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Morphology and classification of the Marchantiophyta

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1.1 Introduction

Liverworts are a diverse phylum of small, herbaceous, terrestrial plants, estimated to comprise about 5000 species in 391 genera. They occupy an assortment of habitats, including disturbed soil along stream banks, road cuts and trails, as well as rocks, logs and trees in natural landscapes. They occur on all continents, including Antarctica, but are most diversified in the montane rain forests of the southern hemisphere. Many species are quite tolerant of repeated cycles of drying and wetting (Clausen 1964, Wood 2007), a feature that has allowed them also to exploit epiphytic substrates, including leaves and branches of the forest canopy. Like mosses and hornworts, they have a heteromorphic life cycle with a sporophyte that is comparatively short-lived and nutritionally dependent on the free-living, usually perennial gametophyte. However, they differ from both of these groups in numerous cytological, biochemical, and anatomical features as detailed by Crandall-Stotler (1984). Significant diagnostic characters of the phylum include the following: they tend to have a flattened appearance, even when leafy, because their leaves are always arranged in rows, never in spiral phyllotaxis; rhizoids are unicellular, thin-walled, and usually hyaline; both leafy and thalloid forms frequently develop endosymbiotic associations with fungi; sporophytes mature completely enclosed by gametophytic tissue and are incapable of self-sustaining photosynthesis; sporophyte setae are parenchymatous and elongate by cell expansion, rather than cell division; and capsules lack the stomates, cuticle, and columella that are common in mosses and hornworts.

Liverworts occupy a critical position in land plant evolution, forming the sister group to all other extant land plants (e.g. Groth-Malonek *et al.* 2005, Qiu

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et al. 2006). Fossil spores that are comparable to liverwort spores date back to 475 million years before present (Wellman *et al.* 2003), and estimates of divergence times based on molecular evidence suggest a Late Ordovician origin for the phylum (Heinrichs *et al.* 2007). Despite rather sparse representation in the fossil record of the Paleozoic, all major (backbone) lineages of hepatics appear to have been established by the Permian (Oostendorp 1987, Heinrichs *et al.* 2007).

Traditionally, liverworts have been subdivided into the marchantioid group, or complex thalloids, and the jungermannioid group, which comprises two morphological subgroups, the anacrogynous, simple thalloids and the acrogynous, leafy hepatics. These groups have been defined in the hierarchy of most classification schemes and have long been viewed as natural phylogenetic units. For example, in Crandall-Stotler & Stotler (2000) they are recognized as classes, Marchantiopsida and Jungermanniopsida, with the latter comprising two subclasses, Metzgeriidae (simple thalloids) and Jungermanniidae (leafy hepatics). A large suite of anatomical and ontogenetic characters differentiates the two classes, including different patterns of gametangial development, spermatid architecture, capsule wall anatomy (Crandall-Stotler & Stotler 2000), and mechanisms involved in defining cytokinetic planes during meiosis (Shimamura *et al.* 2004, Brown & Lemmon 2006). Recent molecular phylogenetic studies (e.g. Heinrichs *et al.* 2005, 2007, Forrest *et al.* 2006, He-Nygrén *et al.* 2006, Qiu *et al.* 2006) have greatly modified this morphology-based concept, especially as regards the simple thalloid group. Whereas the monophyly of the complex thalloids and the leafy hepatics is broadly supported in all of these analyses, the simple thalloids are paraphyletic with representatives in four of the six backbone clades. One of these, comprising the Haplomitriaceae and Treubiaceae, has been identified as the earliest diverging lineage of the hepatics and relegated to a third class, Haplomitriopsida (Forrest *et al.* 2006). Liverworts are unambiguously resolved in these more recent, comprehensive multilocus analyses as monophyletic, in contrast to earlier postulates that they are polyphyletic (Capesius & Bopp 1997, Bopp & Capesius 1998).

This chapter provides a conspectus of liverwort morphology, with emphasis on the defining characters of the major lineages (clades) currently recognized. Although our knowledge of morphological character diversity has changed little since the first edition of this book, many interpretations of character evolution within the group have been modified (e.g. He-Nygrén *et al.* 2004, 2006, Crandall-Stotler *et al.* 2005). A classification scheme that links morphological data with the well-supported relationships generated in recent molecular phylogenetic analyses is provided, with brief morphological diagnoses for the taxon ranks above the level of family. Unless otherwise indicated, class, subclass, ordinal and family names used in the text refer to these ranks as they are defined and circumscribed in this classification.

1.2 Conspectus of liverwort morphology

The foundations for morphological studies in hepatics were laid in the nineteenth century with the seminal publications of Hofmeister (1851) and Leitgeb (1874–1881), whose comparative studies clarified the homologies among embryophytes, and documented the structural diversity and complexity of hepatics, respectively. Later workers, including Goebel (1893, 1895, 1912), Douin (1912), Evans (1912), Knapp (1930), Crandall (1969) and Renzaglia (1982) among others, have contributed additional anatomical descriptions of selected structures across broad groups of hepatics. Nevertheless, many gaps persist in our knowledge, with the vast majority of liverwort taxa known only at the level of a taxonomic description. This conspectus serves to provide a general overview of what is currently known about the structural organization and diversity of liverworts. To date, few reconstructions of morphological character state evolution have been published, so definitive statements about evolutionary trends in many characters cannot yet be made. Comprehensive reviews of the comparative anatomy and morphology of hepatics can be found in Schuster (1966, 1984a) and Crandall-Stotler (1981).

1.2.1 *Apical cells and gametophyte growth*

Whether leafy or thalloid, liverwort gametophytes display modular organization, with each module composed of a series of merophytes that trace their origin back to a single apical cell, the dynamic generative center of the gametophyte (Hallet 1978, Crandall-Stotler 1981). All metamers derived from a single apical cell compose a module that is a single branch or shoot (Mishler & DeLuna 1991). Since branching is common, most plants are composed of more than one module.

Four geometrically different types of apical cell occur within hepatics, namely, tetrahedral (or pyramidal), cuneate (or wedge-shaped), lenticular (or lens-shaped) and hemidiscoid types (Crandall-Stotler 1981: Fig. 1.1). As the name suggests, a tetrahedral apical cell has four somewhat curved, triangular surfaces, one of which forms the external or free surface of the cell. The other three surfaces, referred to as the cutting faces, are surrounded by the ranks of daughter cells generated from division of the apical cell. This type of apical cell has a triangular outline in all planes of section (Fig. 1.1A, B), and produces merophytes in three ranks. A lenticular apical cell has a lens-shaped free surface and two triangular cutting faces. It produces merophytes in two lateral ranks, has a triangular outline in both vertical and horizontal longitudinal sections, and is shaped like a convex lens in transverse section (Fig. 1.1C, D). A cuneate apical cell is wedge-shaped with five surfaces, a narrow, rectangular free surface, two

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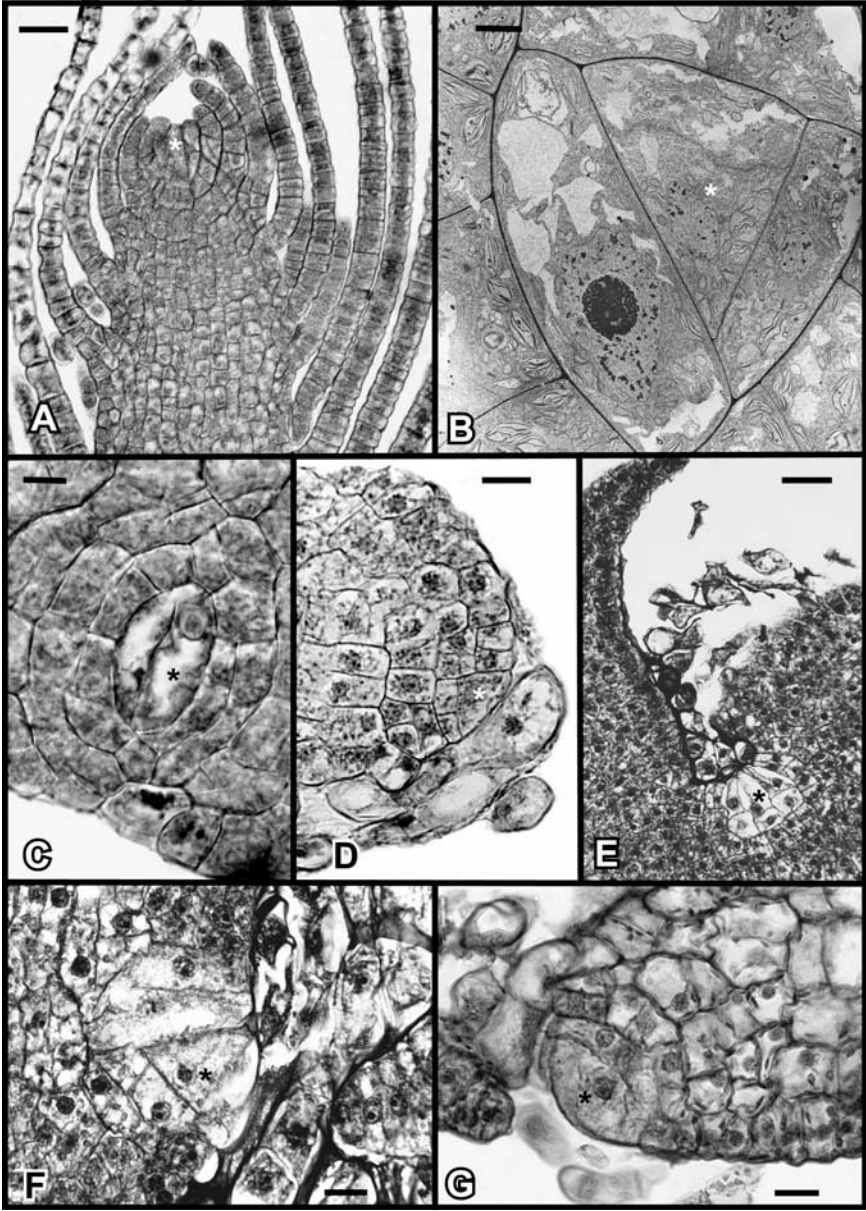


Fig. 1.1. Apical cell diversity in liverworts; apical cells are marked with asterisks. (A, B) Apices with tetrahedral apical cells; (A) *Porella platyphylla*, horizontal longitudinal section, bar = 25 μm ; (B) *Haplomitrium hookeri*, transverse section, bar = 5.4 μm . (C, D) Apices with lenticular apical cells; (C) *Pallavicinia ambigua*, transverse section, bar = 10 μm ; (D) *Aneura pinguis*, vertical longitudinal section, bar = 25 μm . (E, F) Apices with cuneate apical cells, *Phyllothallia nivicola*; (E) horizontal longitudinal section, bar = 50 μm ; (F) vertical longitudinal section, bar = 18 μm . (G) Apex with a hemidiscoid apical cell, *Pellia epiphylla*, vertical longitudinal section, bar = 18 μm . Note the slime cells overarching the apical cell in (D–G); in *Aneura* (D) they form only on the ventral surface, but in *Phyllothallia* and *Pellia* they arise from both dorsal and ventral surfaces.

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vertically aligned, triangular surfaces and two horizontally aligned, broad rectangular surfaces. Apical cells of this type have rectangular outlines in transverse and horizontal longitudinal sectioning planes, but triangular outlines in vertical longitudinal section (Fig. 1.1E, F). They produce merophytes in four ranks: dorsal, ventral, and two lateral. Finally, the rather specialized hemidiscoid apical cell appears rectangular in both transverse and horizontal longitudinal sections, but has a prismatic to semicircular outline in vertical longitudinal section (Fig. 1.1G). This type of apical cell has two lateral cutting faces and a single basal cutting face, rather than a dorsal and a ventral face as in the cuneate form. According to Hutchinson (1915) and Campbell (1913), respectively, in *Pellia epiphylla* and *Sandeothallus radiculosus* (= *Calycularia radiculosa*), the hemidiscoid geometry is developmentally derived from a cuneate form by a rounding out of the dorsal and ventral faces into a single, curved basal face.

Although there is substantial variation in apical cell dimensions as well as pattern and rate of merophyte formation, typically apical cell geometry is conserved within taxa. A tetrahedral apical cell, which has been reconstructed as the plesiomorphic state in hepatics (Crandall-Stotler *et al.* 2005), is characteristic of the Haplomitriopsida and all of the Jungermanniiidae, as well as select genera of the Pelliidae. The assumption by He-Nygrén *et al.* (2004, 2006) that a cuneate geometry is the plesiomorphic state and that tetrahedral geometries have been derived independently in several lineages is not supported by analyses of character evolution. All Marchantiopsida possess cuneate apical cells, often with lenticular types in early stages of ontogeny (Leitgeb 1881). Lenticular apical cells are characteristic of all genera of the Metzgeriidae, with the lenticular apical cell of *Pleurozia*, in fact, providing the sole morphological signal of its relationship with the Metzgeriales. Only the Pelliidae exhibit multiple apical cell types: tetrahedral in *Notoecloada*, *Petalophyllum*, and *Sewardiella*; cuneate in *Makinoa*, *Allisonia*, the *Pellia endiviifolia* species complex, *Phyllohallia*, *Moerckia*, and *Symphyogyna*; and hemidiscoid in *Calycularia*, *Sandeothallus*, and the *Pellia epiphylla* species complex.

There is no absolute correlation between plant form and apical cell type but taxa with tetrahedral apical cells do tend to have “leafy” morphologies, and taxa with hemidiscoid apical cells are always thalloid. Lenticular and cuneate apical cells typically occur in thalloid taxa, but some leafy plants, e.g. *Fossombronia* and *Pleurozia*, possess lenticular apical cells and others, like *Phyllohallia*, have cuneate apical cells (Renzaglia 1982).

Distinctive patterns of early merophyte division lead to the formation of leaves in the Jungermanniiidae and the foliar appendages and thallus wings in the rest of the hepatics (Crandall-Stotler 1981). As verified by many workers (e.g. Leitgeb 1875, Evans 1912, Crandall 1969), in the Jungermanniiidae the first

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division of a lateral merophyte is perpendicular to its free surface (i.e. anticlinal), partitioning the merophyte into two halves. Subsequent divisions that are parallel to the free surface (i.e. periclinal) generate a five-celled merophyte comprising two primary leaf and three primary stem initials; in many groups one or both leaf initials divide again to form three or four secondary leaf initials. Leaf growth occurs first from apical cells delimited from each of the leaf initials, which establish the segments or lobes of the leaf, and then from a basal meristematic zone that forms the undivided lamina (Bopp & Feger 1960). The number and size of lobes that occur in a leaf are dependent on the number of apical cells differentiated and the relative proportion of apical to basal growth that occurs. Leaf apical growth is terminated with the conversion of the apical cells to club-shaped papillae. If apical growth is pronounced, these papillae occur at the tips of the leaf lobes, as in *Lepidozia* or *Lophocolea*; however, if growth is mostly from the basal meristem, they are found near the base of the leaf, as in *Jungermannia* or the dorsal lobe of *Porella* (Bopp & Feger 1960, Fig. 23).

In groups other than the Jungermanniidae, two successive anticlinal divisions partition the lateral merophyte into three cells, the middle one of which forms the single wedge-shaped initial from which the thallus wing or foliar appendages are derived (Renzaglia 1982, Bartholomew-Began 1991). The cells to either side generate the tissues of the stem, midrib, or central portion of the thallus. Since there is only a single foliar (or wing) apical cell per merophyte, leaves in taxa such as *Haplomitrium*, *Noteroclada*, and *Fossombronina* are never deeply lobed although they can be marginally incised owing to the activity of secondarily produced centers of marginal growth (Bartholomew-Began 1991). The basal meristem is established early in leaf or wing ontogeny. Leaves of this type are polystratose at the base, often have scattered marginal papillae, and are homologous to the wings of both simple and complex thalloid taxa. A modification of this pattern occurs in *Treubia* and perhaps *Pleurozia*. In the former, the large lobes or “leaves” of the plant develop from the wedge-shaped central cell of the three-celled merophyte and a small lobule develops from the cell dorsal to it (Renzaglia 1982). In *Pleurozia* early divisions appear to produce a wedge-shaped central cell, but subsequent leaf development is like that of a true leafy liverwort, involving multiple initials and apical cells (Crandall-Stotler 1976).

1.2.2 Oil bodies

Liverworts are distinguished from all other embryophytes by their almost universal production of oil bodies, unique membrane-bound organelles that synthesize and sequester a vast array of terpenoids and other aromatic compounds (Flegel & Becker 2000, Suire *et al.* 2000). Oil bodies are formed during early stages of cell maturation (Crandall-Stotler 1981) as dilatations of the

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endoplasmic reticulum (Duckett & Ligrone 1995, Suire 2000) or from dictyosome vesicle fusion (Galatis *et al.* 1978, Apostolakis & Galatis 1998). The enclosing membrane of the oil body resembles the tonoplast in having an asymmetric, tripartite appearance but differs from it in enzyme composition and transport capabilities (Suire 2000). The oil body interior consists of small osmophilic droplets suspended in a granular stroma that is rich in proteins and carbohydrates (Pihakaski 1972, Suire 2000). Frequently, in addition to oil bodies, cells contain dispersed lipid droplets (oleosomes) in their cytoplasm. These are droplets of triacylglycerides and neither these, nor the plastoglobules common in plastids, are involved in or part of oil body development (Suire 2000).

In Jungermanniopsida and *Haplomitrium*, oil bodies are usually produced in all cells of both the sporophyte and gametophyte generations. In these taxa variations in oil body size, shape, color, number and distribution are taxonomically informative (Pfeffer 1874, Müller 1939, Schuster 1966, 1992a, Gradstein *et al.* 1977), with five broadly defined categories recognized (Fig. 1.2). *Massula*- and *Bazzania*-type oil bodies are shiny, homogeneous, and either very small and abundant (*Massula*-type) (Fig. 1.2B) or larger and fewer per cell (*Bazzania*-type) (Fig. 1.2C). Oil bodies of the *Calypogeia* type are botryoidal, consisting of grape-like clusters of discrete, shiny globules; they can be translucent or pigmented, of small to medium size and usually many per cell (Fig. 1.2D). Oil bodies that are opaque, gray to gray-brown and granulose to papillose in texture (*Jungermannia*-type) are the most common type in the Jungermanniopsida; these can be small and numerous per cell, or very large and solitary as in *Radula* (Fig. 1.2E). In *Treubia* and most genera of the Marchantiopsida, oil bodies occur only in scattered idioblastic cells of the gametophyte; they are large, solitary, granular and opaque, gray to gray-brown (Fig. 1.2F). These idioblastic “oil cells” differ from the surrounding vegetative cells only by the presence of the large oil bodies in them (Suire 2000), in contrast to earlier views that they lack chloroplasts (Schuster 1984b).

Unfortunately, because of the volatility of the oils contained in them, oil bodies rapidly “disappear” in dried specimens. In fact, their morphology is often modified even during short-term storage in the dark, so observations of oil body morphology must be conducted only on freshly collected samples. Ultrastructural evidence confirms that the oil body membrane and internal matrix remain intact for up to six weeks in dark-stored specimens, but the oil droplets within the matrix disappear within a few days (B. Crandall-Stotler, unpublished data).

In phylogenetic reconstructions, the presence of oil bodies is a synapomorphy of the Marchantiophyta, and oil bodies of the *Massula*-type are reconstructed as the plesiomorphic state (Crandall-Stotler *et al.* 2005). Oil bodies have been

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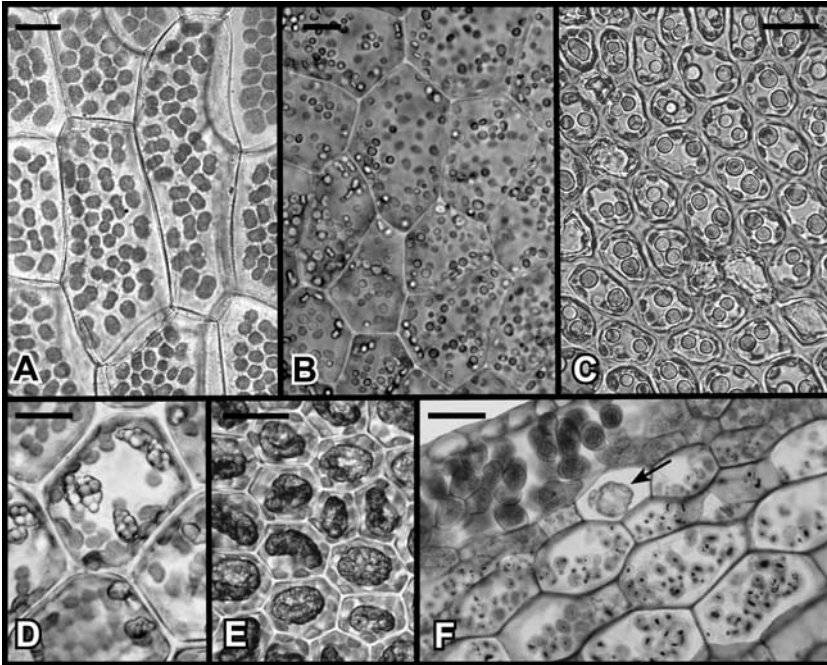


Fig. 1.2. Cells and oil bodies of liverworts. (A) Thallus wing cells of *Blasia pusilla*, thin-walled and lacking oil bodies, bar = 15 μm . (B) Leaf cells of *Austrofossombronia peruviana*, with inconspicuous trigones and numerous small, homogeneous oil bodies of the *Massula*-type, bar = 25 μm . (C) Leaf cells of *Marsupella emarginata*, with large, triangular trigones and large homogeneous oil bodies of the *Bazzania*-type, bar = 20 μm . (D) Leaf cells of *Calypogeia muelleriana*, with medium triangular trigones, and botryoidal oil bodies of the *Calypogeia*-type, bar = 20 μm . (E) Leaf cells of *Radula obconica*, with inconspicuous trigones and large, solitary, papillose oil bodies of the *Jungermannia*-type, bar = 15 μm . (F) Longitudinal section of the thallus of *Marchantia polymorpha*, showing an idioblastic oil cell at the arrow, bar = 15 μm .

independently lost in several families, including the Antheliaceae, Cephaloziaceae, Lepidoziaceae, and Metzgeriaceae of the Jungermanniopsida, and the Blasiaceae (Fig. 1.2A), Sphaerocarpaceae, and Ricciaceae of the Marchantiopsida (Schuster 1966, Crandall-Stotler *et al.* 2005).

Various hypotheses have been formulated regarding the adaptive value of oil bodies, including suggestions that oil bodies deter herbivores and provide cold and/or UV protection (Schuster 1966). Immunolabeling techniques have shown that oil bodies contain enzymes involved in isoprenoid biosynthesis (Suire *et al.* 2000), confirming that they are active metabolic compartments of the liverwort cell. In addition, they sequester terpenoids and other secondary aromatics, much like the secretory glands of vascular plants (Flegel & Becker 2000).

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1.2.3 Gametophyte organizations

Three very different types of gametophyte organization occur within the phylum. The most widespread morphology is the leafy shoot, or nodal type organization, in which the gametophyte is composed of a stem and two or three rows of leaves. This type of organization is distributed across the phylogeny, occurring in all of the subclasses delineated in this work (Figs. 1.3–1.7), and characterizes almost all of the genera of the Jungermanniidae (Fig. 1.7). Simple thalloid morphology is common in the Pelliidae (Fig. 1.5) and Metzgeriidae (Fig. 1.6), but is also found in a few Marchantiopsida (e.g. Blasiales and *Monoclea* and *Monosolenium* in the Marchantiales), and Jungermanniidae (e.g. *Pteropsiella* and *Schiffneria*). In this morphology, plants consist of an unspecialized, planate thallus that is usually composed of a somewhat thickened central midrib and two lateral wings. In contrast, a dorsiventrally differentiated thallus, bearing a system of dorsal air pores and air chambers and a ventral storage zone, characterizes complex thalloid organization. This is the most restricted type of morphology, occurring only in the Marchantiidae (Fig. 1.4). Since the variation that occurs in each of these morphological categories employs a different suite of descriptors, they will be discussed separately. It should be noted, however, that leafy, simple thalloid and complex thalloid categories do not necessarily imply natural groupings, but simply refer to a type of morphological organization.

Variation in leafy morphologies

Multiplicity in the distribution, form, size and insertion of leaves provides many of the characters that define genera and species of foliose liverworts. In *Haplomitrium* (Fig. 1.3) and a few genera of the Jungermanniidae, e.g., *Herbertus* and *Lepicolea*, plants are erect and radially symmetric with three ranks of identical leaves (isophylly). The vast majority of leafy forms, however, display bilateral symmetry in which plants bear two rows of lateral leaves with or without a single row of smaller ventral leaves or underleaves (= amphigastria). In anisophyllous taxa the underleaves can be morphologically like the leaves, but smaller, or differ both in size and morphology. In traditional classification schemes, isophyllous taxa were considered primitive and evolution was presumed to progress toward planation and anisophylly (e.g. Evans 1939, Stotler & Crandall-Stotler 1977, Schuster 1984b). Recent phylogenetic hypotheses derived from sequence data suggest, however, that isophylly is a derived state (Davis 2004, Crandall-Stotler *et al.* 2005, He-Nygrén *et al.* 2006).

With a few exceptions, such as *Pachyglossa* and *Herzogiaria*, leaves in the Jungermanniidae are completely unistratose, whereas those of *Treubia*, *Haplomitrium*, and leafy taxa of the Pelliidae and Marchantiopsida are polystratose for

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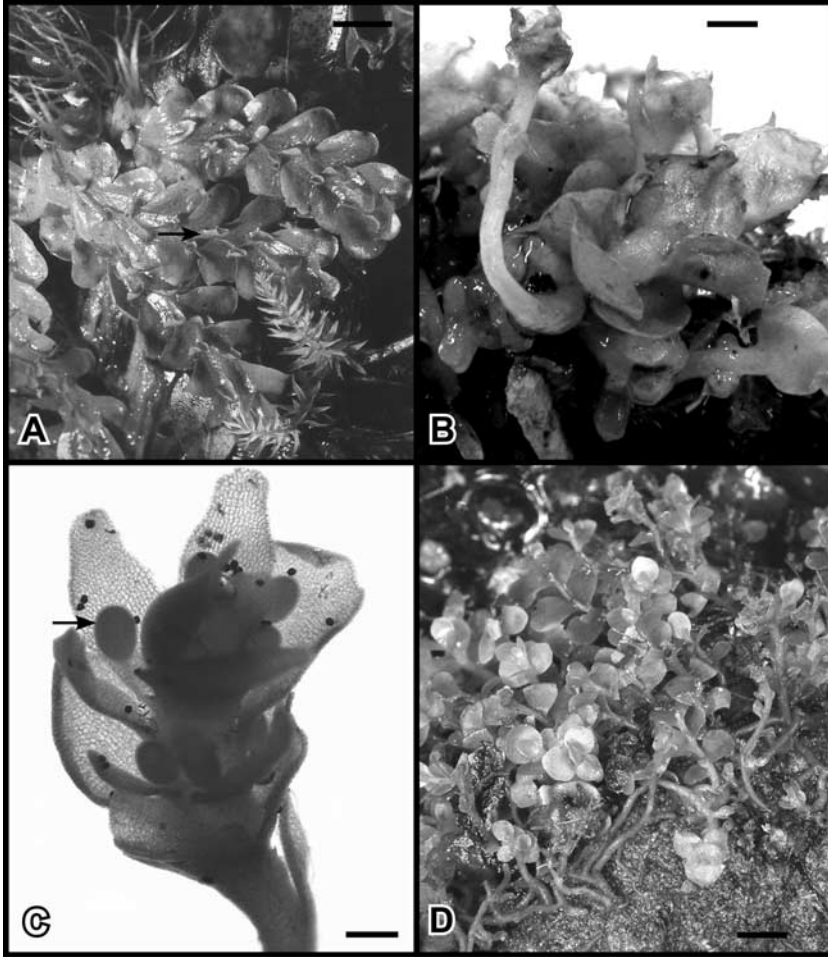


Fig. 1.3. Characters of the Haplomitriopsida. (A) *Treubia lacunosoides*, dorsal view, showing the lobate thallus and small dorsal lobules (at arrow) associated with each thallus lobe, bar = 4 mm. (B–D) Representatives of three lineages of *Haplomitrium*. (B) Male shoots of *Haplomitrium gibbsiae* (subg. *Haplomitrium* sect. *Archibryum*), arising from a slime-covered stolon system; antheridia are clustered at the apices of the leafy shoots. This species is sister to all other species in the genus, bar = 1.5 mm. (C) Male shoot of *Haplomitrium hookeri* (subg. *Haplomitrium* sect. *Haplomitrium*), showing antheridia (arrow) in the axils of unmodified leaves just below the shoot apex, bar = 300 μ m. (D) *Haplomitrium mnioides* (subg. *Calobryum*), dorsal view; note the branched, leafless stolon system and anisophyllous shoots, with the smaller third row of leaves on the dorsal side, bar = 2 mm.