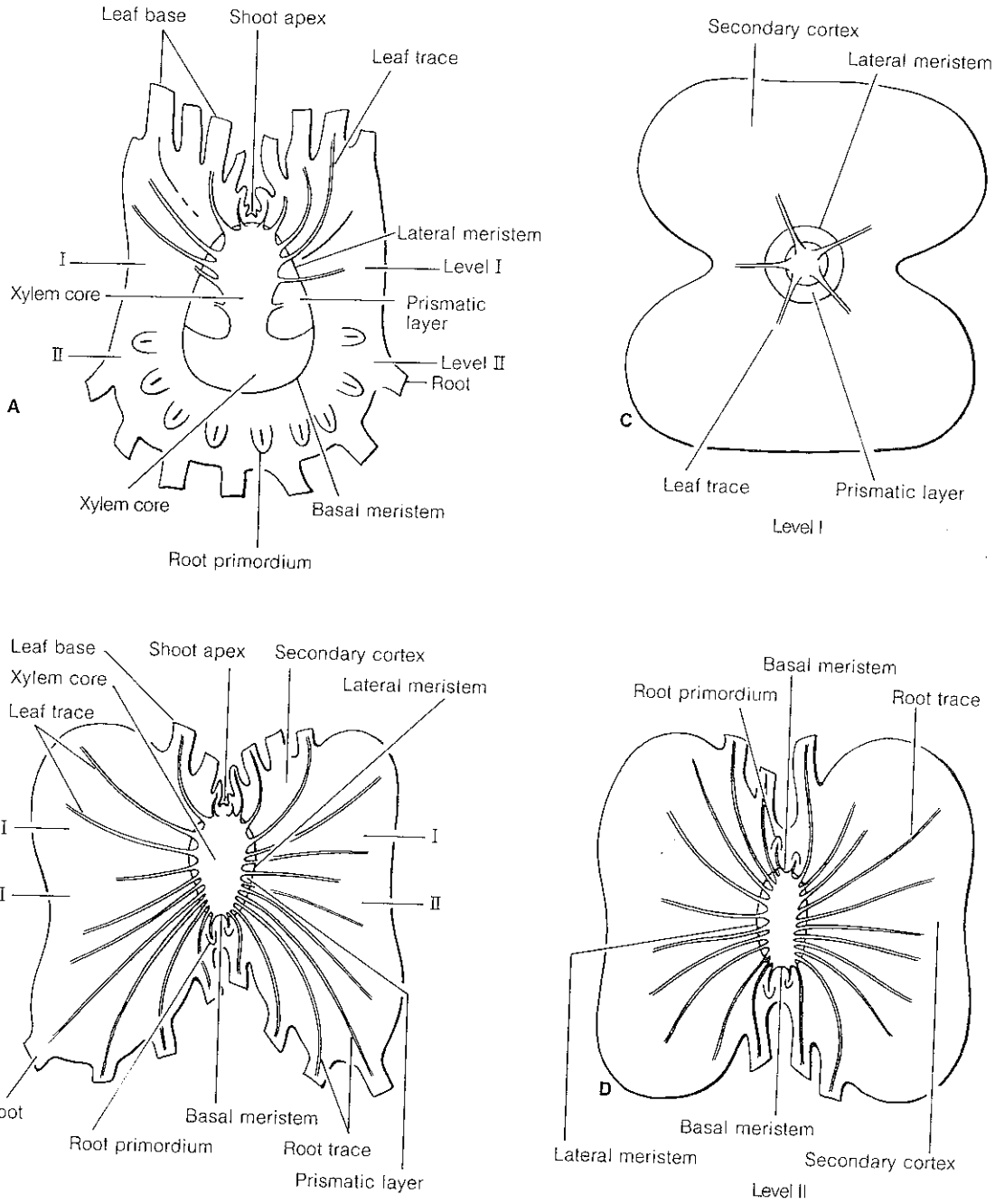



**FIGURE 9-52** *Isoetes howellii.* **A, B,** longitudinal sections of two-lobed plants in the plane of the furrow or groove (**A**) and perpendicular to the furrow (**B**); **C,** transverse section at high level (I-I in **A** and **B**); **D,** transverse section at low level (II-II in **A** and **B**). [Redrawn from Paolillo, *Illinois Biol. Monogr.* 31, 1963.]

tives of the lateral meristem differentiate as sieve elements. In older (larger) plants the inner derivatives of the lateral meristem differentiate as layers of sieve elements, alternating with layers of parenchyma (Fig. 9-51, C). Some or many of the cells in the parenchyma layers may differentiate as tracheids. A comparable type of anatomy occurs in *I. taiwanensis* (Chiang and Chen, 1986).

Recognition of sieve elements is made possible by a combination of features: sieve pores or areas, deposition of callose, and generally thicker walls than those of parenchyma cells (Esau et al., 1953). A degenerated nucleus persists in a functioning sieve element, comparable to other lower vascular plants. In older plants some or many of the cells in the layers of parenchyma may differentiate as tracheids. An ultrastructural study of *I. muricata* detected very little callose lining the pores, although with the approach of dormancy the pores become occluded with copious amounts of callose. The dormancy callose begins to disappear with the resumption of growth, and some sieve elements near the lateral meristem are reactivated before the lateral meristem becomes functional (Kruatrachue and Evert, 1977). This type of reactivation is similar to that of certain woody dicotyledons: *Vitis* (grapevine) and *Tilia* (linden).

The lateral meristem also produces cells centrifugally which retain their meristematic activity, but ultimately mature into parenchyma cells—constituting a broad secondary cortex (Figs. 9-51, B; 9-52, B–D). The secondary cortex is added to regularly and the outer part sloughs off from the "shoulders" of the corm.

The basal meristem, as noted earlier, contributes to the xylem core and also forms ground tissue distally in which root primordia become organized. The roots eventually penetrate the ground tissue. Roots are produced on either side of the furrow—forming series (rows roughly parallel with the furrow) and orthostichies (rows running at right angles to the furrow). Series are more easily seen near the furrow. (See Paolillo, 1963, Karrfalt and Eggert, 1978, for discussions.)

Thus far, organography, basic organization, and functioning of lateral and basal meristems (collectively constituting a cambium) have been described. What constitutes primary growth (and tissues) of the corm? Leaves originate in the surface layer near the base of the small apical cone; the subapical cells produced internally enlarge and divide, producing radiating files. It is within this tissue that axial procambium as well as leaf traces differentiate.

The primary plant body of the shoot, then, consists of a core of primary xylem, primary phloem, primary cortex, leaf traces, and leaves. The lateral meristem arises outside of the primary phloem and gives rise to secondary vascular tissue (prismatic layer) from its inner face and secondary cortex from its outer face. The origin of the lateral meristem outside the primary phloem is quite different from that of the vascular cambium in most woody seed plants, that is, between the primary xylem and phloem.

Paolillo (1963) regards the basal meristem as part of the cambium because the two meristems (lateral and basal) originate together and, during ontogeny, portions of the lateral meristem may be added to the basal meristem by conversion of initials from one function to another. Earlier, it will be remembered, the term xylem core was used to describe the central core of tissue. This term purposely was used because the origin of the central xylary tissue is from two quite different sources—primary xylem derived from the procambium (a primary meristem) of the upper part of the plant axis and the internally produced cells of the basal meristem (part of the cambium). However, Karrfalt and Eggert (1977b, 1978) have presented evidence for their belief that the basal meristem also should be considered a primary meristem.

In summary, one might ask: How is the mature plant form in *Isoetes* related to meristematic activity? Longitudinal growth, accomplished through the functioning of the apical meristem of the shoot apex and of the basal meristem, is very slow, as evidenced by the extremely short axis and the crowding of appendages. The lateral meristem, which surrounds the upper portion of the vascular cylinder and encloses the sides of the basal portion of the xylem core, gives rise yearly to increments of secondary vascular tissue and a large amount of secondary cortical tissue. This accounts for the fact that older plants appear to be more broad than tall.

ROOTS. Roots branch dichotomously (except in one species) after they emerge from the corm ground tissue. For *Isoetes howellii* the root apical

meristem is reported to consist of a layer of initials that gives rise to the cells of the outer cortex, epidermis, and root cap, and a group of initials common to the inner cortex and procambium (Paolillo, 1963).

A transverse section of a mature functioning root reveals a simple type of root of unusual interest. It consists of a cylindrical cortex which surrounds a large air cavity, and a vascular cylinder which is supported in a flange of the cortex in the cavity (Fig. 9-53, A). The primary xylem and phloem are collateral in arrangement, the phloem being oriented toward the cavity. An endodermis, with the usual casparian strips, is present around the primary vascular tissues. The air cavity is formed by a breakdown of cortical cells throughout the length of that portion of the root which has emerged from the corm. Histologically the root of *Isoetes* resembles very closely an appendage on the rhizophore of *Stigmaria* (Fig. 9-53, B). The similarity is conclusive enough to support the belief of some botanists that a phylogenetic relationship between *Isoetes, Stylites,* and some members of the Lepidodendrales is certain.

**THE LEAF.** Actually each foliar appendage is a potential sporophyll, either a microsporophyll or a megasporophyll. (The terms leaf and sporophyll will be used interchangeably.) Each sporophyll has a thickened and expanded base, with a tapering upper portion which is awl-shaped and pointed. In young plants leaf arrangement is distichous (leaves arranged in two rows), but soon becomes helical. With *Isoetes tegetiformans* (Rury, 1978) the distichous arrangement persists (Fig. 9-58). Leaves may be a few centimeters long or 50 centimeters or more *(Isoetes japonica).* The lower parts of the leaves may be buried in the soil and lack chlorophyll, and are commonly a glistening white. Some species have black leaf bases. In many species most of the leaves die and decay at the termination of the growing season. In permanently aquatic species leaves may remain on the plant for some time. The upper portion of a leaf is traversed longitudinally by four large air chambers (Fig. 9-50, B, D) that may be partitioned into compartments by transverse tissue diaphragms — the possession of large air cavities is a feature common to many water plants. Running throughout the length of the leaf (a microphyll) is an unbranched vascular bundle.

Located on the adaxial side near the base of each leaf is a sporangium (Fig. 9-50, A-C). A ligule is present just above the sporangium. Covering or partially covering the sporangium is a protective flap of leaf tissue, the velum (Fig. 9-50). All sporophylls generally have normal sporangia except several of the late-formed sporophylls of a growing season.

**EXPERIMENTAL MORPHOLOGY AND PHYSIOLOGY.** Expression of form in plants is determined largely by the occurrence and distribution of endogenous growth regulators. Perhaps the small, short corm-like form of *Isoetes* is due to a lack of certain endogenous regulating substances. In one experiment the investigator tested the effects of indoleacetic acid and gibberellic acid on *Isoetes*. Applied indoleacetic acid is known to stimulate or inhibit certain growth functions, and treatment with gibberellic

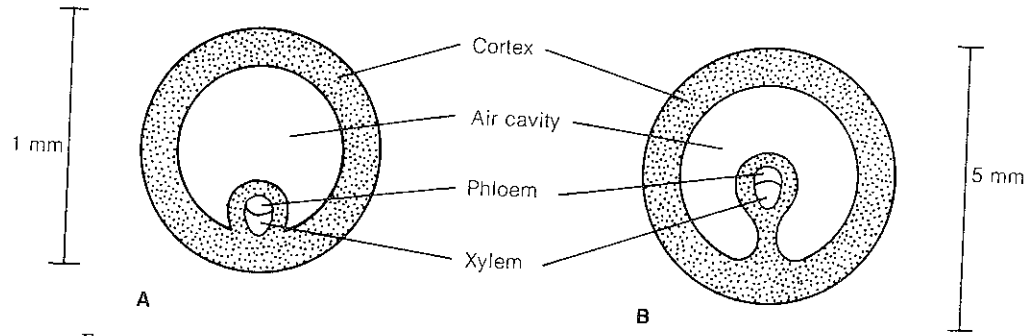



FIGURE 9-53 Schematic representations. **A,** transection of root of *Isoetes macrospora;* **B,** a stigmarian appendage of *Stigmaria.* [Redrawn from Stewart, *Amer. Jour. Bot.* 34:315–324. 1947.]

acid is known to result in stem elongation in many plants. Except for some proliferation of cortical tissue in the upper part of the corm, stimulated by the indoleacetic acid, there were no other obvious effects on growth (Zinda, 1966). Additional experiments, using combinations of certain growth regulators, would be of great interest to the better understanding of control of form in *Isoetes*.

*Isoetes howellii* has been shown to have crassulacean acid metabolism (CAM). It has been shown that when plants are completely submerged in vernal pools, dissolved free carbon dioxide is often totally depleted by noon on sunny days. The submerged leaves fix carbon dioxide into malic acid at night. During daylight hours, the malic acid is broken down and the carbon dioxide is utilized in photosynthesis. As the water level in vernal pools drops and the plants become emergent, the CAM mechanism is largely lost (Keeley et al., 1983).

**SPOROPHYLL AND SPORANGIUM DEVELOPMENT.** Sporophylls originate in a position lateral to the apical cone. Very soon after a sporophyll primordium is initiated, a ligule is produced on the adaxial face of the sporophyll. By repeated divisions the ligule soon overtops the leaf apex. The ligule eventually becomes tongue shaped (Fig. 9-50, A–C) and shows a high degree of histologic specialization comparable to ligules in *Selaginella* (p. 131–132).

In *Isoetes*, the glossopodium is deeply embedded in the leaf tissue and consists of a transverse bar and a pad that connects two side arms (Fig. 9-54); each of the arms may become anchor shaped (Sharma and Singh, 1984). In a median longitudinal section of a leaf only the pad is evident (Fig. 9-50, D). The sporangium and velum have their origin through periclinal divisions in surface cells below the ligule. The velum is first to take form, and is followed by growth of the sporangium. As a result of the first periclinal divisions, which localize the sporangial position, a central mass of potentially sporogenous cells is separated from outer layers of peripheral cells. During development, the cells of the outer layers may continue to add derivatives to the sporogenous mass. Ultimately the outer three or four peripheral layers constitute the sporangium wall. The sporogenous cells divide in all planes.

Microsporangia and megasporangia are indistinguishable during early stages of development, and it is only after the potential microsporocytes or megasporocytes become apparent that the two types of sporangia can be distinguished. In a microsporangium irregular groups of deeply staining cells ultimately become the microsporocytes, and cer-

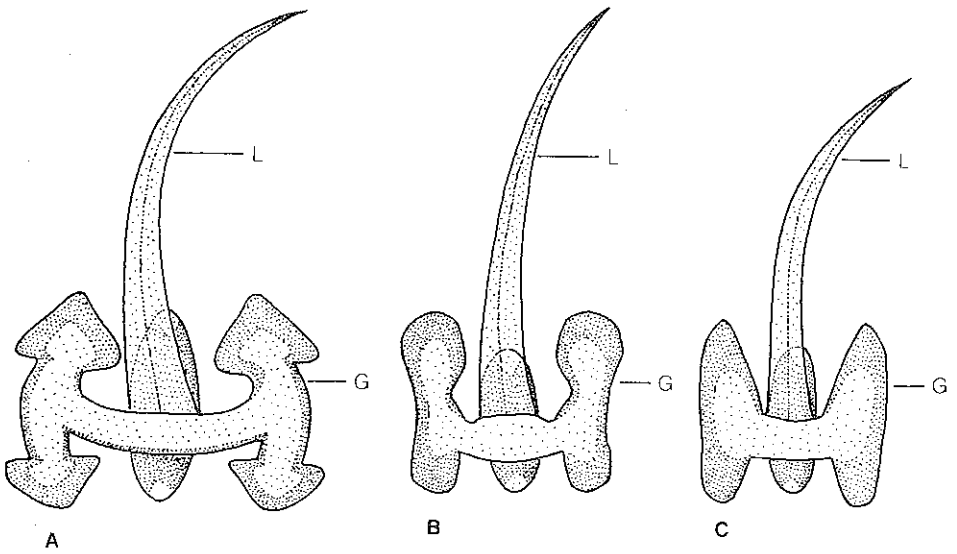



FIGURE 9-54 Reconstructions of *Isoetes* ligules. **A**, *Isoetes coromandelina*. **B**, *I. rajasthanensis*. **C**, *I. reticulata*. G, glossopodium; L, free portion of ligule. [Redrawn from Sharma and Singh, *Amer. Fern Jour.* 74:22–28, 1984.]

tain bands of lightly staining cells (originally potentially sporogenous) become the trabeculae, which traverse the sporogenous mass but do not divide it into compartments or locules (Fig. 9-55). Covering the trabeculae is a tapetum which may be biseriate or multilayered, the cells of which are derived also from potentially sporogenous cells. The tapetum of the trabeculae is continuous with a tapetal layer lining the sporangium wall. The microsporocytes separate from each other prior to meiosis. Estimates of the number of bifacial (monolete) microspores produced by a single sporangium range from 300,000 to 1,000,000. The spore number of each sporangium in *Isoetes* is probably greater than it is in any other vascular plant.

In a megasporangium, even before the trabeculae are distinguishable, certain cells are greatly enlarged over their neighbors and will become the megasporocytes. Not all of the enlarged cells will become megaspore mother cells, but some will degenerate and be resorbed. It is only after megaspore mother cells are in evidence that trabeculae become apparent. As a result of meiosis, each sporangium contains approximately 100 to 300 tetrahedral (trilete) megaspores which may range from 200 to 900 micrometers in diameter (Fig. 9-50, D). Megaspores may be white, gray, or black. The wall may be smooth or have a distinctive ornamentation. Monolete microspores are small, from 20 to 40 micrometers long, and have various characteristic wall patterns.

Sporangia are indehiscent, and liberation of spores is brought about by decay of sporophylls in the fall or winter seasons in cooler latitudes. Certain species of *Isoetes* growing in vernal pools, in which the corm is entirely buried, have a special means of exposing the sporangial contents. In these cases decaying sporophylls of the previous season are forced up by the expansion of mucilage cells at the base of the sporophylls, whereupon the spores are brought to the surface (Osborn, 1922). Distribution of spores is by wind, disturbance of mud in which certain species grow, or wave action in lakes; also, earthworms have been reported as carriers (Duthie, 1929).

**CYTOLOGY AND SYSTEMATICS.** The chromosomes of *Isoetes* range in length from 1 to 2 micrometers,

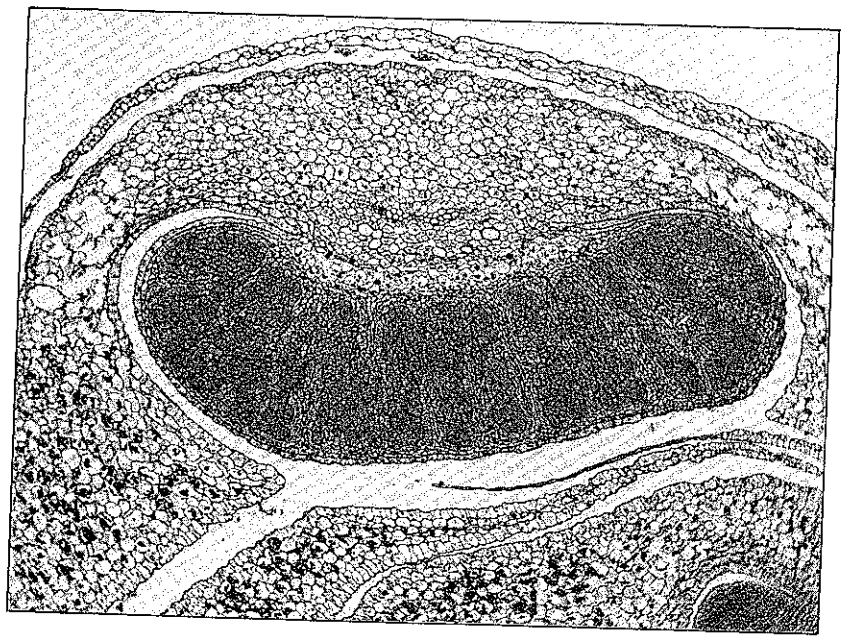


FIGURE 9-55 Transection of microsporophyll and developing microsporangium of *Isoetes howellii*. Trabeculae can be seen traversing the mass of potential microsporocytes. The bases of other sporophylls are evident.

or less, up to 7 to 8 micrometers. The basic chromosome number for the genus is $x = 11$, and polyploidy is common in the genus. A study of six species in northeastern North America revealed diploids $2n = 22$, tetraploids $2n = 44$, and one decaploid $2n = 110$ (Kott and Britton, 1980). A triploid $2n = 33$ has been reported for an apogamous (without fertilization) species from India (Abraham and Ninan, 1958), and a hexaploid $2n = 66$ for *I. japonica* from Japan (Löve et al., 1977). Chromosome counts of $2n = 22$, 44, and 132 have been reported for several neotropical species (Hickey, 1984).

Characters such as spore color and morphology, extent of velum cover, and leaf length have, for example, been used to separate species. The results of certain population studies indicate that these features are quite variable and become greatly modified under certain environmental conditions. For example, *Isoetes* populations associated with granite outcrops of the southeastern Piedmont in the United States range in morphology from those that can be classified as *I. melanospora*, through a series of intermediate populations, to those classified as *I. piedmontana*. Also, the populations have similar polyphenolic patterns (Matthews and Murdy, 1969).

Interspecific hybridization is now known to be common in *Isoetes* and probably accounts for much of the confusion in the past over the delimitations of species. Under natural conditions, species are often isolated geographically or ecologically and they can be distinguished morphologically from one another. However, the ease by which interspecific hybridization can occur between species of different sections of the genus has been demonstrated recently for four species. Crosses were set up in such a manner that the megaspores of each species were brought into contact with the microspores of every other species, under rigorous experimental conditions. Progeny were produced from all crosses (Boom, 1980). Experiments such as this may help to explain the several to many described varieties for certain species when populations impinge on each other or overlap geographically.

**GAMETOPHYTES.** Spores may germinate immediately after being shed from the sporangium, but generally germination does not take place until winter in warm-climate species or spring in cold-climate species, after decay of the sporophylls. The gametophytes, as in *Selaginella*, are endosporic. The microgametophyte is retained entirely within the spore wall, though a portion of the megagametophyte may be exposed.

**MICROGAMETOPHYTE DEVELOPMENT.** The first division of the microspore forms a small prothallial cell, and a large cell that is interpreted as an antheridial initial. By several divisions the antheridial initial is subdivided into a layer of jacket cells and a total of four spermatogenous cells (the actual spermatids, in this case). After a developmental period of about two weeks, the spore wall cracks along the flat surface. The jacket cells and prothallial cell degenerate, and four multiflagellate sperm are liberated (Liebig, 1931). The general scheme of development is comparable to that in *Selaginella* (Fig. 9-30). The possession of sperm with many flagella contrasts strikingly with the biflagellate condition of the sperm of other genera (*Lycopodium* and *Selaginella*) of the Lycophyta.

**MEGAGAMETOPHYTE DEVELOPMENT.** A mature megaspore contains a considerable amount of stored food surrounded by the spore wall. The primary nucleus is quite large and may be at the base or apex (toward the triradiate ridge) (Campbell, 1891; LaMotte, 1933). A period of free nuclear division ensues. Wall formation then takes place rapidly around nuclei at the apical end, proceeding basipetally and centripetally at a slower rate. Stored material is prominent in the basal end of the developing megagametophyte. No large central vacuole, as in *Selaginella*, is present. The megagametophyte may not become entirely cellular until the embryo is quite advanced in development (LaMotte, 1933). With an increase in volume of the megagametophyte, the megaspore wall breaks along the triradiate ridge (Fig. 9-56). The first archegonium appears at the apex, and at maturity consists of four tiers of neck cells, a neck-canal cell, a ventral canal cell, and an egg cell. If fertilization does not occur, many more archegonia may be formed among rhizoids that extend above the surface of the gametophyte (LaMotte, 1933).

**THE EMBRYO.** After fertilization, the first division of the zygote is transverse or oblique to the long

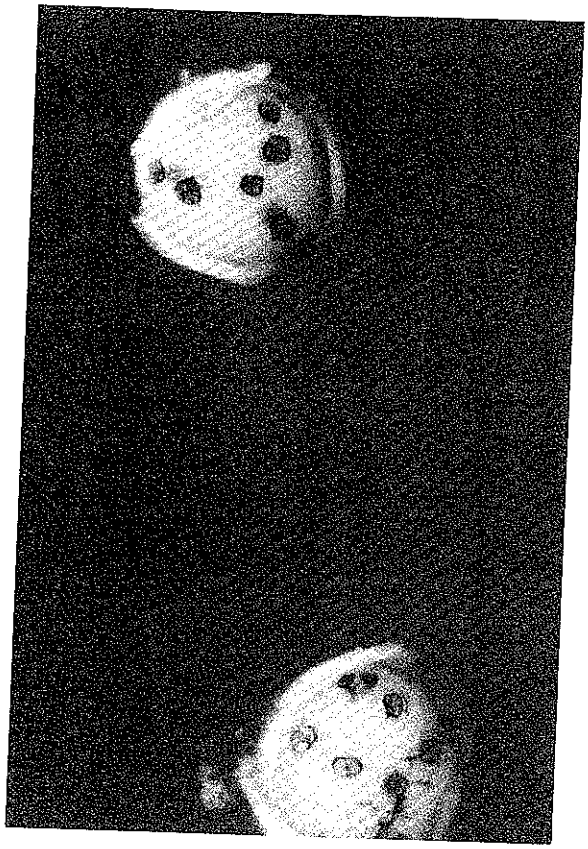


FIGURE 9-56 Megagametophytes of *Isoetes howellii* protruding through cracked spore walls. In each case, the megaspore wall was ruptured along the triradiate ridge, and three portions of the wall are visible. The dark areas are archegonia, and one tier of four neck cells is apparent in an archegonium of the gametophyte to the right.

axis of the archegonium (Fig. 9-57, A). The embryo becomes globose, and in some instances quadrants (Fig. 9-57, B) are recognizable (Campbell, 1891; LaMotte, 1937). The upper half (hypobasal) of the embryo (toward the neck of the archegonium) gives rise to the foot and root, and the lower half (epibasal) produces the first leaf and shoot apex. The embryo is thus endoscopic, but no suspensor is formed as in *Lycopodium* and *Selaginella*. Interpretation of subsequent embryonic development is indeed difficult, though the following description may represent a reasonably accurate account (LaMotte, 1933, 1937).

That portion of the embryo which will become the foot grows downward obliquely and into the storage tissue of the megagametophyte. At the same time, that portion of the embryo which will produce the first leaf and shoot apex grows laterally or perpendicular to the long axis of the archegonium. The primary root grows in the same plane but in the direction opposite from the leaf. Reorientation of the embryo is thereby achieved (Fig. 9-57, C, D). With further development the first leaf of the sporophyte breaks through the gametophytic sheath; the root emerges and turns downward. The young sporophyte may become firmly established on the substratum, but it remains attached for some time to the gametophyte and surrounding megaspore wall.

NEWLY DISCOVERED FORMS. Our study of *Isoetes* would be incomplete without a discussion of the recently described *Isoetes tegetiformans* (Rury, 1978), a mat-forming species that occurs in Georgia in the United States, and *Stylites* that occurs in Peru.

The corm of *I. tegetiformans* is bilobed, and the lobes exhibit intercalary extension from the median furrow (Fig. 9-58). Phyllotaxy is distichous (two rows of leaves) throughout the life of a plant, contrary to the usual helical arrangement in adult forms of other *Isoetes* species. The stem vasculature is a sympodium of leaf and root traces. Another distinctive feature is the presence of three rows of nondichotomizing roots. Also, adventitious buds arise on the elongate lobes of the corm.

The family Isoetaceae was considered to be monotypic until 1957 when another genus, *Stylites*, was established for plants which were found growing in dense cushions around the boggy margins of a small glacial lake high in the Andes of Peru (Amstutz, 1957). The plants resemble certain species of *Isoetes*, but the corms are much more elongate, up to several centimeters long, and branch dichotomously (Fig. 9-59, A, B).

The morphology, anatomy, and life history of the originally described species *(Stylites andicola)* and of another species *(S. gemmifera)* are now well known (Rauh and Falk, 1959a, b). Secondary growth occurs, as in *Isoetes*, but the root-producing meristem is upturned and the nondichotomizing roots, generally present only on one side of each axis, appear to be more lateral in position than basal. In *S. gemmifera* adventitious buds can arise at the base of sporophylls as has been reported for

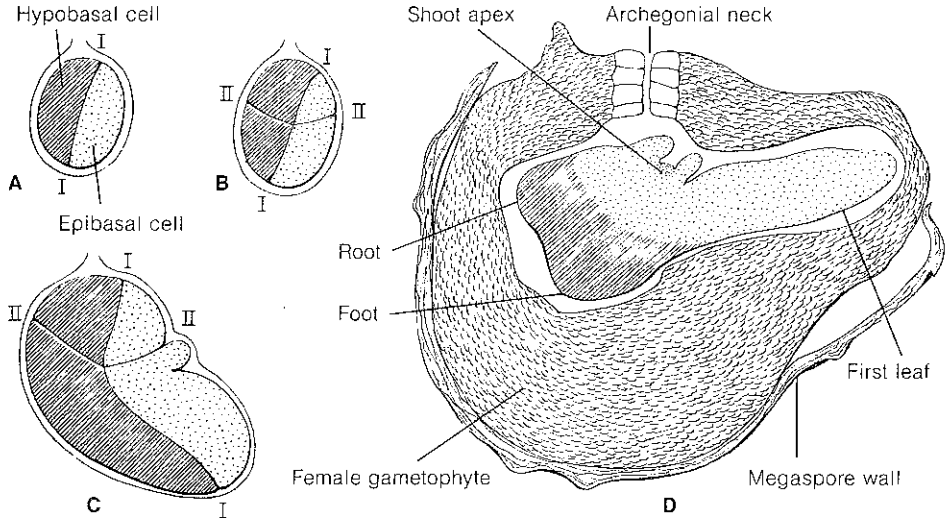



FIGURE 9-57 Early cleavages and subsequent growth of the embryo in *Isoetes lithophila*. **A,** the first cleavage (I-I) in this species is usually as shown, only rarely being at right angles to the neck of the archegonium; **B,** quadrant stage; **C, D,** later stages, each segment being multicellular, but not indicated by cells. More rapid growth occurs in the lower two quadrants, resulting in an apparent rotation of the embryo. [Redrawn from La Motte, *Ann. Bot.* n.s. 1:695, 1937; *Amer. Jour. Bot.* 20:217, 1933.]

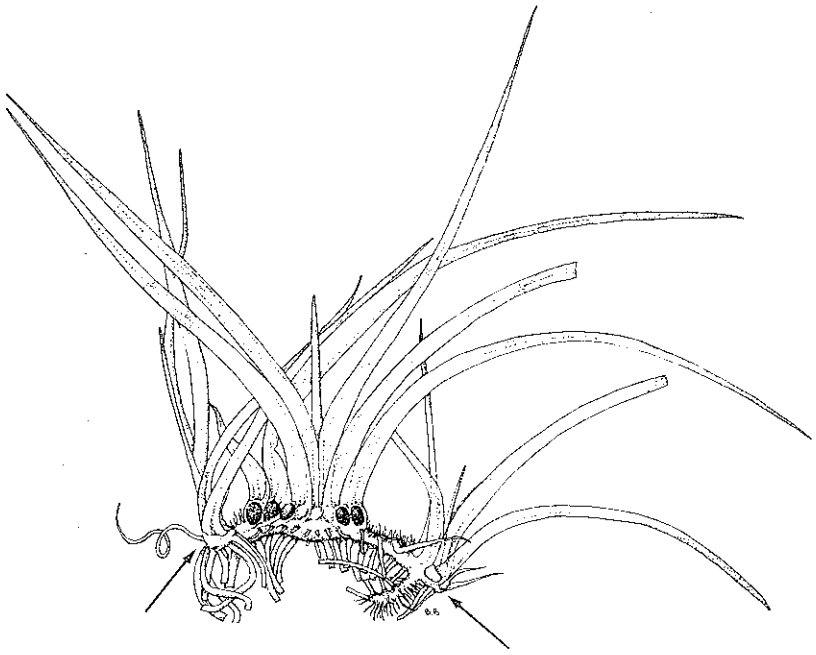


FIGURE 9-58 *Isoetes tegetiformans*. Arrows indicate the adventitious buds that arise on the elongate lobes of the corm. [Courtesy of Dr. P. M. Rury.]

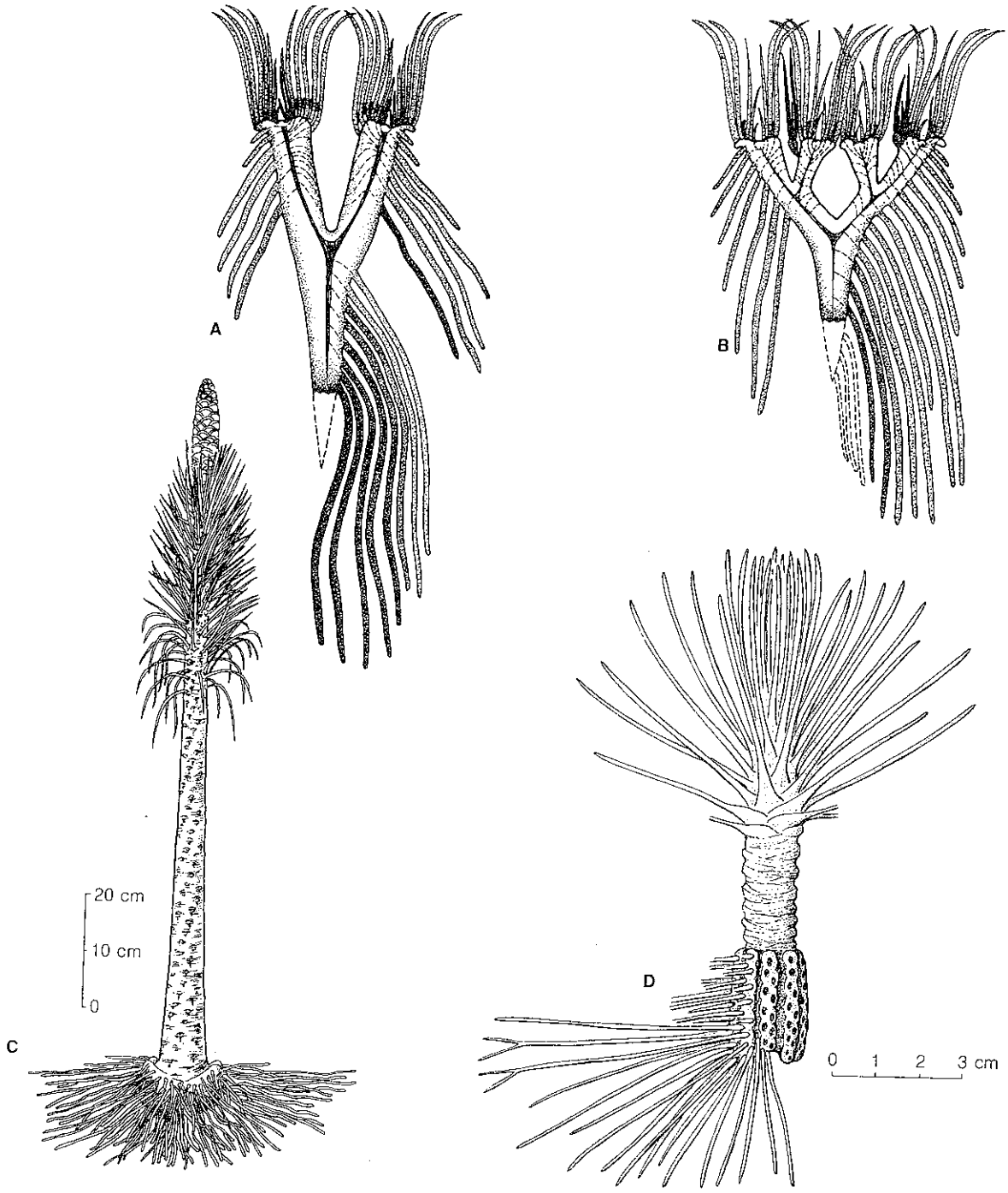



FIGURE 9-59 Schematic representation of growth form and type of branching in *Stylites andicola* (A) and *Stylites gemmifera* (B). C, reconstruction of *Pleuromeia sternbergii*. D, reconstruction of *Nathorstiana arborea*. [A, B redrawn from Rauh and Falk, *Sitzb. Heidelb. Akad. Wiss. Jahrgang* 1–83, 1959; C redrawn from Hirmer, *Palaeontographica* 78(B):47–56, 1933; D redrawn from *Paläobiologie der Pflanzen* by K. Magdefrau. Gustav Fischer Verlag, Stuttgart. 1968.]

certain species of *Isoetes*. An interesting physiological feature of *S. andicola* has been reported by Keeley et al. (1984). *Stylites* lacks stomata and derives nearly all of its photosynthetic carbon through its roots. This species possesses characteristics of crassulacean acid metabolism (CAM) comparable to *Isoetes howellii* (p. 161).

A reinvestigation of *Isoetes triquetra* from the Andes of Colombia revealed many similarities to *Stylites* (Kubitzki and Borchert, 1964). Enough information has been accumulated to convince some botanists that the validity of the genus *Stylites* should be questioned. Perhaps the two species of *Stylites* should be considered only as extremes in the morphological variation of *Isoetes*, or, at best, *Stylites* should be recognized only as a subgenus of *Isoetes*. (For discussions, see Rury, 1978, and Gomez, 1980.)

### Pleuromeiales

A representative member of the order Pleuromeiales is the extinct Triassic genus *Pleuromeia* (Fig. 9-59, C). The plant body consisted of an erect, unbranched stem about 1 meter or more in height and 10 centimeters in diameter. The stem axis terminated in a strobilus composed of closely overlapping sporophylls with apparently adaxial sporangia (Emberger, 1968; Delevoryas, 1962). Spirally arranged vegetative leaves were present beneath the strobilus. A very remarkable structural feature of the genus was the enlargement of the stem base. This basal part was divided commonly into four lobes (a lobed rhizophore) upon which roots were produced in an orderly fashion.

On the basis of a careful morphological study of *Pleuromeia*, Paolillo (1982) has presented evidence that root initiation was similar to that of *Isoetes*. Anatomically, the roots were of the "stigmarian" type (Fig. 9-53, B), and similar to *Isoetes*.

Another genus, *Nathorstiana*, of the Cretaceous, is often included in this order. The plants were probably about 10 to 30 centimeters tall, had a crown of leaves, and had an enlarged lobed basal rhizophore (Fig. 9-59, D). Paolillo (1982) has suggested that root initiation may have been comparable to that of *Isoetes* although, on the basis of a study of young unlobed rhizophores of *Nathorstiana*, Karrfalt (1984) and Rothwell (1984) believe that the basal meristems of the lobed types of *Nathorstiana* and of *Isoetes* are primary meristems rather than secondary meristems. According to their view a homology exists among the root-producing meristems of the rhizophores of lepidodendrids, *Nathorstiana*, and *Isoetes*.

Considerable evidence points toward a phylogenetic relationship among the three genera. The *Isoetes*-type plant body is probably a miniature form of the *Pleuromeia-Nathorstiana* type, brought about by the telescoping of the entire axis. Plants with certain features of a modern *Isoetes* occurred in the Triassic; a more convincing fossil, *Isoetites serratus*, has been described from the Upper Cretaceous. (See Taylor, 1981, for additional details.)

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