

PHYLOGENY AND DIVERSIFICATION OF BRYOPHYTES¹

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The bryophytes comprise three phyla of embryophytes that are well established to occupy the first nodes among extant lineages in the land-plant tree of life. The three bryophyte groups (hornworts, liverworts, mosses) may not form a monophyletic clade, but they share life history features including dominant free-living gametophytes and matrotrophic monosporangiate sporophytes. Because of their unique vegetative and reproductive innovations and their critical position in embryophyte phylogeny, studies of bryophytes are crucial to understanding the evolution of land plant morphology and genomes. This review focuses on phylogenetic relationships within each of the three divisions of bryophytes and relates morphological diversity to new insights about those relationships. Most previous work has been on the mosses, but progress on understanding the phylogeny of hornworts and liverworts is advancing at a rapid pace. Multilocus multigenome studies have been successful at resolving deep relationships within the mosses and liverworts, whereas single-gene analyses have advanced understanding of hornwort evolution.

Key words: Anthocerophyta; Bryophyta; bryophyte phylogeny; hornworts; liverworts; Marchantiophyta; mosses; tree of life.

As the only land plants with a dominant gametophyte generation, liverworts, mosses, and hornworts exhibit structural and reproductive attributes that are exclusive, unifying, and innovative. Their persistent gametophyte is responsible for exploratory growth as well as for proliferation of a new generation through either sexual or asexual processes. As a consequence, bryophyte gametophytes exhibit a degree of diversity and complexity unparalleled in tracheophytes. They are characterized by modular growth (repeated patterns) from a generative apex, range in habit from upright to procumbent, and include thalloid to leafy forms (Mishler and DeLuna, 1991). Within mosses and liverworts, leafy gametophytes are the norm, rivaling the leafy sporophytic growth forms of some tracheophytes, especially lycophytes (Renzaglia et al., 2000). However, because they depend on water for sexual reproduction, the gametophytes of bryophytes are small relative to most vascular plant sporophytes. Sexual reproduction in bryophytes involves release of motile male gametes into the environment and requires successful navigation of these naked cells from the male to the female sex organs via an external water source.

Sporophytes of bryophytes are without exception monosporangiate and matrotrophic throughout their life span (Graham and Wilcox, 2000). Ephemeral and dependent on the gametophyte for nutrition and protection, they never exhibit the modular, indeterminate growth form of the gametophyte generation. In their greatest structural complexity, bryophyte sporophytes consist of a nutritive foot, elongating pedicel or seta, and a single terminal sporangium or capsule. Formative divisions in the embryo produce all precursor components of the sporophyte; i.e., distinct embryonic regions are determined to develop into the three organographic zones of the mature spo-

rophyte: sporangium, seta, and foot. In contrast, an apical meristem initial develops in the embryo of tracheophytes and is subsequently responsible for continuous production of repeated shoot and root modules in these plants (Bierhorst, 1971; Kato and Imaichi, 1997). Capsules of bryophytes are structurally elaborate and, in some instances, exhibit complicated mechanisms for spore production and dispersal. Basal sporophyte elongation with nonsynchronized spore production in hornworts, elaters in liverworts, and peristomes of mosses provide examples of this complexity.

General treatments of bryophyte morphology can be found in Leitgeb (1874–1881), Campbell (1895), Goebel (1905), Smith (1955), Parihar (1965), Watson (1971), Puri (1973), Richardson (1981), Schofield (1985), and Crum (2001). The *Manual of Bryology*, edited by Verdoorn (1932), contains authoritative treatments of selected bryology topics that summarized the state of our knowledge at that time, and the *New Manual of Bryology*, edited by Schuster (1984), provided expanded updates more than fifty years later. Both manuals are still useful. Other edited volumes on various aspects of bryophyte biology especially relevant to the tree of life include Clarke and Duckett (1979), Smith (1982), and Shaw and Goffinet (2000).

The crucial position of bryophytes in embryophyte evolution—An unambiguous conclusion from the multitude of contemporary phylogenetic investigations of streptophytes is that bryophytes are the first green plants to successfully radiate into terrestrial niches. These small, inconspicuous plants have existed for several hundreds of millions of years and have played a prominent role in shaping atmospheric and edaphic change and the subsequent evolution of all forms of plant life on land. Explorations of life history phenomena in bryophytes and a solid understanding of interrelationships among them are necessary to reconstruct the early evolution of embryophytes.

The concept that the embryo/sporophyte evolved in land plants through intercalation of mitotic divisions between fertilization and meiosis is widely accepted (Graham, 1993; Graham and Wilcox, 2000). Based on this axiom, land plant evolution proceeded in the direction of progressively more elaborate sporophytes. Although generally true, unconditional acceptance of this trend leads to conclusions that ignore

¹ Manuscript received 5 January 2004; revision accepted 15 June 2004.

Various aspects of the research presented here were supported by NSF grants from the Systematic Biology and Assembling the Tree-of-Life Programs (DEB-0089131 to AJS and DEB-0235985 and DEB-0228679 to KSR). We are grateful to Christine Cargill, Cymon Cox, Barbara Crandall-Stotler, Christine Davis, R. Joel Duff, Laura Forrest, Bernard Goffinet, Xiaolan He-Nygrén, and Juan Carlos Villarreal for providing data and sharing unpublished manuscripts. We are also indebted to Andrew Blackwell and Scott Schuette for contributions of images and expert technical assistance. Molly McMullen kindly edited an earlier manuscript draft.

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processes of reduction and parallel/convergent evolution, phenomena that have occurred repeatedly during bryophyte diversification (Schuster, 1992; Niklas, 1997; Boisselier-Dubayle et al., 2002). A defining characteristic of embryophytes is the meiotic production of spores in tetrads and sporopollenin-impregnated spore walls. Because of their resistance to degradation, fossil spores have provided valuable clues to the initial stages of land colonization (Taylor, 1995; Wellman and Gray, 2000; Wellman et al., 2003). The earliest confirmed land plant fossils are spores, speculated to be from an ancient liverwort dating to the middle Ordovician, some 475 million years ago (mya) (Wellman et al., 2003).

Gametophytes of bryophytes also provide critical clues about land plant evolution. Thalloid and filamentous growth forms are shared with pteridophytes, but the completely subterranean and nonphotosynthetic life histories found in many lycophytes and some ferns show no homology in bryophytes (Bierhorst, 1971). The achlorophyllous gametophyte of the liverwort *Cryptothallus* is a recent acquisition within a strictly photosynthetic lineage (Renzaglia, 1982). Unlike pteridophytes, bryophyte gametophytes frequently show organ development (leaf, stem, and rhizome) and extensive tissue differentiation, including conducting and supportive tissues (Héban, 1977; Ligrone et al., 2000). Production of multicellular gametangia was an innovation in embryophytes that was a necessary precursor to embryo development (Graham and Wilcox, 2000). Among land plants, only mosses and liverworts produce superficial gametangia, which are variously protected by elaborate appendages, including leaves. Hornworts sequester vulnerable organs in internal compartments (Renzaglia et al., 2000; Renzaglia and Vaughn, 2000).

A lack of intermediate forms in both life history phases and the potential to interpret morphological transitions in opposite directions have obscured relationships among bryophytes and pteridophytes. Understanding morphological evolution requires unambiguous establishment of phylogenetic relationships among and within bryophyte lineages. Over the past decade, great strides have been made toward reaching this goal; however, fundamental questions remain.

In addition to elucidating early patterns of morphological diversification in embryophytes, bryophytes are crucial to understanding plant genome evolution. Approximately 66% of genes identified from expressed sequence tag analyses of gene expression in gametophytes of *Physcomitrella patens* have homologues in the *Arabidopsis* genome, consistent with the hypothesis that genes expressed in the diploid plant body of angiosperms were expressed in the gametophytes of early land plants and were recruited for sporophytic morphogenesis later in plant phylogeny (Nishiyama et al., 2003). Phylogenetic and functional analyses of genes expressed in *Physcomitrella* gametophytes have clarified the phylogenetic history of several important gene families, including MIKC-type MADS-box genes (Krogan and Ashton, 2000; Henschel et al., 2002; Hohe et al., 2002) and homeobox genes (Champagne and Ashton, 2001). Phylogenetic analyses of the KNOX (homeobox) gene family across the land plant tree of life have provided insights into the history of gene duplication and functional divergence during embryophyte history (Champagne and Ashton, 2001). Because KNOX genes are involved in expression of meristematic activity in vascular plant sporophytes, functional analyses of KNOX genes in mosses, liverworts, and hornworts are central to understanding evolution of plant development in embryophytes. Comparable studies of genes involved in flower

development are underway, and, in the context of phylogenetic analyses of bryophytes, the early evolution of these genes is now a tractable problem for investigation (Himi et al., 2001).

Relationships among the three lineages—Relationships among the three lineages of bryophytes remain one of the major unresolved questions in plant evolutionary biology (Goffinet, 2000). Virtually every conceivable hypothesis has been put forth in regards to primary branching patterns at the base of embryophytes. Most commonly, bryophytes are viewed as a grade of three monophyletic lineages, with an uncertain branching order (Mishler et al., 1994; Qiu et al., 1998). Controversy often focuses on which bryophyte group is sister to all other embryophytes, with two hypotheses most frequently supported: liverworts as sister to other embryophytes vs. hornworts as the sister group (Mishler et al., 1994; Hedderson et al., 1996, 1998; Malek et al., 1996; Garbary and Renzaglia, 1998; Qiu et al., 1998; Beckert et al., 1999; Duff and Nickrent, 1999; Nishiyama and Kato, 1999; Soltis et al., 1999; Nickrent et al., 2000; Renzaglia et al., 2000; Stech et al., 2003). A moss-plus-liverwort clade has been recovered in several of these analyses (Hedderson et al., 1996, 1998; Nishiyama and Kato, 1999; Nickrent et al., 2000; Renzaglia et al., 2000). Recently, it was postulated that hornworts, not mosses, are the closest living relative of tracheophytes. This speculation finds support in sequence data as well as in structural genomic features (Samigullin et al., 2002; Kelch et al., in press). In contrast, recent analyses of amino acid sequences based on entire plastid genomes provided support for a monophyletic bryophyte assemblage; however, these results must be viewed with caution because of severe limitations in taxon sampling (Nishiyama et al., in press).

The focus of this review is to present the current state of knowledge on phylogenetic relationships within, not among, hornworts, liverworts, and mosses. Emphasis is placed on synthesizing results of recent molecular investigations that have revolutionized interpretations of genetic and morphological diversification within each of these groups. Intriguing new perspectives on character evolution have emerged from these studies.

ANTHOCEROTOPHYTA

Hornwort classification and relationships—For centuries, botanists have marveled at the structural peculiarities of hornworts (Hofmeister, 1851; Leitgeb, 1879; Campbell, 1895, 1917, 1924; Goebel, 1905; Lang, 1907; Bower, 1935). In no other branch of the green tree of life does extension of each sporophyte involve continuous, presumably indeterminate, basipetal growth of a single elongated sporangium. All stages of spore development, from undifferentiated cells through premeiotic/meiotic spore mother cells to sequentially more mature spores, can be found in a single hornwort sporangium. A constant production of spores therefore ensures dispersal throughout the growing season for as long as the gametophyte persists. This mode of sporophyte development has no counterpart in other plant groups, thus obscuring the phylogenetic position of hornworts among green plants.

Hornworts have remained relatively unexplored at all levels of phylogenetic inquiry (Renzaglia and Vaughn, 2000; Stech et al., 2003; Duff et al., in press). The perception that hornworts are invariable, elusive, and difficult to identify has contributed to the paucity of systematic studies within the group.

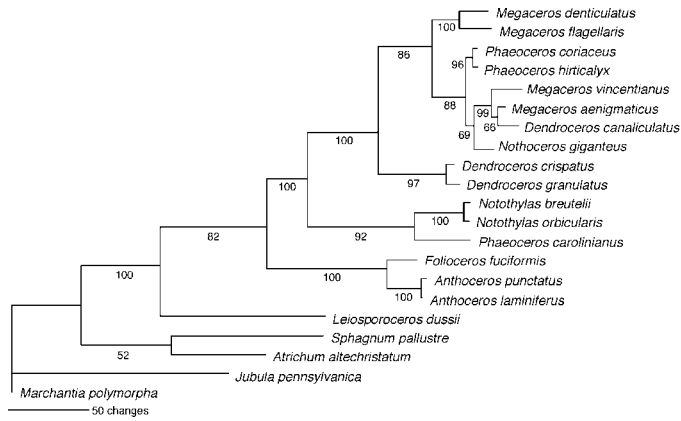


Fig. 1. “Backbone” phylogeny of the one most parsimonious tree based on *rbcL* sequences from seven of the 11 named genera of hornworts (Anthocerophyta). Bootstrap percentages are shown below branches. Tree provided by R. Joel Duff, University of Akron.

This small, homogeneous assemblage contains 100–150 poorly delineated species (Schuster, 1992). Concepts of interrelationships among hornworts based on morphology, and the resulting classification schemes, show virtually no consensus at the generic, familial, and ordinal levels (Mishler and Churchill, 1984; Hasegawa, 1988; Hässel de Menéndez, 1988; Schuster, 1992; Hyvönen and Piippo, 1993; Renzaglia and Vaughn, 2000). Twelve genera of hornworts have been named, *Anthoceros*, *Dendroceros*, *Folioceros*, *Notothylas*, *Megaceros*, *Phaeoceros*, *Aspiromitus*, *Hattorioceros*, *Leiosporoceros*, *Nothoceros*, *Mesoceros*, and *Sphaerosporoceros*, of which only the first six are widely recognized.

Even with the advent of molecular systematics and a renewed interest in early land plant phylogeny, hornwort sampling has been sparse, with one to three taxa included in most analyses (Katoh et al., 1983; van de Peer et al., 1990; Mishler et al., 1994; Bopp and Capiesius, 1996, 1998; Hedderson et al., 1996, 1998; Malek et al., 1996; Qiu et al., 1998; Beckert et al., 1999; Duff and Nickrent, 1999; Nishiyama and Kato, 1999; Soltis et al., 1999; Nickrent et al., 2000). Among the dozens of papers on bryophyte phylogeny over the past ten years, there is only one comprehensive molecular analysis of within-hornwort relationships, based on *rbcL* gene sequences from 20 hornworts (Duff et al., in press). A second study utilizing the plastid *trnL* intron sampled nine hornworts but focused on the position of the group among land plants (Stech et al., 2003). Results of these analyses are congruent and reveal novel but intuitive relationships. The *rbcL* analysis provided much greater resolution of hornwort interrelations because of more extensive sampling, including additional species of the five genera included in the *trnL* study and representatives of three other genera (*Folioceros*, *Leiosporoceros*, and *Nothoceros*). The discussion that follows will focus on taxonomic inferences and morphological character evolution that emerge from scrutiny of the consensus phylogenetic pattern supported by these pioneering studies (Fig. 1).

Diagnostic characters of hornworts are found in both life history generations and are variably emphasized by systematists (Cargill et al., in press). Growth form (Fig. 2), chloroplast structure and number (Fig. 3), antheridial number and jacket cell organization, *Nostoc* colony organization, and presence of mucilage canals and thallus outgrowths are taxonomically useful gametophytic characters. Taxonomically informative features of the sporophyte include degree of development of his-

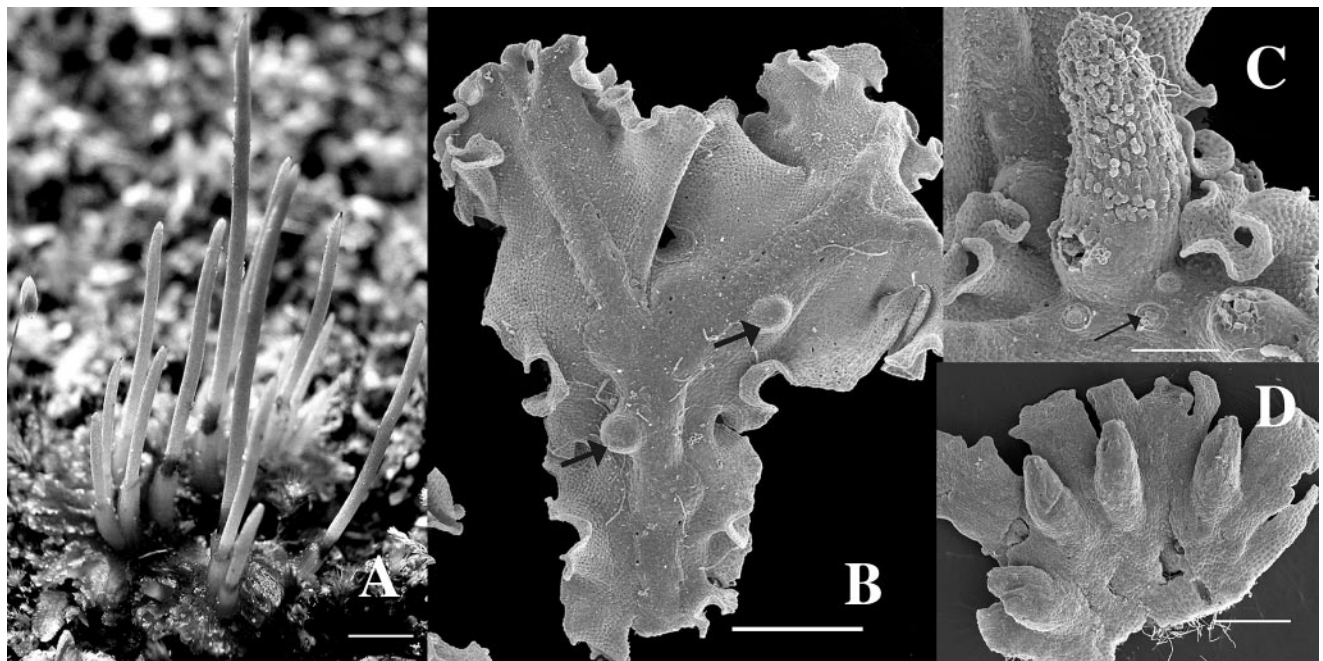


Fig. 2. Diversity in growth forms among hornworts. **A**. Photograph of *Anthoceros punctatus* L. Small orbicular gametophyte with both immature and almost ripe sporophytes, growing on soil. Image provided by Christine Cargill. **B** and **C** Scanning electron micrographs (SEM) of gametophyte of *Dendroceros crispatus* (Hook.) Nees. **B**. Ventral surface showing monostromatic wings and thickened midrib with bulging *Nostoc* colonies (arrow). Note the numerous small pores (mucilage clefts) along either side of the midrib. **C**. Dorsal surface showing sunken archegonia (arrow) on the midrib and developing sporophytes enclosed within gametophytic involucre. **D**. SEM of *Notothylas orbicularis* (Schwein.) Sull. Small orbicular gametophytes growing on bare soil; note the numerous small, horizontally oriented sporophytes enclosed in involucre. Bar = 0.2 mm, except in A, bar = 3 mm.

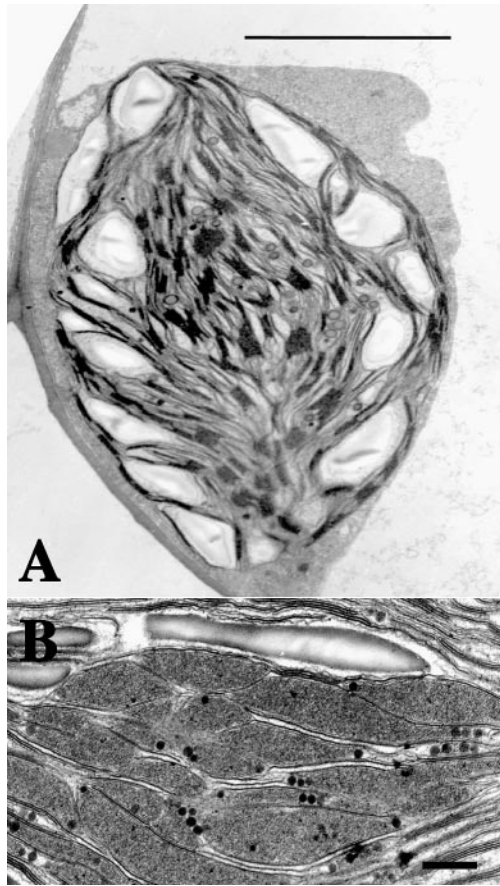


Fig. 3. Transmission electron micrographs of chloroplasts in hornworts. **A.** *Leiosporoceros dussii* (Steph.) Hässel. Chloroplast in the assimilative layer of the sporophytes showing peripheral starch and centralized grana. **B.** *Folioceros fuciformis* Baradw. Central pyrenoid with lens-shaped subunits separated by narrow grana and surrounded by starch grains. Bar = 0.5 μm .

togenic regions (Fig. 4), spore and pseudoelater architecture and ultrastructure (Fig. 5), and the presence of columella and stomata (Fig. 6).

The backbone of hornwort phylogenetic relationships—Molecular evidence corroborates morphological inferences that the hornworts are monophyletic (Fig. 1). The genus *Leiosporoceros*, which was named for its unusually small, monolete, smooth spores produced in isobilateral tetrads (Figs. 4A, 5A), is sister to the remaining hornworts. The position of *Leiosporoceros* among hornworts has been controversial. Hässel de Menéndez (1986, 1988) segregated it into an autonomous family and order, whereas Hasegawa (1988) and Schuster (1992) recognized it as a subgenus of *Phaeoceros*. In contrast, Hyvönen and Piippo (1993) supported a sister relationship between *Leiosporoceros* and *Folioceros*, based primarily on morphologically similar pseudoelaters (Fig. 5A). More detailed examination of *Leiosporoceros dussii* reveals morphological and molecular features heretofore undescribed in any hornwort. Moreover, *Leiosporoceros* gene sequences have extremely low levels of RNA editing (J. Duff, University of Akron, unpublished data) and thus differ from other hornworts that have been shown to have extensive editing (Yoshinga et al., 1996; Duff et al., in press). The gametophyte resembles that of *Phaeoceros* and *Megaceros* in that it is fleshy and lacks

internal mucilage canals. Number of antheridia per cavity is greater than 20, a feature shared only with *Anthoceros* and *Folioceros* (Cargill et al., in press). However, unlike other hornworts in which *Nostoc* is in discrete spherical colonies within the ventral thallus (Fig. 2B), those in *Leiosporoceros* occur in branching strands that run longitudinally and are sequestered in the thallus midregion. Ventral mucilage clefts that enable *Nostoc* to enter and establish colonies in other taxa (Fig. 6B) are lacking in *Leiosporoceros*. Chloroplasts of *Leiosporoceros* are also readily differentiated from those in other hornworts. Starch is neatly aggregated around the periphery of the organelle, with a central elaboration of photosynthetic membranes; the chloroplasts have no pyrenoids (Fig. 3A).

The sporophyte of *Leiosporoceros* is elongated and robust, and its anatomy departs significantly from that of other hornworts (Fig. 4A). The suture is highly differentiated and visible as a deep longitudinal groove. The assimilative and sporogenous regions are massive when compared with other hornworts. Several layers of small spore tetrads are surrounded by mucilage and interspersed with groups of large, elongated pseudoelaters (Fig. 5A). Stomata are abundant and appear similar to those in more derived taxa (Fig. 6A). Clearly, the shared traits between *Leiosporoceros* and other hornworts provide insight about plesiomorphies within the group. For example, stomata and large numbers of antheridia are best interpreted as ancestral hornwort traits. On the other hand, unique morphological traits that characterize *Leiosporoceros* are presumed autapomorphies and likely reflect the deep evolutionary separation of this genus from other hornworts. Further molecular and morphological studies are required to evaluate these hypotheses.

After *Leiosporoceros*, *Anthoceros* plus *Folioceros* form a clade sister to other hornworts (Fig. 1). Taxonomic treatments have generally recognized a sister relationship between *Anthoceros* (including *Folioceros*) and *Phaeoceros*, placing them in the same family or subfamily. Thus genetic divergence between *Anthoceros* plus *Folioceros* and the remaining taxa appears problematic at first glance. Similarities between *Anthoceros*, *Folioceros*, and *Phaeoceros* include rosette-like habits (Fig. 2A), large solitary chloroplasts with well-developed pyrenoids (Fig. 3B), and comparable sporophyte anatomy (Fig. 4B). However, clearly defined features distinguish *Anthoceros* and *Folioceros* from other hornworts; these include dorsal lamellae, schizogenous mucilage cavities, and antheridia in large groups of up to 50 per cavity, as compared to 1–4 (–6) antheridia per cavity in other hornworts. Darkly pigmented spores with well-defined trilete marks also serve to differentiate these taxa from other hornworts (Fig. 5B). Molecular evidence that *Anthoceros* plus *Folioceros* form a clade sister to all other hornworts (except *Leiosporoceros*) has reinforced the taxonomic value of diagnostic morphological features that are restricted to these two genera.

Close affinity between *Folioceros* and *Anthoceros* is well supported by *rbcL* data and reflected in most current classifications (Hasegawa, 1988, 1994; Schuster, 1992; Hyvönen and Piippo, 1993). In contrast, Hässel de Menéndez (1988) segregated *Folioceros* into a monotypic family and order based on spore ornamentation and pseudoelaters. *Folioceros* has thick-walled, reddish brown, highly elongated pseudoelaters, whereas *Anthoceros* has short, thin-walled multicellular pseudoelaters similar to those of *Phaeoceros* (Fig. 5D). Additional differences are found in the placenta and chloroplasts of these two taxa (Vaughn et al., 1992; Vaughn and Hasegawa, 1993).

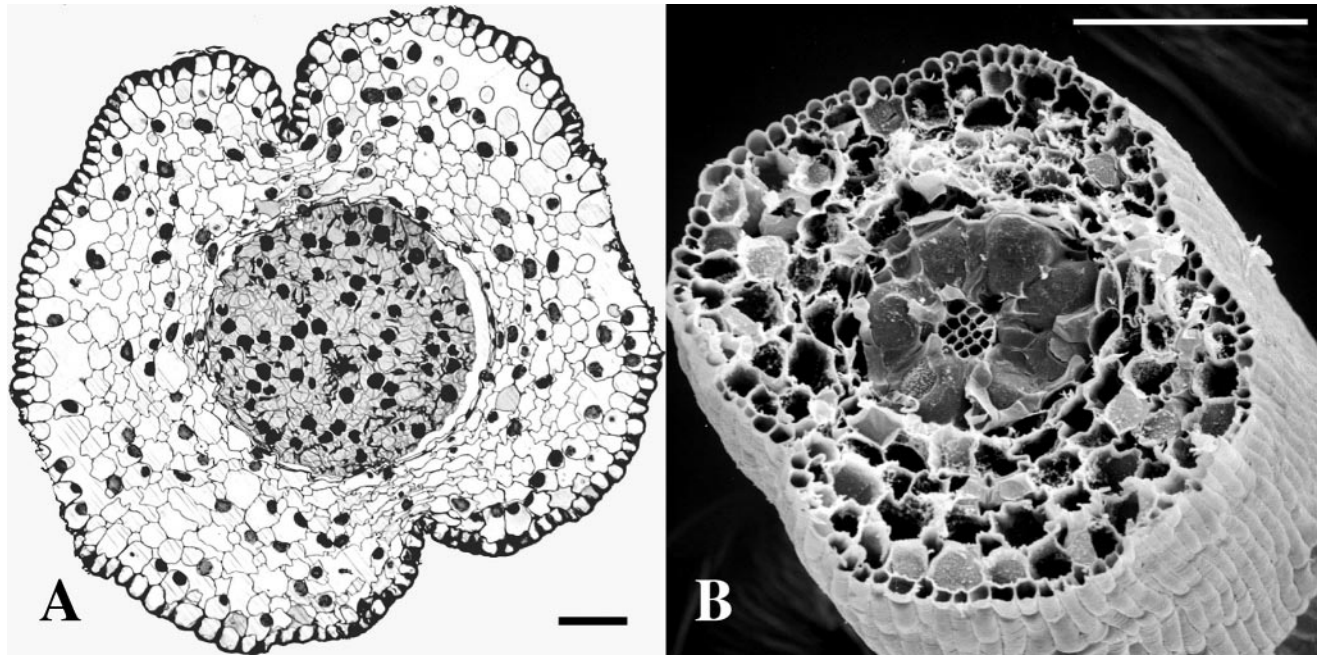


Fig. 4. Cross sections of hornwort sporophytes. **A.** Light micrograph of *Leiosporoceros dussii* (Steph.) Hässel. Tissue is differentiated from outside to inside as follows: single-layered epidermis, 9–10 layers of assimilative cells, abundant sporogenous tissue with several layers of tetrads intermixed with elaters, and an indistinct columella. The suture is clearly defined as a longitudinal groove that extends nearly to the sporogenous tissue. Bar = 100 μm . **B.** Scanning electron micrograph of *Phaeoceros carolinianus* (Michx.) Prosk. In contrast to Fig. 4A, this sporophyte contains an assimilative zone of four cell layers, sporogenous tissue with one layer of large tetrads intermixed with small elaters, and a columella of 16 cells. Bar = 1 mm.

With only a single species included in the *rbcL* sequence analysis, it is not possible to evaluate monophyly of *Folioceros*.

The remaining hornworts form a monophyletic group that includes two well-supported assemblages: *Phaeoceros laevis* sensu lato (represented in Fig. 1 by *P. carolinianus*) plus *Notothylas* and *Megaceros* plus *Dendroceros*. A close affinity between *Phaeoceros* and *Notothylas* was suggested by Hässel de Menéndez (1988), who placed these two genera in the family Notothyladaceae. Both genera have chloroplasts with prominent pyrenoids, spores with an equatorial girdle (Fig. 5C), and 2–4 (–6) antheridia per chamber. However, because of the distinctive sporophyte of *Notothylas* (Fig. 2D), most systematists have segregated this genus into a monotypic subfamily, family, or order (Singh, 2002). *Notothylas* is the only hornwort taxon in which growth of the sporophyte is abbreviated, spore production appears synchronized, stomata are absent, and the columella is normally absent to poorly developed, a combination of characters that indicate affinities with liverworts. Consequently, it has been suggested that the *Notothylas* sporophyte is plesiomorphic, representing a structural “link” with other bryophytes. Under this interpretation, hornwort radiation involved an elaboration of sporophytes in more derived taxa (Campbell, 1895; Mishler and Churchill, 1984; Graham, 1993; Hyvönen and Piippo, 1993; Hasegawa, 1994). An alternative hypothesis, supported by molecular data, is that sporophytes in *Notothylas* are not representative of the ancestral condition in hornworts but are highly reduced and specialized (Lang, 1907; Bartlett, 1928; Proskauer, 1960; Renzaglia, 1978; Schofield, 1985; Schuster, 1992). Features such as the existence of a relictual and largely nonfunctional suture in some species support the derived nature of the *Notothylas* sporophyte. If parallel reduction in sporophyte complexity occurred among hornwort genera, *Notothylas* may be polyphyletic

(Lange, 1907; Proskauer, 1960). An evaluation of this hypothesis requires increased taxon sampling across the hornworts.

Diversity within *Phaeoceros* is particularly evident in spore morphology (Schuster, 1992). *Phaeoceros laevis* s. l., includes species with spiny papillate spores, whereas ornamentation in the remaining species varies from vermiculate to blunt, wart-like projections. As described later, the three representatives of *Phaeoceros* with vermiculate spores included in molecular analyses are more closely related to *Megaceros* than to *P. laevis* s. l.

A close relationship between *Megaceros* and *Dendroceros* is evident in morphological characters such as spiraled pseudoelaters (Fig. 5E, F), absence of stomata, and solitary antheridia. The only epiphytic hornwort, *Dendroceros*, has a thickened, central midrib with perforated wings (Fig. 2B, C); large, central pyrenoids in each plastid; and multicellular spores (Fig. 5E) (Hasegawa, 1980; Renzaglia and Vaughn, 2000). Diagnostic features of *Megaceros* include unicellular green spores with distal mammilla (Fig. 5F), the absence of a pyrenoid, and multiple plastids per cell (Hasegawa, 1983; Valentine et al., 1986; Vaughn et al., 1992). However, as discussed next, the demarcation between *Megaceros* and *Dendroceros* is not always well defined, especially with regard to growth form (Proskauer, 1953; Hässel de Menéndez, 1962).

A clade containing two species of *Dendroceros* is sister to a monophyletic assemblage that includes species previously placed in *Megaceros*, *Phaeoceros*, *Nothoceros*, and *Dendroceros* (Fig. 1). This taxonomically heterogeneous group in turn consists of two clades: the first includes two Old World species of *Megaceros*, the Austral-Asian *M. flagellaris* and *M. denticulatus* (Hasegawa, 1983; Glenn, 1998), and the second is an assemblage of species from four generic segregates. Three *Phaeoceros* species, *P. coriaceus* (Steph.) Campbell, *P. hirti-*

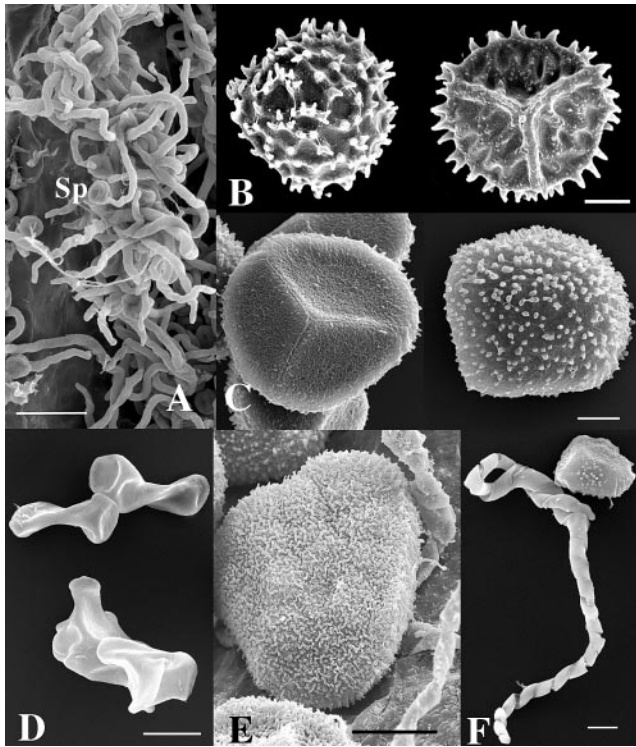


Fig. 5. Scanning electron micrographs (SEM) of spores and pseudoelaters of hornworts. **A.** *Leiosporoceros dussii* (Steph.) Hässel. Abundant elongated, slightly spiraled-thickened pseudoelaters with scattered small, smooth, isobilateral spore tetrads (Sp). **B.** *Anthoceros* spores showing spinelike echinae on the distal surface (left) and a well-developed triradiate ridge on the proximal face. Image provided by Christine Cargill. **C.** *Phaeoceros* spores showing papillose distal surface (right) and proximal surface with an indistinct trilete mark similar to the equatorial girdle (not visible). **D.** Short multicellular pseudoelaters of *Phaeoceros*. **E.** *Dendroceros crispatus* (Hook.) Nees. Multicellular spores and spiraled pseudoelaters. **F.** *Megaceros gracilis* (Reich.) Steph. Spiraled pseudoelater and spore with proximal surface facing downward and distal surface with mamilla upward. Bar = 10 μm , except in A, bar = 50 μm .

calyx Steph., and *Phaeoceros chiloensis* (Steph.) Hässel de Menéndez, are included within the second *Megaceros* clade (Stech et al., 2003; Duff et al., in press). All these species have spores with markedly different architecture from those of other *Phaeoceros* (Fig. 5C). Moreover, these species have monoandrous androecia and multiple chloroplasts that lack pyrenoids, two diagnostic characters of *Megaceros* (Duff et al., in press). However, lack of spiraled pseudoelaters, existence of stomata, and yellow, not green, spores are defining features of *Phaeoceros*. Bartlett (1928) and Proskauer (1951) noted that morphological boundaries between *Megaceros* and *Phaeoceros* are blurred and that similarities in growth form and chloroplast structure suggest a close relationship between the two. Molecular analyses have indicated that *Phaeoceros* with vermiculate spores and mamillae on the distal faces are more closely related to *Megaceros* than to *Phaeoceros* with papillate spores (*P. laevis* s. l.) (Fig. 5B).

The crown group of *Megaceros* consists of *M. aenigmaticus*, the only North American representative of the genus; *M. vincentianus*, the only species from the Neotropics; *Dendroceros canaliculatus* (= *M. canaliculatus*), and *Nothoceros giganteus* (= *M. giganteus*). The last two species have thickened midribs and wings, which accounts for previous, and appar-

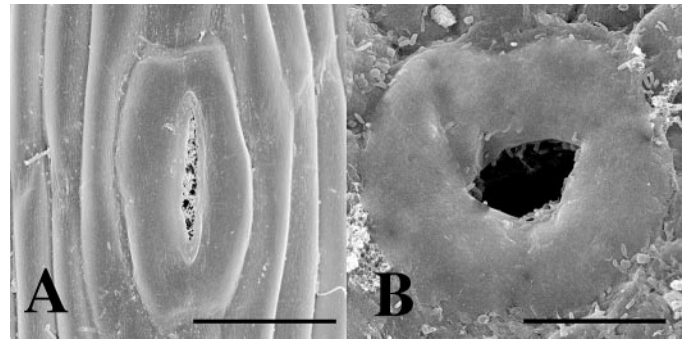


Fig. 6. Scanning electron micrographs of pores in hornworts. **A.** Stoma in the sporophyte epidermis of *Phaeoceros carolinianus* (Michx.) Prosk. **B.** Ventral mucilage cleft in the gametophyte epidermis of *Dendroceros crispatus* (Hook.) Nees. Bars = 20 μm .

ently inappropriate, placements in *Dendroceros* or the newly delineated *Nothoceros*. The existence of unicellular, mamillate spores, and plastids devoid of pyrenoids clearly place these species in *Megaceros*. Thus, scrutiny of the morphology of these seemingly disparate hornwort species reveals features that solidify their inclusion in the *Megaceros* clade. The well-developed costa and monostromatic wings in these taxa were likely a result of parallel evolution with *Dendroceros*.

Inferences about morphological evolution in hornworts from molecular analyses—One intriguing feature of hornworts is the large, solitary chloroplast with a prominent pyrenoid, which is shared with green algae but has no parallel in any other embryophyte group. Within hornworts, pyrenoids appear to have been lost multiple times. Similar pyrenoid losses (and gains) have been described in several algal lineages (Hoham et al., 2002; Nozaki et al., 2002). In hornworts, chloroplast compartmentalization characterizes several taxa, including *Leiosporoceros* and certain species of *Phaeoceros*, *Anthoceros*, and *Megaceros* (Burr, 1970; Valentine et al., 1986; Vaughn et al., 1992; Duff et al., in press). This arrangement is consistent with a carbon-concentrating mechanism typical of organisms with pyrenoids, including other hornworts (Smith and Griffiths, 1996, 2000; Hansen et al., 2002). It has been speculated that the “pyrenoid-like” area evident in certain hornworts represents a transitional state from presence to complete absence of the pyrenoid (Burr, 1970). An evolutionary inference supported by this interpretation, in addition to the phylogenetic topology presented in Fig. 1, is that a solitary plastid with a pyrenoid is plesiomorphic in hornworts. In *Leiosporoceros*, the plastid is solitary but without a pyrenoid, the remnant of which is a compartmentalized organelle with peripherally aggregated starch and centralized grana and plastoglobuli (Fig. 3A). Independent losses of the pyrenoid with or without organellar compartmentalization occurred at least once each in *Megaceros*, *Notothylas*, and *Anthoceros* (Vaughn et al., 1992; Singh, 2002; Duff et al., in press). In *Phaeoceros hirticalyx*, *P. coriaceus*, and *P. chiloensis*, species that are probably better placed in *Megaceros*, loss of the pyrenoid may be interpreted as preceding the evolution of spiraled pseudoelaters and stomatal loss.

As structures that facilitate gas exchange, stomata are important innovations in the diversification of land plants. Their presence in hornworts has been viewed either as a synapomorphy with mosses and tracheophytes or as a homoplastic

acquisition within hornworts (Mishler and Churchill, 1984; Kenrick and Crane, 1997; Renzaglia et al., 2000). The presence of stomata in *Leiosporoceros*, *Anthoceros*, and *Folioceros* supports the contention that these structures are plesiomorphic in hornworts and may be homologous to those in mosses and/or tracheophytes. A clear case of homoplasy is the loss of stomata in at least three, possibly four, hornwort lineages: *Notothylas*, *Dendroceros*, and *Megaceros*. Stomatal loss may have accompanied modifications in sporophyte development, e.g., maturation of the sporophyte within the protective gametophytic involucre where gas exchange is limited (*Notothylas*, Fig. 2D and *Dendroceros*, Fig. 2C). Stomatal loss in *Megaceros* is associated with occurrence of these species in periodically inundated habitats. The existence of *P. coriaceus*, *P. hirticalyx*, and *P. chilensis*, three terrestrial species with stomata, supports this hypothesis. The topology presented in Fig. 1 necessitates at least two losses of stomata in the *Megaceros* clade.

The interpretation set forth by Proskauer (1951) and Schuster (1992) that mucilage clefts on the ventral side of the gametophyte in hornworts (Fig. 6B) are homologous to sporophytic stomata (Fig. 6A) is not supported by molecular analysis. Absence of mucilage clefts in *Leiosporoceros* and the specialized function of these structures in all other hornworts indicate that gametophytic “stomata” evolved after hornwort diversification simply as an entryway for the cyanobacterium, *Nostoc*.

It is reasonable to hypothesize that the habit of extant members of *Anthoceros*, *Leiosporoceros*, and *Phaeoceros* represent the ancestral condition in hornworts and may be related to their common occurrence on exposed soil. Morphological diversity in other taxa likely results from radiation into and consequent adaptations to specialized habitats; e.g., *Dendroceros* is an epiphyte, and *Megaceros* is restricted to tropical or temperate sites where it often occurs submerged in streams. Diversification of *Dendroceros* may be correlated with the evolution of angiosperms, which provided abundant new bark and leaf habitats (Ahoen et al., 2003). *Notothylas* is an ephemeral hornwort that grows as a pioneer on soil. Unlike other genera in which spores are wind dispersed, *Notothylas* spores are dispersed by water or facultatively by insects or other animals, thus eliminating the “need” for vertical elongation of the sporophyte.

MARCHANTIOPHYTA

Liverwort classification and relationships—The immense morphological diversity among the 377 genera and 6000–8000 species of liverworts has presented significant challenges to systematists (Schljakov, 1972; Schuster, 1984; Crandall-Stotler and Stotler, 2000). Within this monophyletic assemblage are several morphologically isolated elements that represent products of deep divergences (Garbary and Renzaglia, 1998; Renzaglia et al., 2000). Morphological heterogeneity in the group is particularly evident in growth form of the gametophyte, which shows the greatest range of variability among bryophytes. Since the starting point of liverwort nomenclature (Linnaeus, 1753) and the beginning of their systematic treatment (Endlicher, 1841), hepatics have been organized into three groups based on growth form: (1) complex thalloids, (2) simple thalloids, and (3) leafy liverworts. Conflicting concepts of diversification have led to opposing views on the directionality of change within liverworts; that is, whether thalloid or

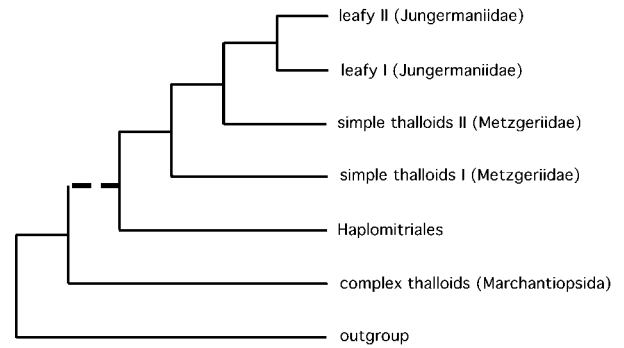
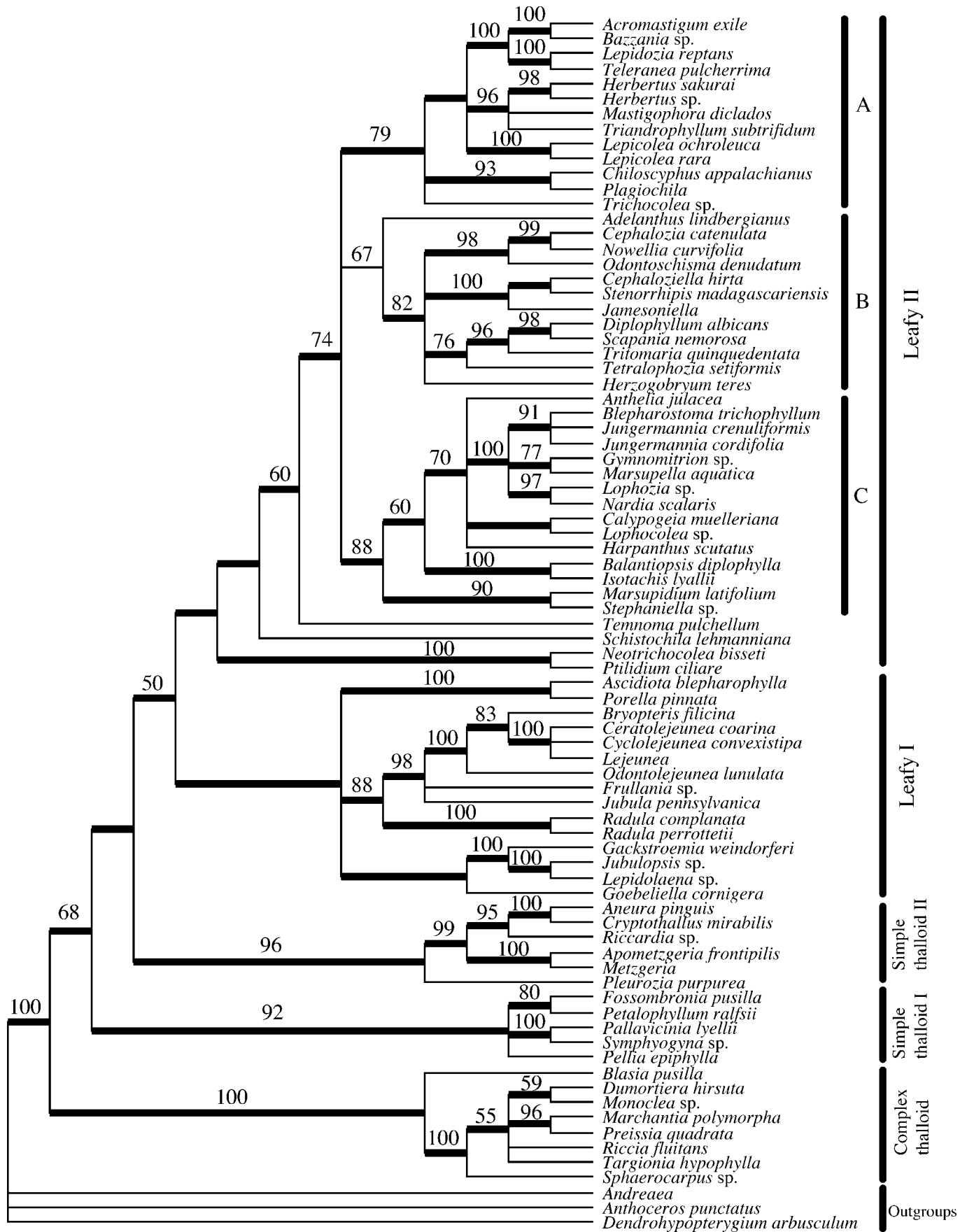


Fig. 7. “Backbone” tree showing phylogenetic relationships among the major clades of liverworts, redrawn from Davis (in press). The topology is from a maximum likelihood analysis of 12 nuclear, plastid, and mitochondrial genes. Broken-bold branch indicates uncertainty in the placement of *Haplomitrium*.

leafy forms are viewed as ancestral (see literature review in Crandall-Stotler and Stotler, 2000 and Davis, in press [Figs. 7, 8]). Morphological studies supported the concept that simple thalloid liverworts are more closely related to leafy types than to complex thalloids. Classification schemes reflect this interpretation with hepatics typically divided into two groups: marchantioid or complex thalloid liverworts (Marchantiopsida, Marchantiidae) and jungermannioid liverworts, including the leafy (Jungermanniopsida, Jungermanniidae) and simple thalloid taxa (Jungermanniopsida, Metzgeriidae). Complex thalloid types usually have air chambers with dorsal pores and differentiated internal tissues (Fig. 9A). Less commonly, the thallus resembles the simple thalloid type in the lack of internal or epidermal differentiation (e.g., *Sphaerocarpos*, *Monoclea*, and *Dumortiera*). Gametophytes of leafy liverworts range from radially symmetrical with three rows of morphologically similar leaves (isophyllous) to dorsiventral with two rows of lateral leaves and an additional row of reduced (to absent) ventral underleaves or amphigastria (anisophyllous; Fig. 9C). Simple thalloid (metzgerialean) organizations show less variability, from fleshy undifferentiated thalli to those with prominent midribs and monostromatic wings (Fig. 9B). Leaf-like lobes or lobules in some taxa blur the distinction between leafy and simple thalloid forms. Internal differentiation of water-conducting tissue is restricted to *Haplomitrium* and certain simple thalloid taxa, whereas conducting parenchyma is widespread among both complex and simple thalloid forms, but not leafy taxa (Héban, 1977; Kobiyama and Crandall-Stotler, 1999; Ligrone et al., 2000).

Liverworts are distinguished from hornworts and mosses by the possession of oil bodies, unique organelles in which terpenoids accumulate (Fig. 9D). All other embryophytes, including mosses and hornworts, produce cytoplasmic oil droplets (usually triglycerides), but they are not sequestered in specialized organelles. Although the function of the oil body is controversial, these single-membrane-bound organelles are restricted to hepatics and occur in approximately 90% of taxa. Derived from endoplasmic reticulum in meristematic cells (Duckett and Ligrone, 1995), oil bodies provide valuable taxonomic information because their size, shape, number, and color are taxon specific (Crandall-Stotler and Stotler, 2000).

Unlike hornworts, but comparable to mosses, is the production of a variety of organized external appendages, most of which function in protecting fragile tissues. For example, var-



ious mucilage papillae, hairs, scales, bracts, cups, or flask-shaped structures protect the meristem, gemmae, and other vegetative organs. Especially vulnerable are the superficial sex organs that often occur in clusters protected by flaps of tissue, leaf lobes, young leaves, or modified branches (Fig. 9B).

The uniformity and uniqueness of liverwort sporophytes provide compelling evidence for monophyly of hepatics. Unlike mosses and hornworts, sporophytes of liverworts reach maturity within the confines of protective gametophytic tissue that develops from the shoot/thallus (= perigynium or coelocaulis) and/or archegonium (= calyptra). Additional gametophytic structures such as perianths, pseudoperianths, bracts, scales, and involucre flaps may further surround the sporophyte and associated protective tissue. In such a milieu, photosynthesis is limited, and the sporophyte derives nourishment from the gametophyte through a placenta. The seta is pale to hyaline, and the capsule is devoid of stomata. The majority of liverwort sporophytes are differentiated into foot, seta, and capsule; in the occasional marchantioid taxon (e.g., *Riccia*, *Corsinia*), the seta and/or foot is vestigial or absent. At completion of meiosis and spore development, cells of the seta typically undergo rapid elongation through water imbibition and thus elevate the capsule away from the substrate. Sterile, elongated elaters have hygroscopic, spiraled, inner-wall thickenings, that are strategically interspersed among spores to facilitate their separation and dispersal (Fig. 9E). Capsule dehiscence normally entails a patterned separation into four longitudinal valves, but variations range from two valves through irregular fragments or plates to cleistocarpous capsules.

The backbone of liverwort phylogenetic relationships—Crandall-Stotler and Stotler (2000) used morphological characters in a cladistic analysis of liverworts. Their analyses included 34 taxa and 61 characters, and they resolved two main lineages: complex thalloids (Marchantiopsida) and simple thalloids plus leafies (Jungermanniopsida: Metzgeriidae, Jungermanniidae, respectively). However, their sampling was not extensive enough to address phylogenetic issues within any of the major clades. There are a few taxa for which placement relative to the three large groups is ambiguous on the basis of morphological, ultrastructural, and chemical features. These include *Treubia* and *Apotreubia* (Treubiales), *Monoclea* (Monocleales), *Sphaerocarpos*, *Geothallus* and *Riella* (Sphaerocarpaceales), *Blasia* and *Cavicularia* (Blasiales), and *Haplomitrium* (Haplomitriales [Calobryales]). Early molecular analyses of the liverworts were limited to single genomic regions with limited taxon sampling (e.g., Lewis et al., 1997; Bopp and Capesius, 1998; Beckert et al., 1999; Stech and Frey, 2001) but recent multigene analyses with increased sampling have begun to clarify phylogenetic relations among (and within) the major groups of liverworts. Phylogenetic relationships within Marchantiopsida (complex thalloids) from DNA sequence data were analyzed by Bischler (1998), Wheeler (2000), and Boisselier-Dubayle et al. (2002). Forrest and Crandall-Stotler (in press) focused on Metzgeriidae (simple thalloids), whereas HeNygrén et al. (in press) sampled a wide diversity of liverwort

taxa. Davis (in press) provided the most extensive analysis of relationships among leafy liverwort genera available to date.

Davis (in press) reconstructed “backbone” relationships among liverworts based on a combined data set including two nuclear, three mitochondrial, and eight loci sequenced from 20 liverworts and three outgroup mosses (Fig. 7). The data were analyzed using maximum parsimony, maximum likelihood, and Bayesian inference, and most of the results were robust to these alternative methods. The liverworts are resolved as monophyletic, as are class Marchantiopsida (complex thalloids) and Jungermanniidae (leafies). Metzgeriidae are resolved as a grade paraphyletic to Jungermanniidae, in agreement with earlier studies. Although Forrest and Crandall-Stotler (in press) sampled different species, results of their analysis of five plastid loci are congruent with those of Davis (in press).

Although *Haplomitrium* has generally been regarded as an early-diverging lineage within the liverworts (Smith, 1955; Schuster, 1984; Renzaglia et al., 1994), the precise placement of this genus remains problematic. The gametophyte of *Haplomitrium* is erect and radially symmetrical and therefore reminiscent of both jungermannian liverworts and mosses. Prior to the discovery of antheridia and sporophytes in *Takakia* (Smith and Davison, 1993; Renzaglia et al., 1997), *Haplomitrium* was considered closely related to *Takakia* because of gametophytic similarities (Schuster, 1972, 1984). More recent molecular and morphological data have come together to solidify the placement of *Takakia* among mosses (see later). Divergent opinions have been expressed with regard to the relationship of *Haplomitrium* to other hepatics. A conclusion from Bartholomew-Began’s (1990, 1991) extensive morphogenetic reevaluation of *Haplomitrium* was that the genus is a member of the simple thalloid lineage. In their analysis of land plant relationships based on *rbcL* sequences, Lewis et al. (1997) noted that the precise position of the genus depended on the data set analyzed (1st and 2nd vs. 3rd positions, all positions, “ts/tv” weighting); *Haplomitrium* fell out sister to all other embryophytes, sister to all other liverworts, or nested within the liverworts and sister to the leafy taxa. Nuclear 18S rDNA sequences resolved *Haplomitrium* (without bootstrap support) as sister to the class Jungermanniopsida (i.e., leafies plus simple thalloids; Hedderson et al., 1996).

Recent multigene analyses have focused on two hypotheses: *Haplomitrium* is either sister to Jungermanniopsida or sister to all other liverworts. In contrast to almost all other nodes on her tree, Davis (in press) reported that the placement of *Haplomitrium* varied among analyses. Under parsimony, likelihood, and Bayesian methods, *Haplomitrium* is resolved with strong support as sister to Jungermanniopsida (simple thalloids plus leafies), and this inclusive clade is in turn sister to Marchantiopsida (complex thalloids; Fig. 7). However, the most complex heterogeneous Bayesian substitution model, with 21 partitions, yielded *Haplomitrium* as the sister group to all other liverworts. Forrest and Crandall-Stotler (in press) and Qiu (2003) reported that *Haplomitrium* plus *Treubia* form a clade sister to all other hepatics. However, the sister-group relation-

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Fig. 8. Phylogenetic relationships among liverworts, especially the Jungermanniidae (leafies). Homogeneous Bayesian 95% majority rule tree from a four-gene data matrix (Davis, in press). Bold branches indicate significant support for the clade in all Bayesian analyses (homogeneous and heterogeneous posterior probabilities ≥ 95). Parsimony bootstrap values ≥ 50 are shown on the tree.

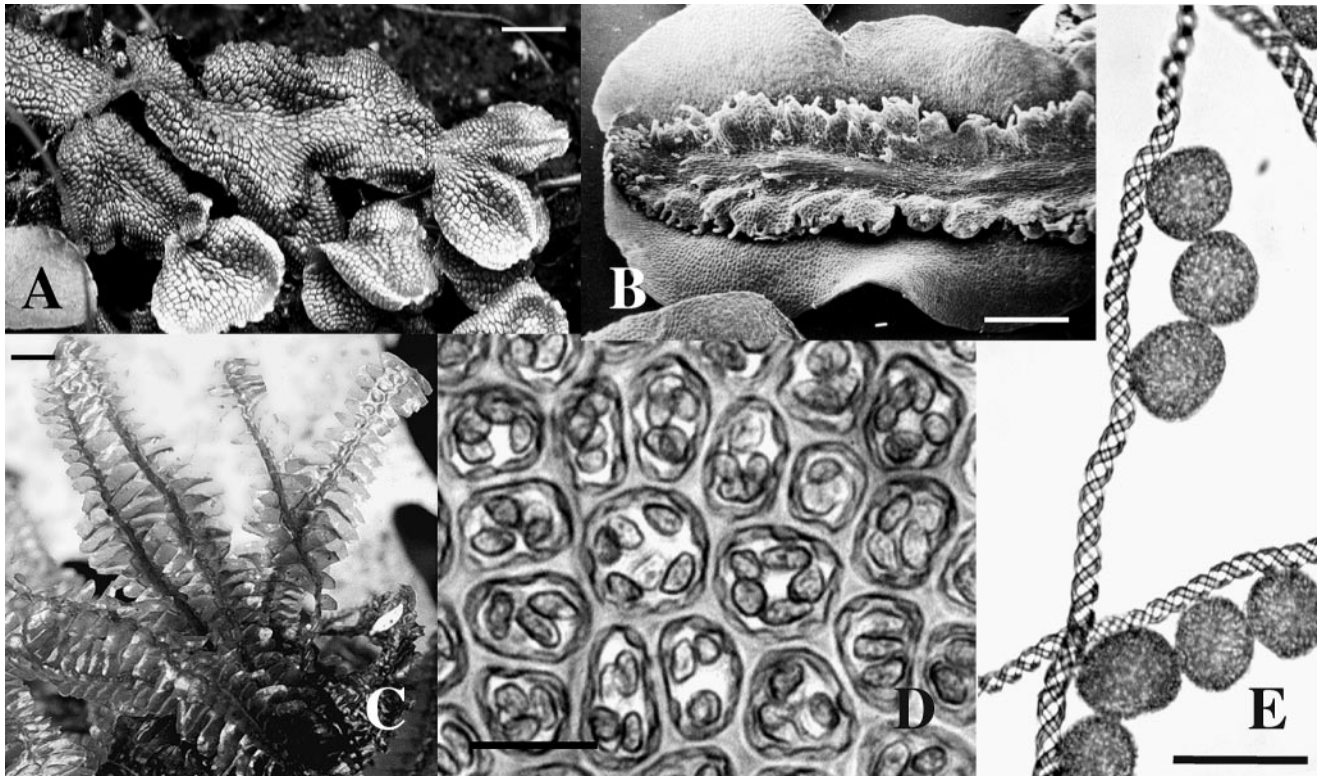


Fig. 9. Morphological diversity in liverworts. **A.** Photograph of complex thalloid gametophyte of *Conocephalum conicum* (L.) Lindb. Note polygonal air chambers on the dorsal surface. Bar = 1.0 cm. **B.** SEM of simple thalloid gametophyte of *Pallavicinia lyellii* (Hook.) Gray showing monostromatic wings and thickened midrib. Flaps of tissue on either side of the midrib cover protect antheridia in this male plant. Bar = 1.0 mm. **C.** Photograph of leafy gametophyte of *Bazzania trilobata* (L.) S. Gray showing incubous leafy insertion from dorsal aspect. The shoot on the right is seen from the ventral side revealing the small row of underleaves. Bar = 1.0 mm. **D.** Light micrograph of large oil bodies in the leaves of *Calypogeia muelleriana* (Schiffn.) K. Muell. Bar = 50 μm . **E.** Light micrograph of spores and elongated spiraled elaters in *Pallavicinia lyellii* (Hook.) Gray. Image provided by Scott Schuette. Bar = 50 μm .

ship was unsupported. When *Treubia* was excluded from the analysis by Forrest and Crandall-Stotler (in press), the position of *Haplomitrium* was unresolved. Thus, the affinities of *Haplomitrium* are not yet satisfactorily resolved; Davis (in press) felt that the weight of the current evidence supports a position for the genus as sister to the class Jungermanniopsida, whereas Qiu (2003) and Forrest and Crandall-Stotler (in press) favor a position as sister to all other hepatics.

Although unexpected, the affinity between *Treubia* and *Haplomitrium* finds support in morphology. Both are "leafy" taxa with gametangia situated in leaf axils or lobules. *Treubia* is decisively more dorsiventral, with an oblique to transverse leaf insertion (succubous) and small dorsal lobules (Renzaglia, 1982), whereas some species of *Haplomitrium* tend toward anisophylly and succubous insertion (Bartholomew-Begin, 1991). In both genera, a tetrahedral apical cell is responsible for shoot growth. Perhaps the most compelling evidence for a close relationship between these two genera comes from the peculiar yet similar sperm cells that they produce. Cladistic analyses based on spermatogenesis consistently recovered a *Treubia* plus *Haplomitrium* clade that is sister to the remaining liverworts (Garbary et al., 1993; Renzaglia and Garbary, 2001). Stech et al. (2000) elevated *Treubia* to class Treubiopsida based on *trnL* intron sequence divergences between it and other liverworts.

Systematics and phylogeny of the Marchantiopsida (complex thalloid liverworts)—Unlike other hepatic groups, the

complex thalloid liverworts include relatively drought-resistant species. Many morphological features of Marchantiopsida indicate xeromorphic adaptations (Schuster, 1992; Wheeler, 2000). In addition to air chambers in the dorsal part of the thallus (Fig. 9A), marchantioid liverworts are characterized by two types of rhizoids (smooth and pegged), archegonial involucre, unlobed spore mother cells, four primary androgones in the antheridium, six rows of neck cells in the archegonium, idioblastic oil body cells, ventral thallus scales, unistratose capsule walls, and a simple locomotory apparatus in the small biflagellated sperm cell (Schuster, 1966, 1992; Renzaglia et al., 2000; Renzaglia and Garbary, 2001). Of these, only features of the sperm appear to be universal in all species.

Although Marchantiopsida are resolved as monophyletic, traditional relationships among taxa generally are not supported by molecular data. The classical morphological separation of this liverwort class into three orders; i.e., Monocleales, Sphaerocarpaceales, and Marchantiales, is challenged by nucleotide sequence data (Wheeler, 2000; Boisselier-Dubayle et al., 2002). Incongruence between morphological and molecular patterns may be attributed to parallel changes in multiple lineages (Boisselier-Dubayle et al., 2002).

The multigene analyses of Davis (in press, Fig. 8) and Forrest and Crandall-Stotler (in press) provided strong support for the placement of *Blasia* as a member of the complex thalloids, a result that conflicts with the traditional placement of this liverwort within the simple thalloids (Renzaglia, 1982). Sperm cell features, persistent ventral scales, a small wedge-shaped

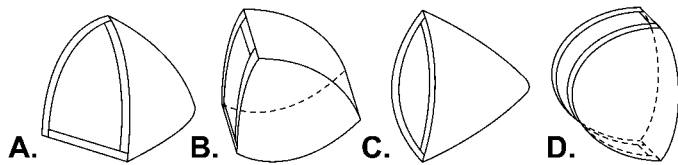


Fig. 10. Diagrammatic representation of apical cell shapes in liverworts as oriented in plants growing horizontally. The shoot tip is directed toward the left. **A.** Tetrahedral cell with three cutting faces. **B.** Wedge-shaped or cuneate cell with two lateral, one dorsal and one ventral cutting face. **C.** Lenticular or lens-shaped cell with two lateral and one dorsal cutting faces. **D.** Hemidiscoid cell with two lateral and one posterior cutting face. This cell is rare in liverworts and was developmentally and evolutionarily derived from a wedge-shaped cell.

apical cell, and a *Monoclea*-like female involucre provide morphological evidence for the inclusion of *Blasia* in the complex thalloid lineage (Renzaglia and Duckett, 1987; Pass and Renzaglia, 1995; Renzaglia and Garbary, 2001). Previous molecular analyses based on one or two gene sequences do not agree in the placement of *Blasia*. Stech and Frey (2001) resolved *Blasia* as sister to Jungermanniopsida (simple thalloids plus leafies) and described the new class, Blasiopsida. Their study was based solely on *trnL* intron sequences (ca. 500 bp), and the relationship was without bootstrap support. Wheeler (2000) found that *Blasia* grouped with the simple thalloids (Metzgeriidae) based on 26S nrDNA (also without bootstrap support), and He-Nygrén et al. (in press) resolved *Blasia* as sister to the remaining liverworts.

After *Blasia*, *Sphaerocarpos* is the next divergent taxon (Fig. 8). A position for Sphaerocarpaceae (*Sphaerocarpos*, *Rielia*, *Geothallus*) among complex thalloids is generally supported by morphology (Smith, 1955; Bishler, 1998; Crandall-Stotler and Stotler, 2000; Boisselier-Dubayle et al., 2002). However, with additional taxon sampling, the sister relationship between Sphaerocarpaceae and the remaining Marchantiopsida is called into question. Based on LSU rDNA sequences, Wheeler (2000) and Boisselier-Dubayle et al. (2002) reported that Sphaerocarpaceae were placed within Marchantiaceae. Similarly, *Sphaerocarpos* nested between *Neohodgsonia* and *Marchantia* in the five-gene analysis of Forrest and Crandall-Stotler (in press). The implication from these results is that the relatively simple morphology of both generations in Sphaerocarpaceae may not be plesiomorphic but rather the product of extreme simplification in ephemeral or aquatic habitats.

Air chambers are found in the crown group taxa (*Marchantia*, *Preissia*, *Targionia*, *Riccia*) (Wheeler, 2000). One lineage, *Monoclea* plus *Dumortiera*, has secondarily reverted to a morphologically simple thallus devoid of chambers, perhaps adaptations to the semi-aquatic habit of these plants (Wheeler, 2000). The production of archegoniophores (carpocephala) that elevate sporophytes above the gametophyte also evolved within the crown Marchantiopsida group. Independent losses of these structures occurred in riccioid taxa (*Riccia*, *Riccio-carpos*, *Oxymitra*) and *Monoclea* (Wheeler, 2000). Reduction in sporophyte complexity is likewise a derived feature of riccioid liverworts (Renzaglia et al., 2000; Boisselier-Dubayle et al., 2002). With a jungermannioid-like sporophyte elevated on a fragile and highly elongated seta, *Monoclea* seems inappropriately placed within this crown group. Additional characters of the genus, including a free nuclear embryo and monoplas-tidic meiosis in some species (Schofield, 1985; Renzaglia et al., 1994), support a more traditional placement of *Monoclea*

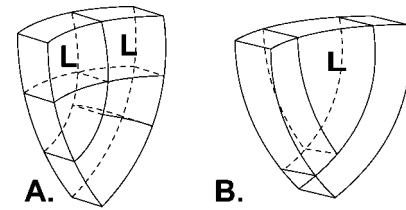


Fig. 11. Formative divisions in the lateral derivative from apical cells of liverworts. Apices are vertically oriented so that the shoot tip is facing up. **A.** Lateral thallus, wing and "leaf" development from a central wedge-shaped cell (single initial) (L) in a three-celled derivative. This type of development characterizes all simple thalloid and complex thalloid taxa and occurs in derivatives from all four types of apical cells. **B.** "True" leaf development in leafy liverworts from two initials. In this five-celled derivative, two leaf initials (L) are determined to develop into the bifid and complicate-bilobed leaves.

in Marchantiopsida. However, congruence among the multi-gene analyses provided support for *Monoclea* close to *Dumortiera*.

Systematics and phylogeny of Metzgeriidae (simple thalloid liverworts)—Clearly not a monophyletic group, Metzgeriidae traditionally include some 30 highly diverse genera of "leafy" and thalloid forms. Although four apical cell types are found in the group (Fig. 10), a unifying feature of apical growth in these plants is development of wings and leaves from a central wedge cell (single initial) that forms in the newly produced apical derivative (Fig. 11A) (Renzaglia, 1982). Simple thalloid genera are distinguished from leafy liverworts (Jungermanniidae) in that they are anacrogynous: archegonia are produced along the mid-thallus of either the main, lateral, or ventral shoots. Consequently, the apical cell is not transformed into permanent tissue after archegonial development, and sporophytes do not terminate the shoot as in acrogynous Jungermanniidae. Additional features that unify the simple thalloid taxa, but are also found in leafy liverworts, are the development of antheridia from two primary androgones, oil bodies in all cells, lobed sporocytes, smooth rhizoids, and five rows of neck cells per archegonium.

Two assemblages of simple thalloid taxa are paraphyletic (simple thalloid I and II in Fig. 8) within Jungermanniopsida. The first group (simple thalloid I) is the most diverse and includes *Phyllohallia*, generally placed in Treubiales, most members of Fossombroniales, and suborder Pallaviciniineae of Metzgeriales (classification according to Crandall-Stotler and Stotler, 2000). Placement of *Phyllohallia*, *Pellia*, *Calycularia*, and *Noterochlada* is not resolved in the five-gene analysis of Forrest and Crandall-Stotler (in press); all represent genetically and morphologically divergent taxa. *Phyllohallia* and *Noterochlada* are distinctly "leafy" in habit, but development is from a wedge-shaped cell (Fig. 10B) in the former and a tetrahedral apical cell (Fig. 10A) in the latter (Renzaglia, 1982). *Pellia* and *Calycularia* are fleshy thalloid types, both with wedge (Fig. 10B) and hemidiscoid apical cells (Fig. 10D). Although support for an assemblage that includes *Fossombronina*, *Austrofossombronina*, *Petalophyllum*, and *Allisonia* is weak, this group includes most of the genera traditionally placed in Fossombroniales (Crandall-Stotler and Stotler, 2000). All have spheroidal capsules, which are typically irregular or nonvalvate in dehiscence. Most exhibit a "leafy" growth form with either lens-shaped (Fig. 10C) or tetrahedral (Fig. 10A) apical cells. Suborder Pallaviciniineae of Metzgeriales are recovered

as monophyletic and include *Hymenophyton*, *Moerckia*, *Hattorianthus*, *Podomitrium*, *Pallavicinia*, *Jensenia*, *Xenothallus*, and *Symphyogyna* (Crandall-Stotler and Stotler, 2000; Forrest and Crandall-Stotler, in press). This morphologically uniform group contains upright or procumbent taxa, most with prominent midribs and monostromatic wings (e.g., *Pallavicinia*, Fig. 9B). Lens-shaped apical cells (Fig. 10C) are responsible for vegetative growth. An autapomorphy of this group is the production of specialized strands of dead, water-conducting cells that predominate in most taxa (Ligrone et al., 2000). Extensive variability is seen in position and type of protective structure associated with gametangia and sporophytes (Renzaglia, 1982; Fig. 9B).

The apparent affinity between suborder Metzgeriinae (simple thalloid II) and Jungermanniidae (“true” leafy liverworts) in the multigene analyses of both Davis (Fig. 8) and Forrest and Crandall-Stotler (in press) was unexpected. Members of Metzgeriinae epitomize the simple thalloid condition, with fleshy (Aneuraceae) and midrib-plus-wing (Metzgeriaceae) organizations. All of these thalli develop from a lens-shaped apical cell (Fig. 10C), and no “leafy” forms exist (except perhaps *Pleurozia*, discussed next). Endogenous branches in Metzgeriaceae are reminiscent of those in leafy liverworts (Renzaglia, 1982).

One of the most surprising results from the Davis (in press) analyses was the placement of *Pleurozia* in Metzgeriinae (simple thalloid II) rather than among the “true” leafy liverworts (Figs. 7, 8). *Pleurozia* is composed of about 11 species distributed primarily in the tropics. Leaves are complicate-bilobed, and for that reason, *Pleurozia* has traditionally been included in or near Porellales within the leafy liverworts (Schuster, 1984; Crandall-Stotler and Stotler, 2000). However, leaf morphology in *Pleurozia* is unique in that the leaf lobule is dorsal in orientation, not ventral (Thiers, 1993), and the plants grow from a lenticular apical cell (Crandall-Stotler, 1976) rather than a tetrahedral cell as in all “true” leafy liverworts. The placement of *Pleurozia* in the metzgerioid liverworts indicates that the “leafy” gametophytes of *Pleurozia*, with their complicate-bilobed leaves, may have evolved convergently in a group otherwise characterized by thalloid gametophytes. In contrast to the single leaf initial in simple thalloids that have “leafy” gametophytes (Fig. 11A), leaves of *Pleurozia* develop from two initial cells, as is typical of leafy liverworts (Fig. 11B; Crandall-Stotler, 1976). The phylogenetic position of *Pleurozia* should be further investigated, although its placement within subclass Metzgeriidae is strongly supported by both the 12- and four-locus analyses of Davis (in press).

Systematics and phylogeny of the Jungermanniidae (leafy liverworts)—The leafy liverworts, with some 4000–6000 species, are by far the largest of the liverwort groups. They occur in most terrestrial and aquatic habitats but are especially diverse in high-moisture environments. Many species are epiphytic on bark, and in the tropics, epiphyllous liverworts may cover the leaves of angiosperm trees and shrubs in shaded, high-humidity forests. More than 75% of the liverworts of tropical lowland forests and almost all the epiphylls belong to Lejeuneaceae (Gradstein, 1994, 1997). Lejeuneaceae comprise approximately 93 of the 307 genera (30%) of leafy liverworts, and well over 1000 species (Gradstein, 1979, 1994, 1997; Crandall-Stotler and Stotler, 2000). In lowland equatorial forests, as many as 20 species of Lejeuneaceae may co-occur on

a single leaf (Zartman, 2003). These organisms are important components of tropical forest diversity, and the diversity of epiphylls (almost exclusively Lejeuneaceae) is a sensitive indicator of habitat change associated with forest fragmentation (Zartman, 2003).

Jungermanniidae are distinguished from Metzgeriidae in having tetrahedral apical cells, gametophytic shoots with (usually) well-differentiated stems and leaves, leaves formed from two initial cells, acrogynous perichaetia (terminating the main stem or branch), bracts and perianths (modified, fused leaves) associated with the perichaetium, and capsules that regularly dehisce into four valves (Crandall-Stotler and Stotler, 2000). The perianths of leafy liverworts are diverse and provide important taxonomic characters in many genera and families.

Leaves of leafy liverworts may be entire or more often have two large lobes or teeth. They are most commonly differentiated as two rows of lateral leaves and a single row of ventral underleaves (amphigastria; Fig. 9C). Underleaves are frequently small or lacking. Insertion of the lateral leaves may be transverse, or, more commonly, they are oblique and the plants are more or less flattened because the leaves overlap. In plants with incubous leaf orientation, the forward leaf margin overlaps the trailing margin of the next younger leaf, resembling the arrangement of roof shingles (Fig. 9C). In succubous orientation, forward margins of older leaves are covered by overlapping trailing margins of the younger leaves. In species with complicate-bilobed leaves, lateral leaves are each folded to form ventral and dorsal lobes. The dorsal lobe is larger in most taxa, and the ventral lobe may be highly modified into the form of a pouch or helmet-shaped lobule that holds water.

Schuster (1966, 1984) assumed that the most primitive liverworts would be the most mosslike, with leafy, radially symmetric gametophytes and therefore placed leafy taxa at the base of his subjectively derived “phylogenetic trees” (e.g., Schuster, 1966, pp. 406, 696). He considered leafy taxa with radial symmetry and three rows of transversely (or nearly so) inserted leaves (e.g., Herbertineae) to be early diverging groups, and from these he showed the branching of lineages or clusters of lineages with increased anisophylly and more obliquely inserted leaves (Schuster, 1966, 1972, 1984). One group includes Schistochilaceae, Cephaloziaceae, Lepidoziaceae, and Pleuroziaceae, whereas the other progresses through Ptidaceae to Jungermanniaceae, Frullaniaceae and Lejeuneaceae. (His diagram shows extant families ancestral to other families.) The classification of Crandall-Stotler and Stotler (2000) has a sequence of families in five orders, Lepicoleales (including Ptilidiaceae, Lepicoleaceae, Schistochilaceae, and Lepidolaenaceae), Jungermanniales (including Herbertaceae, Balantiopsidaceae, Geocalycaceae, Lepidoziaceae, Cephaloziaceae, Jungermanniaceae, and Gymnomitriaceae), Porellales (including Porellaceae, Jubulaceae, and Lejeuneaceae), and the monotypic Radulales and Pleuroziales. Their classification implies similar concepts of evolution in leafy liverworts to those of Schuster.

In a liverwort backbone tree based on 12 loci, Davis (in press) resolved two major clades within subclass Jungermanniidae (Fig. 7). One clade contains most of the taxa with complicate-bilobed, incubous (or transverse) leaves (mainly Porellaceae, Jubulaceae, Radulaceae, and Lejeuneaceae), whereas the other contains the remaining families of leafy liverworts. In a more taxon-extensive analysis that included 81 liverworts, two mosses, and a hornwort, based on sequences from 26S nrDNA, two plastid loci (*psbA* and *rps4*), and mitochondrial

nad5, the same two leafy liverwort clades were resolved (Fig. 8). The noncomplicate-bilobed group consists of three subclades for which sister group relationships are ambiguous (A, B, and C in Fig. 8). Species in clade A have incubous or transverse leaf insertion, well-developed underleaves, and multilobed lateral leaves (Davis, in press). *Herbertus*, assumed by Schuster (1984) to be primitive among leafy liverworts, is resolved in a derived position within clade A (Fig. 8). Moreover, other isophyllous taxa (e.g., *Anthelia*, *Triandrophyllum*) are also resolved in relatively derived phylogenetic positions. Taxa in clade B have succubous or transverse leaves and generally lack underleaves; however, lateral leaf shape is variable. Leaf shape, insertion, and underleaf development are highly variable in clade C, but many of the species are characterized by having perichaetia formed in fleshy perigynia or marsupia, which do not occur elsewhere in the leafy liverworts.

Among suborders of leafy liverworts recognized by Schuster (1984), only Radulineae and Balantiopsidaceae are monophyletic based on the four-locus analysis of Davis (in press). The classification of Crandall-Stotler and Stotler (2000) is also in conflict with many of the phylogenetic inferences from Davis's analysis (Fig. 8). Notably, Lepicoleales are extensively polyphyletic, and Radulales are nested within Porellales. Herbertaceae, Lepidoziaceae, Balantiopsidaceae, Cephaloziaceae, Porellaceae, and Radulaceae are supported as monophyletic. Lejeuniaceae are monophyletic only if *Bryopteris* is included within them (Bryopteridaceae, fide Crandall-Stotler and Stotler, 2000). Jungermanniaceae, Gymnomitriaceae, Geocalycaceae, Cephaloziaceae, Lepidolaenaceae are paraphyletic (Davis, in press).

Inferences about morphological evolution in liverworts from molecular analyses—Leaves or leaflike lobes have evolved in every major group of hepatics. *Haplomitrium* and *Treubia* have leafy appendages. *Blasia* and *Sphaerocarpos*, taxa within the marchantioid line, have leafy habits. *Phyllohallia*, *Noteroclada*, and *Pleurozia*, with leafy gametophytes, are scattered among simple thalloid taxa. The leaves of these plants are typically succubous to transversely inserted and may be formed from any one of three apical cell types: wedge-shaped, lenticular, or tetrahedral. In *Phyllohallia* and *Noteroclada* (Fig. 10A), each leaf develops from a single initial, whereas in *Pleurozia* there are two initials. A single initial is also responsible for development of wings and lateral thallus in all simple thalloids, complex thalloids, hornworts, and many pteridophyte gametophytes and is thus best viewed as plesiomorphic.

An autapomorphy of the Jungermanniidae is the production of bifid leaves from two leaf initials in a derivative from a tetrahedral apical cell (Fig. 10B). Once "locked" into this pattern of cell divisions, a number of variations on the "typical" bifid leaf of hepatics evolved, including complicate-bilobed leaves. A narrower ventral cutting face in the apical cell is associated with a smaller size or absence of underleaves that originate from it. Incubous leaf insertion results from a ventral (downward) tilt of the apical cell (Crandall-Stotler and Stotler, 2000), a feature that is often correlated with taxa that grow on vertical substrates such as tree bark (e.g., Lejeuniaceae). Conversely, succubous leaf arrangements are correlated with a dorsal (upward) tilt of the growing tip.

Few conclusions can be drawn at present about the evolution of apical cell shapes and growth forms in liverworts. Transformation from one apical cell type to another readily

occurs during development, and this plasticity may have provided fuel for evolutionary change (Renzaglia et al., 2000). Depending on the position of *Haplomitrium* in the trees, either a tetrahedral or wedge-shaped cell is plesiomorphic. Similarly, either an upright "leafy" habit or a flattened thallus is ancestral in hepatics; both hypotheses have garnered support (Schuster, 1992; Mishler and Churchill, 1984). Outgroup comparisons provide no further resolution of this issue as hornwort and pteridophyte gametophytes are thalloid with wedge-shaped apical cells, whereas mosses are leafy with tetrahedral cells.

Within liverworts, significant evolutionary changes can be inferred at the cellular level based on the consensus topology of recent molecular analyses. Monoplastidic meiosis occurs in all mosses and hornworts. However, it is restricted in liverworts to *Haplomitrium*, *Blasia*, and *Monoclea* (Renzaglia et al., 1994) and is best interpreted as plesiomorphic. Monoplastidic meiosis involves precise control of plastid division and migration prior to chromosomal separation (Brown and Lemmon, 1990). Polyplastidic meiosis predominates in liverworts and is a derived state. Similarly, lobed spore mother cells that occur in liverworts such as *Haplomitrium*, *Treubia*, and *Blasia* are shared with other bryophytes and represent a plesiomorphic condition (Brown and Lemmon, 1988). Sporocyte lobing was lost within Marchantiopsida, whereas spores united in permanent tetrads are viewed as derived within Sphaerocarpaceae. Among bryophytes, pre-meiotic patterning of spore wall ornamentation occurs in *Apotreubia* and *Haplomitrium* and presumably has been lost in more derived liverwort lineages (Brown et al., 1986). Further ultrastructural studies across a range of hepatic groups are likely to provide new insights into the nature and direction of changes in cellular processes during early land plant evolution.

BRYOPHYTA

Moss classification and relationships—Division Bryophyta, or mosses, include about 10000 species (Crosby et al., 2000). Systematic knowledge about the mosses has grown steadily since Hedwig (1801), the starting point for moss nomenclature (excluding *Sphagnum*), recognized 32 genera. Most classifications of the 19th century depended on gametophyte characters for defining the major groups of mosses (e.g., Bruch et al., 1851–1855; Kindberg, 1897). Mosses (excluding *Sphagnum* and *Andreaea*) were divided into acrocarpous and pleurocarpous taxa (Mitten, 1859). Acrocarpous mosses have archegonia terminating the main stems, which tend to be sparsely if at all branched. Pleurocarpous mosses, in contrast, have archegonia borne laterally along relatively highly branched, generally procumbent or pendent, extensively interwoven stems. The two forms of gametophyte architecture are often obvious, but some taxa are confusingly intermediate (e.g., Rhizogoniaeae, Orthotrichaceae, Hedwigiaceae) because they have moderately branched stems with archegonia terminating short to long lateral branches. La Farge-England (1996) clarified the definitions of these forms of gametophytic architecture and discussed possible phylogenetic relations between taxa characterized by acrocarpous, pleurocarpous, and cladocarpous gametophyte architecture (the latter including the seemingly intermediate forms).

Philibert (1884–1902) published a series of seminal papers describing variation in the structure of the moss peristome (sporophytic) and distinguished several basic types character-

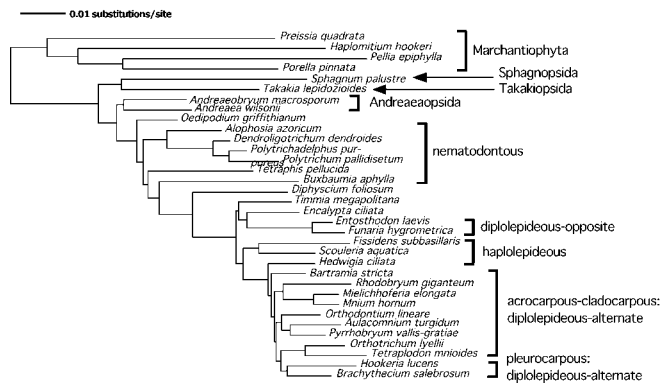


Fig. 12. "Backbone" phylogenetic tree of mosses showing relationships among major clades characterized by different peristome types. This is the single optimal tree under maximum likelihood (Cox et al., in press).

izing large groups of taxa. Fleischer (1923) developed a radically new classification of moss diversity based on Philibert's peristome observations for his flora of Java and adjacent regions, and variations on this classification were utilized through almost all of the 20th century. The most influential classification utilizing Philibert's observations and Fleischer's taxonomic concepts was Brotherus' (1924–1925) worldwide synopsis of mosses for Engler and Prantl's *Die natürlichen Pflanzenfamilien*. With minor modifications, the Brotherus system formed the basis for moss classification (e.g., Vitt, 1984) until the last five years, during which insights from molecular analyses have accumulated (Buck and Goffinet, 2000) (Fig. 12).

Toward the end of the 20th century, Edwards (1979) and Vitt (1984) provided refined insights into differences between the basic peristome types in mosses. Evans and Hooker (1913), Blomquist and Robertson (1941), Shaw and Anderson (1988), Shaw et al. (1987, 1989a, b), and Goffinet et al. (1999) documented developmental characteristics of the peristome types and differences between them.

Two basic types of peristome, nematodontous and arthrodontous, are distinguished by whether the teeth are formed from whole dead cells or just remnants of cell walls, respectively. Nematodontous peristomes are heterogeneous in both development and mature structure. The so-called *Polytrichum* type (Shaw and Robinson, 1984) consists of 32 or 64 teeth united at their tips by a membranous epiphragm (Fig. 13A–E). The teeth are formed from whole cells derived from the innermost four to eight layers of the amphithecium. (Endothecium and amphithecium are embryonic tissues that differentiate early in the ontogeny of bryophyte capsules.) *Polytrichum*-type peristomes are uniquely characterized by a series of early anticlinal divisions in the amphithecium, and, because peristome development involves remarkably regular alternating anticlinal and periclinal divisions, the amphithecium ends up having double the number of cells compared to arthrodontous and other nematodontous types (Fig. 13B). The *Polytrichum*-type peristome is further characterized by complex patterns of cell deformation during development (Fig. 13C). The other form of nematodontous peristome, the *Tetraphis*-type, is simpler in development, including the absence of the additional anticlinal divisions found in the *Polytrichum*-type (Shaw and Anderson, 1988), and consists at maturity of four massive teeth derived from the entire amphithecium (Fig. 13D).

Arthrodontous peristomes consist of (mainly) periclinal

plates of cell wall material; most of the anticlinal walls are resorbed prior to maturity. Arthrodontous peristomes may have one or two rings of teeth. Diplolepeidous peristomes typically consist of an outer ring of 16 teeth, the exostome, and an inner more delicate ring, the endostome (Fig. 13F, K). Endostome teeth, when present, are referred to as segments and may be united below as a basal membrane. Diplolepeidous peristomes take their name from the fact that a vertical line extends down the outer surface of each exostome tooth because each consists of two vertical rows of cell wall plates on the dorsal surface. These plates are derived from two columns of periclinal cell walls (Fig. 13F). Haplolepeidous peristomes, in contrast, (typically) consist of a single ring of 16 teeth, and each tooth lacks a median vertical line extending down its dorsal surface (Fig. 13I). The "haplo" of haplolepeidous refers to the single column of cell wall plates on the outer (dorsal) surface, not the fact that most haplolepeidous peristomes consist of a single ring of teeth. In fact, some haplolepeidous peristomes have additional irregular teeth formed external to the main peristome. Arthrodontous peristomes are derived from the innermost three layers of the amphithecium: inner peristomial layer (IPL), primary peristomial layer (PPL), and outer peristomial layer (OPL). Whereas haplolepeidous peristomes are formed from the IPL and PPL, diplolepeidous peristomes are derived from all three peristomial layers. Exostomes form from adjacent OPL and PPL cells, and endostomes are formed from adjacent PPL and IPL cells.

Diplolepeidous peristomes may have endostome segments that lie opposite the exostome teeth (the *Funaria*- or diplolepeidous-opposite type; Fig. 13K) or alternate with them (*Bryum* or diplolepeidous-alternate type; Fig. 13F). Endostomes in the *Funaria*-type consist of relatively massive teeth (reduced or absent in some taxa) without a basal membrane. The endostomes in (well-developed) *Bryum*-types are more membranous and consist of a basal membrane and 16-keeled segments. Narrow cilia may occur between the endostome segments. Vitt (1981) argued that peristomes found in Orthotrichaceae constitute another basic type, but this interpretation was not supported by morphological studies of Shaw (1986) or Goffinet et al. (1999).

Peristomes characterizing Buxbaumiaceae and Diphyssiaceae have been interpreted as intermediate between nematodontous and arthrodontous types (Edwards, 1979, 1984; Vitt, 1984). In *Buxbaumia*, outer teeth are derived from whole cells, whereas inner teeth are arthrodontous (Fig. 13E). More than three amphithecial layers contribute to peristomes, as in nematodontous types. The *Diphyscium* peristome is entirely arthrodontous but is a pleated cone unlike any other arthrodontous peristome (Fig. 13H).

Peristomial formulae describe the numbers of cells in the three peristomial layers as revealed by patterns of vertical and horizontal lines visible on mature peristome teeth. These lines represent remnants of anticlinal walls from cells in peristomial layers and thus, numbers of cells in the layers. Formulae specify numbers of cells in the IPL, PPL, and OPL in 1/8 of the capsule's circumference (Edwards, 1984). Haplolepeidous peristomes are characterized by a 4 : 2 : 3 (OPL : PPL : IPL) formula (rarely 4 : 2 : 1), and diplolepeidous peristomes by formulae of 4 : 2 : 4–12 (Edwards, 1984; Shaw and Rohrer, 1984). Another significant feature distinguishing peristome types is whether anticlinal walls in IPL are laterally offset with regard to anticlinal walls in PPL (Fig. 13G, J). Offset walls characterize haplolepeidous and diplolepeidous-alternate peri-

stomes but not the diplolepidous-opposite (*Funaria*-) type. Anticlinal walls in the *Polytrichum*-type nematodontous peristomes are not offset (Wenderoth, 1931), and there is little if any offsetting of the walls in *Tetraphis*-type nematodontous peristomes (Shaw and Anderson, 1988). The peristome of *Diphyscium* has a developmental pattern that conforms in all details to the haplolepidous peristomial type and has a 4 : 2 : 3 formula at maturity despite the unique structure of the mature peristome (Shaw et al., 1987).

Developmental studies have succeeded (even if based on few taxa) in defining when and how the basic peristome types differ from one another. These studies do not, however, clarify phylogenetic relationships among the types. One of the central goals of higher-level phylogenetic analyses for mosses has been to resolve these relationships. Much progress has been made, but a full resolution is still forthcoming.

The backbone of moss phylogenetic relationships—Various approaches to resolving relationships among mosses have included taxon-extensive analyses using a single plastid gene (*rps4*: Goffinet et al., 2001; *rbcL*: Tsubota et al., 2002), and analyses of multigene, multigenomic data sets with more synoptic taxon sampling (Cox et al., 2004). Both approaches have their merits, but it is clear that resolution of well-supported relationships among the major groups of mosses will not be accomplished using one or a few genes, even if such analyses succeed in placing more genera into monophyletic groups. The best-supported “backbone” for mosses was derived from an analysis of eight genes representing the mitochondrial, plastid, and nuclear genomes of 30 exemplars that represent major lineages based on previous studies (Cox et al., 2004). The following synopsis is based on that analysis, with discussion of supportive and/or contradictory evidence when appropriate.

With sequences from four species of liverworts as the outgroup, the Bayesian reconstruction presented by Cox et al. (2004) indicated that *Sphagnum* and *Takakia* form a clade sister to all remaining mosses (Fig. 12). A close relationship between *Sphagnum* and *Takakia* was also resolved by Hedderson et al. (1998), Newton et al. (2000), and Yatsenyuk (2001) from nucleotide sequences, although Newton et al. (2000) were not able to identify any morphological synapomorphies uniting the two genera. Gametophytes of *Sphagnum* and *Takakia* could not be more divergent: those of *Takakia* are tiny, simple in structure, and reminiscent of liverworts, whereas those of *Sphagnum* are large and characterized by a number of autapomorphies. The sporophyte of *Takakia* is mosslike in development (Renzaglia et al., 1997) with a well-developed seta, a cylindrical capsule, and spiraled dehiscence (Fig. 14), whereas capsules of *Sphagnum* are ovoid in shape, open by an apical operculum, and are elevated on gametophytic pseudopodia. In the analyses of Cox et al. (2004), support for the clade containing *Sphagnum* and *Takakia* was lower when substitution patterns were modeled separately for each of the eight genomic regions than when a single model was applied, thus raising the possibility that resolution of the *Sphagnum*-*Takakia* clade may be an artifact. Phylogenetic relationships among species within Sphagnopsida (*Sphagnum* and *Ambuchanania*) have been described by Shaw (2000) and Shaw et al. (2003a).

After *Sphagnum* and *Takakia*, the next diverging clade of mosses contains the two genera, *Andreaea* and *Andreaeobryum*. These two mosses, although similar in gross morphology, differ in several seemingly fundamental morphological features including development of a seta (absent in *Andreaea*

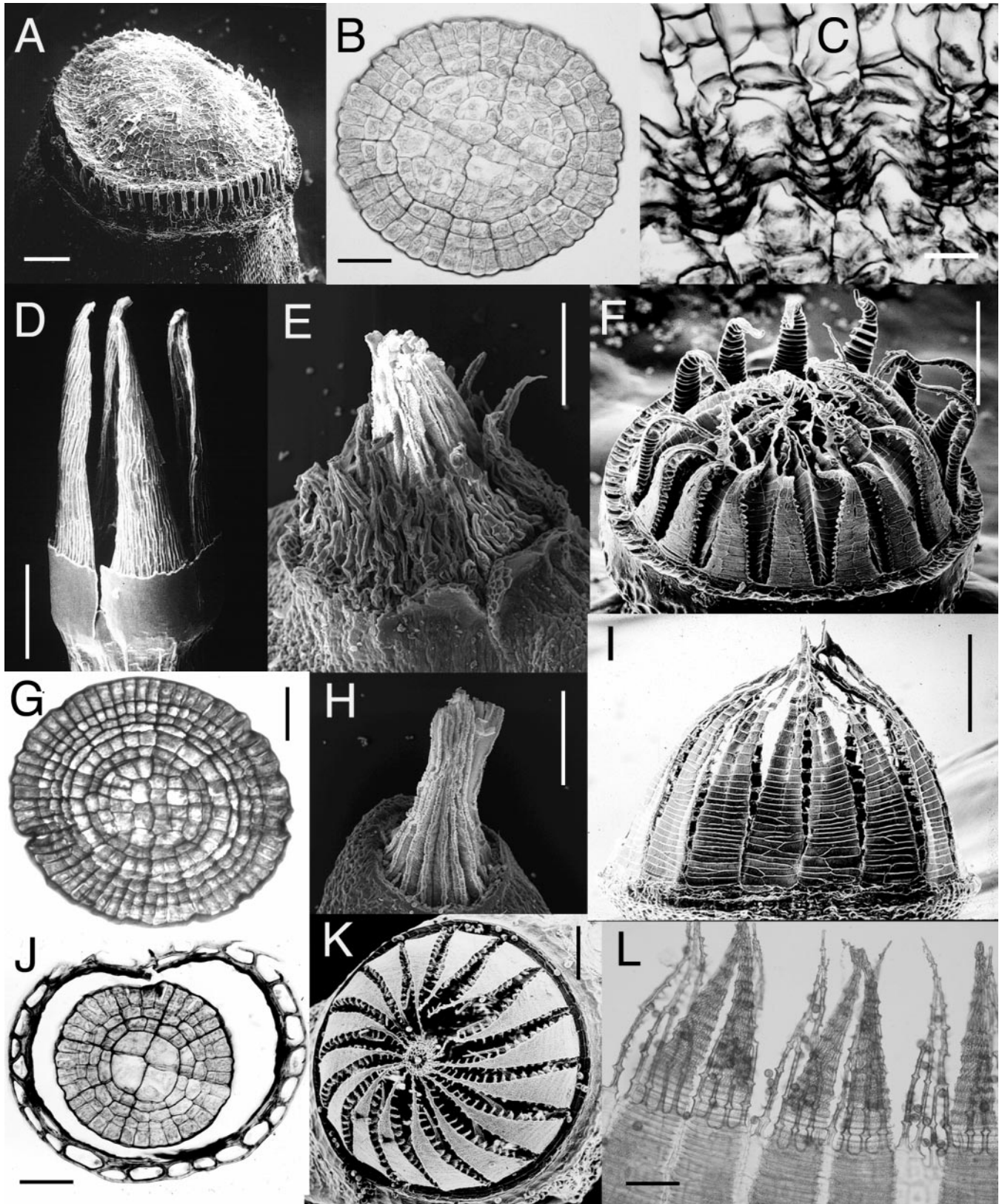
but present in *Andreaeobryum*), mode of capsule dehiscence, and timing of perichaetium differentiation relative to sporophyte development. Newton et al. (2000), Goffinet et al. (2001), and Cox et al. (2004) resolved *Andreaea* and *Andreaeobryum* in a single clade, but without impressive support from the bootstrap, Bremer support indices, and Bayesian posterior probabilities. It remains possible, though not likely, that *Andreaea* and *Andreaeobryum* form a paraphyletic grade leading to the “true” (peristomate) mosses. Murray (1988) noted morphological similarities that might link *Andreaeobryum* with *Takakia*.

One of the most exciting insights from phylogenetic analyses of mosses is that the monospecific genus, *Oedopodium*, appears to be sister to all remaining peristomate taxa (Fig. 15). A critical position for *Oedopodium* corroborates the results of Newton et al. (2000) from combined analyses of morphology and four plastid DNA regions and of Goffinet et al. (2001) based on a taxon-extensive analysis of plastid *rps4* sequences. Goffinet et al. (2001) resolved *Oedopodium* as sister to a clade containing Tetraphidaceae and Polytrichaceae at or near the base of peristomate mosses, but relationships were not fully resolved and without bootstrap support. Newton et al. (2000) and Magombo (2003), based on four plastid DNA regions, resolved *Oedopodium*, both with moderate to strong bootstrap support, as sister to all peristomate mosses.

Oedopodium griffitheanum (Dicks.) Schwaegr. is a small acrocarpous moss with soft obovate to spatulate leaves, thin-walled hexagonal leaf cells, erect capsules with a well-developed, long-tapered sterile neck region, a well-developed operculum but no peristome (Fig. 15). Stalked multicellular gemmae are sometimes formed in the leaf axils (Smith, 1978). The species is uncommon, but reported from Alaska, Greenland, Britain, Scandinavia, and Japan, and it is disjunct in the Southern Hemisphere on the Falkland Islands, where it grows on peaty soil, typically in rock crevices (Smith, 1978; Mahú, 1979; Noguchi, 1988). *Oedopodium* has previously been classified near Funariaceae, mainly because of similarities in gametophyte morphology (especially the broad, soft-textured leaves with large, thin-walled cells). The absence of a peristome in *Oedopodium* may well be plesiotypic, although the possibility of secondary loss cannot be eliminated (Cox et al., 2004).

Mosses characterized by nematodontous peristomes form a grade paraphyletic to the arthrodontous taxa (Fig. 12). Polytrichales form a monophyletic group sister to the rest of the peristomate mosses; *Tetraphis* (representing the small family, Tetraphidaceae) is next diverging, then *Buxbaumia*, and *Diphyscium*. Monophyly of Polytrichales based on the eight-gene data set corroborated earlier results of Hyvönen et al. (1998), Newton et al. (2000), and Magombo (2003). Tetraphidaceae, characterized by four massive, nematodontous peristome teeth, are not part of the monophyletic Polytrichaceae. This result makes sense in terms of peristome structure and development; *Tetraphis* does not have the “extra” anticlinal division that characterizes the amphithecial layers of Polytrichaceae (Shaw and Anderson, 1988) nor the complex pattern of cell malformation that occurs during peristome development in Polytrichaceae. Aside from their nematodontous structure, peristomes of Tetraphidaceae and Polytrichaceae have little in common.

Molecular phylogenetic analyses, as well as peristome structure and development, support the interpretation that *Buxbaumia* and *Diphyscium* are intermediate between nematodontous



and arthodontous mosses. The two families are paraphyletic to the rest of the arthodontous clade, forming a bridge from taxa with nematodontous peristomes (Fig. 12). Newton et al. (2000) resolved *Buxbaumia* and *Tetraphis* as a monophyletic group between Polytrichaceae and *Diphyscium*, but their topology was otherwise similar in the intermediate placement of these taxa between nematodonts and arthodonts. Goffinet et al. (2001) resolved *Buxbaumia* in an unsupported clade with Tetraphidaceae and *Oedopodium* (in contrast to Fig. 12), but their analysis resolved *Diphyscium* as sister to arthodontous taxa (as in Fig. 12). With more extensive taxon sampling within Diphysiaceae, Magombo (2003) confirmed that the family is monophyletic and corroborated its phylogenetic position between Buxbaumiaceae and arthodontous mosses. A placement of *Buxbaumia* near the basal node of the *Diphyscium*-arthodontous clade is supported by a shared deletion of approximately 200 nucleotides in the *rps4* gene (Goffinet et al., 2001; Cox et al., 2004). The deletion is absent in nematodonts (Polytrichaceae, Tetraphidaceae) as well as in *Oedipodium*, *Sphagnum*, *Takakia*, *Andreaea*, and *Andreaebryum*.

Relationships within arthodontous mosses are less well established, and internal branches down the backbone of the arthodonts in the eight-gene tree are notably short (Fig. 12). If these short branches reflect time rather than a shift in substitution rate, the shape of the tree indicates a rapid radiation of arthodontous mosses. Arthodontous taxa are resolved in two lineages. One includes the genus *Timmia* plus the Encalyptaceae and Funariaceae. Timmiaceae are a small family of Northern Hemisphere mosses with one genus and fewer than 10 species (Brassard, 1979). The peristome of *Timmia* is unique, although unambiguously arthodontous. It has typical diplolepidous exostome teeth, but the endostome consists of a membranous basal membrane from which approximately 64 cilia arise (Fig. 13L); normal endostome segments are not formed. There has been much speculation about homology of cilia in the *Timmia* endostome (Vitt, 1984) but little consensus. In the eight-gene tree in Fig. 12, *Timmia* is sister to a clade containing Encalyptaceae and Funariaceae, which is consistent with the topology recovered from analyses of plastid and mitochondrial sequences (Beckert et al., 1999, 2001; Goffinet and Cox, 2000; Magombo, 2003). It is possible that sequences from the nuclear genome produce a different position for *Timmia* as sister to all arthodontous mosses (Cox and Hedderson, 1999; Newton et al., 2000), but support for this potential conflict is weak at present.

Interpretations of peristome structure in *Encalypta* (Encalyptaceae) have been controversial. Vitt (1984) suggested that the genus encompasses characteristics of nematodontous and arthodontous peristomes, including species with diplolepidous-alternate and diplolepidous-opposite types (Vitt, 1984). Developmental patterns in the sporophytes of and phylogenetic relationships among the peristomially diverse species of *En-*

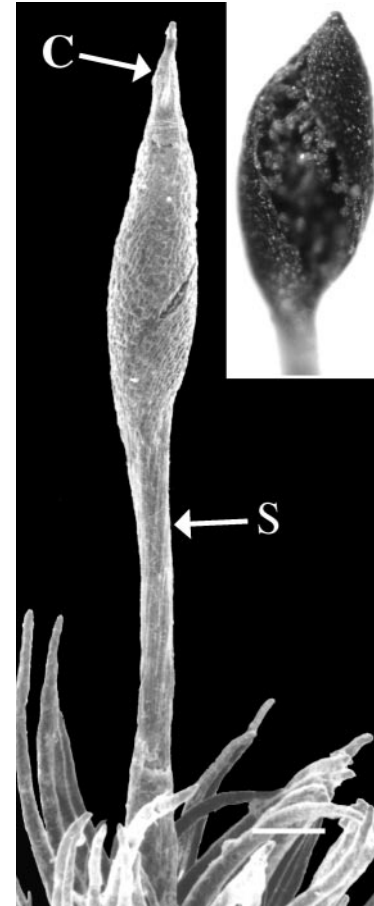


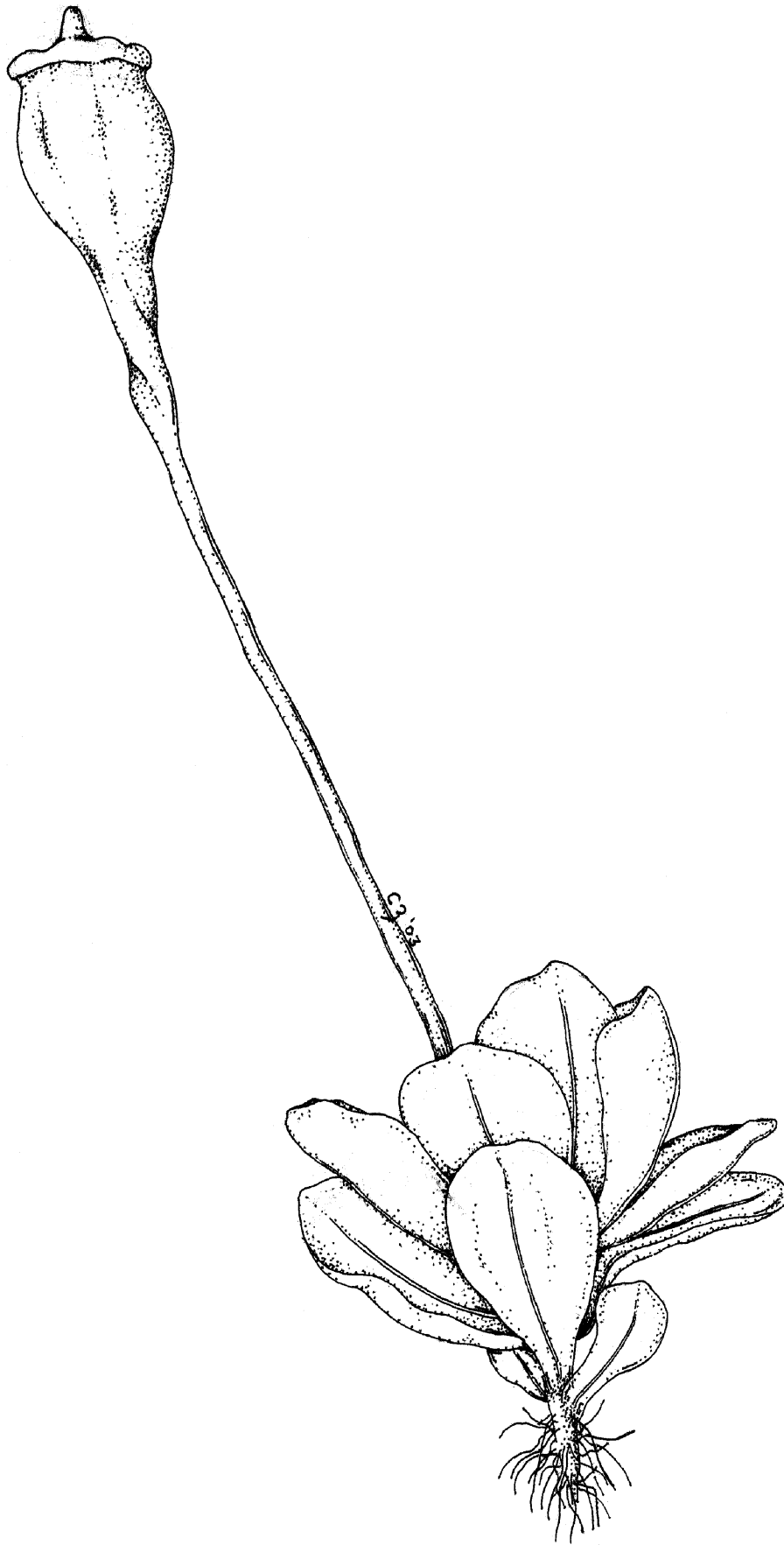
Fig. 14. Sporophyte of *Takakia ceratophylla* (Mitt.) Grolle. Scanning electron micrograph showing cylindrical capsule with a spiraled suture (S) and persistent terminal calyptra (C). At the base of the sporophyte is the remnant of the archegonium and spirally inserted terete phyllids (leaves) of the gametophyte. Inset (light micrograph) shows dehiscence capsule with spores adhering along the spiraled opening. Bar = 0.2 mm.

calyptra have not been investigated but might offer insights into peristome evolution. Funariaceae are also diverse in peristome structure, ranging from well developed to absent. When present, however, they consistently have a *Funaria*-type diplolepidous-opposite morphology. The absence of peristomes in some Funariaceae is generally interpreted as secondary reduction, and phylogenetic analyses do not contradict this conclusion. Some Funariaceae, including the “model organism” *Physcomitrella patens* (Hedw.) Bruch & Schimp., have cleistocarpous capsules (i.e., no differentiated operculum or peristome).

The eight-gene tree in Fig. 12 indicates a sister-group re-

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Fig. 13. Anatomy and development of moss peristomes. **A.** *Polytrichum juniperinum* Hedw. Bar = 45 μ m. **B.** Transverse section of a young *Polytrichum* capsule showing two innermost amphithecial layers of 32 cells (arrows) resulting from “extra” periclinal divisions early in development. Bar = 100 μ m. **C.** Transverse section of an older *Polytrichum* capsule showing cell deformation. Bar = 30 μ m. **D.** *Tetraphis* peristome. Bar = 350 μ m. **E.** *Buxbaumia aphylla* Hedw. peristome. Bar = 425 μ m. **F.** *Bryum pseudotriquetrum* (Hedw.) Gaertn. et al. (diplolepidous-alternate) peristome showing 16 outer exostome teeth surrounding a keeled endostome. Bar = 200 μ m. **G.** Transverse section of a young *Funaria hygrometrica* Hedw. capsule showing symmetric anticlinal divisions (arrows) in the inner peristomial layer (IPL). Bar = 100 μ m. **H.** *Diphyscium* peristome. Bar = 450 μ m. **I.** *Dicranum scoparium* Hedw. (haplolepidous) peristome. Bar = 75 μ m. **J.** Transverse section of a young *Bryum* peristome showing asymmetric anticlinal divisions (arrows) in the IPL. Bar = 100 μ m. **K.** *Funaria* peristome (diplolepidous-opposite). Bar = 125 μ m. **L.** *Timmia* peristome, viewed from the inside, showing the endostome consisting of cilia but no segments. Bar = 55 μ m.



relationship between taxa characterized by haplolepidous and diplolepidous-alternate peristomes but with weak bootstrap and/or Bayesian support. This is an important relationship in the context of understanding peristome evolution. As noted before, Newton et al. (2000) found evidence of a close relationship between Encalyptaceae and haplolepidous taxa, but they nevertheless resolved the haplolepidous plus Encalyptaceae clade as sister to the diplolepidous-alternate mosses. Within the diplolepidous-alternate clade, acrocarpous taxa form a paraphyletic grade leading to pleurocarps (Fig. 12). Pleurocarpous mosses form a strongly supported monophyletic group derived from an acrocarpous grade in all analyses with sufficient sampling conducted to date (Buck et al., 2000; De Luna et al., 2000; Tsubota et al., 2002).

Phylogenetic relationships within acrocarpous and cladocarpous mosses—Cox and Hedderson (1999) reconstructed relationships among acrocarpous mosses with diplolepidous-alternate peristomes based on nuclear 26S rDNA and plastid *rps4*, and *trnL-trnF* sequences. Their study upset many long-established taxonomic concepts. In particular, they showed that the large family, Bryaceae, is phylogenetically heterogeneous. *Leptobryum*, always previously classified in Bryaceae, turned out to be in Meesiaceae, a conclusion corroborated by subsequent studies (Cox et al., 2000; Goffinet and Cox, 2000; Goffinet et al., 2001). *Orthodontium* was removed from Bryaceae in favor of a placement among (largely unresolved) acrocarpous genera near the base of the pleurocarps. Most striking, however, was their finding that even the core bryaceous genera, *Bryum*, *Brachymerium*, *Pohlia*, *Mielichhoferia*, do not form a monophyletic group. *Pohlia* and related genera (e.g., *Mielichhoferia*, *Mniobryum*) are part of a clade including taxa traditionally classified in Mniaceae, leaving only *Bryum* and related genera to form a more restricted Bryaceae. *Pohlia* has relatively narrow, nonbordered leaves and long leaf cells; *Bryum* and relatives have broader, frequently bordered leaves and shorter hexagonal or rhombic leaf cells; and Mniaceae are characterized by broader still, sometimes elliptical leaves generally with a strong border and isodiametric cells. The unequivocal placement of *Pohlia* in Mniaceae could never have been predicted from morphological observations and showed clearly how misleading morphological patterns can be about phylogenetic relationships (notwithstanding many congruent patterns of relationship inferred from morphology and molecular data in the mosses). Moreover, phylogenetic insights gained from molecular analyses raise questions about the nature of large morphological transitions within monophyletic groups such as Mniaceae.

Cladocarpous taxa have archegonia borne on lateral branches, seemingly intermediate between acrocarpous and pleurocarpous architectures (La Farge-England, 1996). Diverse groups of cladocarps include Hedwigiaceae, Orthotrichaceae, and Rhizogoniaceae. Placement of Orthotrichaceae is also important in the context of interpreting basic peristome types in mosses (discussed earlier). Unfortunately, relationships of Orthotrichaceae are still unresolved, although all studies to date have indicated that the family is nested within groups characterized by diplolepidous-alternate peristomes (Goffinet et al., 2001), possibly among a group of relatively derived ac-

rocarps from which pleurocarps evolved (Cox and Hedderson, 1999; Cox et al., 2000; Tsubota et al., 2002). Goffinet et al. (2001) and Cox et al. (2004) resolved Orthotrichaceae as sister to Splachnaceae (the dung mosses). This result has significant support (i.e., >95% posterior probability) in the analyses of Cox et al. (2004).

The traditional family Rhizogoniaceae (e.g., Brothrus, 1924) are consistently resolved as nonmonophyletic by molecular data (Goffinet et al., 2001). Members of Rhizogoniaceae, however, along with the genera *Orthodontium* (traditionally placed in Bryaceae; Brothrus, 1924; Vitt, 1984) and *Aulacomnium*, appear to be close to the ancestral acrocarps from which pleurocarpous mosses originated (Cox et al., 2000; De Luna et al., 2000; Goffinet et al., 2001; Tsubota et al., 2002). These taxa are critical to questions about the origins of pleurocarpy, and progress is being made resolving relationships among taxa traditionally classified in Rhizogoniaceae (A. Newton, British Museum, Natural History, personal communication).

Phylogenetic relationships among pleurocarpous mosses—The pleurocarpous mosses include some 5000 species, approximately 50% of all mosses. Pleurocarps are diverse in tropical forests, although they are also well represented in Northern and Southern Hemisphere temperate regions. It is well established that the pleurocarps are monophyletic and evolved from acrocarpous ancestors (De Luna et al., 2000; Newton et al., 2000; Goffinet et al., 2001; Cox et al., 2004).

Pleurocarpous mosses have traditionally been classified in three orders: Hookeriales, Hypnales, and Leucodontales. There is now little question that Leucodontales, defined primarily on the basis of reduced peristomes (Brothrus, 1924–1925; Vitt, 1984; Buck, 1991), are nonmonophyletic (Buck et al., 2000; De Luna et al., 2000; Tsubota et al., 2002). Relationships within Hypnales have been recalcitrant to phylogenetic resolution because of short branch lengths at the base of the hypnalian clade (Buck et al., 2000). Shaw et al. (2003a) provided molecular evidence that Hypnales underwent a rapid radiation early in their history. Consequently, resolution of family relationships within Hypnales is likely to require a tremendous amount of nucleotide sequence data and/or comparative information about genome structure. Although relationships among hypnalian families are largely unresolved at present, some apparently monophyletic groups have been identified and generic relationships within them investigated (Chiang and Schaal, 2000; Quandt et al., 2000; Tsubota et al., 2001a, b; Blöcher and Capesius, 2002; Pedersen and Hedenäs, 2002; Stech et al., 2002; Vanderpoorten et al., 2002a, b). A difficult but critical issue confronting phylogenetic analyses of generic relationships within families of hypnalian pleurocarps has been the identification of well-supported monophyletic groups appropriate for detailed investigations.

Buck et al. (in press) resolved ordinal relationships in the pleurocarps based on four genes (nuclear 26S rDNA, plastid *rps4*, *trnL-trnF*, and mitochondrial *nad4*). They found (with strong support) that a clade including traditional Garovagliaceae and Ptychomniaceae is sister to Hookeriales plus Hypnales. These orders are also supported as monophyletic. On this basis, Buck et al. (in press) reclassified the pleurocarps in

two superorders, the Ptychomniana and Hypnanae. Ptychomniana include the single order, Ptychomniales (with one family: Ptychomniaceae), whereas Hypnanae encompass Hookeriales and Hypnales. They also resolved familial and generic relationships within the Hookeriales, recognizing seven families, and reconstructed the evolution of morphological characters on the basis of their results. Obtaining phylogenetic resolution within Hookeriales proved less problematic than in Hypnales because Hookeriales do not appear to have undergone the sort of rapid radiation that characterizes Hypnales (Shaw et al., 2003b). Branch lengths along Hookerialean backbone are substantially longer than in Hypnales.

Inferences about morphological evolution in mosses from molecular phylogenies—Cox et al. (2004) conservatively stated the morphological implications of their phylogenetic results for the mosses. These inferences are briefly summarized here.

Taxa near the base of the moss tree have the capsule elevated on a gametophytic pseudopodium rather than on a sporophytic seta (i.e., *Sphagnum* and *Andreaea*), but the ancestral condition in mosses is ambiguous because *Takakia* and *Andreaeobryum* have a seta. Cox et al. (2004) concluded that the pseudopodium evolved independently in *Sphagnum* and *Andreaea*. Because stomata are absent in *Takakia*, *Andreaea*, and *Andreaeobryum* and those of *Sphagnum* are nonfunctional, Cox et al. also concluded that stomata-like structures in *Sphagnum* may not be homologous with stomata of more derived mosses or to those of tracheophytes and hornworts. Their presence in some hornworts indicates, to the contrary, that stomata may be homologous in mosses and hornworts, which implies multiple losses in mosses. Alternatively, stomata could have been lost once in the early evolution of mosses and regained in class Bryopsida.

The acrocarpus habit is clearly pleisiotypic in peristomate mosses, but acrocarps are a paraphyletic group within which pleurocarps are nested. Although resolution among cladocarpous taxa is poor, it appears that cladocarpy evolved several times. It may be that hypnalean pleurocarps evolved from a cladocarpous ancestor, but additional resolution among acrocarps near the origin of pleurocarps is needed.

Absence of a peristome in *Oedopodium* makes the phylogenetic node at which peristomes originated ambiguous, and it is not clear whether nematodontous peristomes of the *Polytrichum*-type evolved independently of arthrodontous peristomes. The unique anticlinal divisions in the IPL of Polytrichaceae, leading to twice the “normal” number of cells in this layer, may be an apomorphy for that clade. These divisions appear to be characteristic not only of *Polytrichum*, but also *Atrichum* and *Pogonatum*, also in Polytrichaceae (Shaw, L. Anderson, Duke University, and B. Mishler, University of California, Berkeley, unpublished data). These divisions do not occur in the developing sporophyte of *Sphagnum* or *Andreaea* (Shaw, unpublished data). Phylogenetic considerations lend support to the hypothesis of Vitt (1984) that the *Funaria*-type arthrodontous peristome with opposite exostome and endostome teeth is primitive in arthrodonts. Cox et al. (2004) noted that although *Timmia* lacks endostome segments, the most parsimonious interpretation of its endostome (which consists of a basal membrane and cilia), given its phylogenetic position, is that it conforms to the opposite type. Developmental studies of peristomial layers in *Timmia* are sorely needed. Of critical importance is whether or not anticlinal walls in the PPL and IPL are offset.

The teeth of haplolepidous peristomes develop in positions that would be opposite exostome teeth were the latter formed (clearly shown in Vitt, 1981). Thus, in terms of development, the single row of teeth in haplolepidous peristomes are homologous with the opposite endostome segments of the *Funaria*-type. Like *Funaria*-type endostomes, haplolepidous peristomes also lack a basal membrane and are relatively massive. Groups characterized by haplolepidous and diplolepidous-alternate peristomes appear to be sister groups, implying that asymmetric anticlinal cell divisions in the IPL during peristome development may be a synapomorphy for that clade. Diplolepidous-alternate peristomes have additional anticlinal IPL walls that become offset during development relative to those in the PPL. This pattern is likely a synapomorphy for the clade characterized by such peristomes.

CONSPECTUS

We are currently in a period of exponential change in our understanding of bryophyte phylogeny. Relationships among the major moss clades are relatively well resolved in comparison to the liverworts and hornworts. However, current work on all three groups is progressing at such a fast pace that even by the time this review is in print, new discoveries and insights are likely.

The molecular hypothesis presented here on hornworts (Fig. 1) is a critical first step toward a modern phylogenetic understanding for the group. Comprehensive analyses using genes from all three genomes in combination with morphological data, with sampling from all 12 genera of hornworts, are required to verify the novel relationships described earlier. Systematic studies of hornworts have lagged so far behind those of other land plants that any molecular analysis must first begin with a clear delineation of morphological characteristics in the specimens/species that are examined. Worldwide collecting and basic taxonomic evaluations are essential. With their unique adaptations to land, including basal elongation of the sporophyte and internalization of vulnerable tissues, hornworts will continue to provide essential information about early land plant evolution.

A general understanding of liverwort relationships has emerged from recent molecular studies, and this has led to major reinterpretations of evolutionary changes in the group. For example, isophylly in leafy liverworts and conducting tissue in simple thalloid taxa are now clearly seen as derived, not ancestral. However, critical unanswered questions in liverwort phylogeny remain, and these include the position of *Haplomitrium* (and *Treubia*), the placement of *Pleurozia* among the simple thalloid vs. leafy clades, and the precise positions of *Sphaerocarpos* and *Lunularia* within the complex thalloid lineage. Relationships among simple thalloid taxa, especially *Pellia*, *Phyllothallia*, *Calycularia*, and *Cavicularia* (the last sister to *Blasia*, Renzaglia, 1982) have not been resolved using multilocus studies to date and will require additional sequences and more taxon sampling. Most striking is the lack of representation of critical genera and families in any one study and the need for a concerted, collaborative effort to obtain and share specimens of poorly known taxa.

Still outstanding questions with regard to moss phylogeny include the relationship among *Takakia*, *Sphagnum*, and *Andreaea* (do they form a monophyletic group sister to all other mosses?), the origin and evolution of the major peristome types, and the nature of the acrocarpus ancestors of pleuro-

carpous taxa. Fundamental morphological data on *Takakia*, including embryology, sporophyte development, and apical growth are necessary to identify structural changes within mosses. Additional work on developmental anatomy of peristomes is also needed in conjunction with ongoing phylogenetic work. Development of peristomial cell layers in *Oedipodium* (which lacks a peristome) is critical, as is the development of the unique *Timmia* peristome. Resolution of family-level relationships in mosses, especially in closely related pleurocarps, will require expanded data sets based on not just one or two genes, nor even five, but probably 15 to 20. Availability of primers for such multilocus analyses are probably not far off, given the intensive genomic work currently underway on a wide diversity of land plants, including mosses.

Molecular technologies are improving at a rate that is unpredictable and incomprehensible; we speculate that within the next decade most of the phylogenetic questions raised in this review will be resolved. In addition to sequences from multiple genes, complete organellar genomes will soon be available for representatives of each major lineage within bryophytes. Structural features of genomes, including intron presence, and gene order and deletions, likewise will continue to provide informative phylogenetic evidence. Further understanding of the existence and expression of developmental genes, especially homeobox and MADS-box genes, in all three groups of bryophytes will provide clues to the evolution of structural complexity within these plants and evolutionary relationships to more complicated organ systems of tracheophytes. Comparative morphological/ultrastructural studies of living and fossil taxa are required to fill in the gaps in knowledge as well as to fully comprehend structural changes. In addition to perfecting data collection methodologies, the primary challenge that lies ahead is in developing methods of analyzing and combining the large and diverse data sets that are rapidly materializing. Only then will the intricate details of early land plant interrelationships be clearly illuminated.

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