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Anatomy, development and classification of hornworts

1.1. Introduction

Recently implicated as the oldest extant lineage of land plants, the anthocerotes hold many clues to the early diversification of terrestrial organisms (Malek *et al.* 1996, Garbary & Renzaglia 1998, Hedderson *et al.* 1998, Vaughn & Renzaglia 1998, Beckert *et al.* 1999, Nishiyama & Kato 1999, Renzaglia *et al.* 2000). Insights into adaptive strategies that enabled plants to survive during early land radiation and to persist through the millennia are gained through exploration of the morphology and reproductive biology of this ancient plant lineage (Renzaglia *et al.* in press). Such information also provides an essential foundation for future comparative studies among bryophytes and with basal groups of tracheophytes. In this chapter, we overview the current state of our knowledge on the morphology, ultrastructure, and developmental diversity within the anthocerotes. This information is evaluated from a comparative point of view in a broader context of relationships among streptophyte lineages. Throughout our discussion, we identify future lines of investigation that will provide significant new phylogenetic information on hornworts. Finally, we briefly review the prevalent ideas on the classification of anthocerotes.

1.2 Anatomy and development

The description that follows is intended to provide 1) an overview of the unifying morphological features of anthocerotes, 2) a brief survey of diversity in structure among hornwort taxa, and 3) a synthesis of published and unpublished data derived from recent ultrastructural studies.

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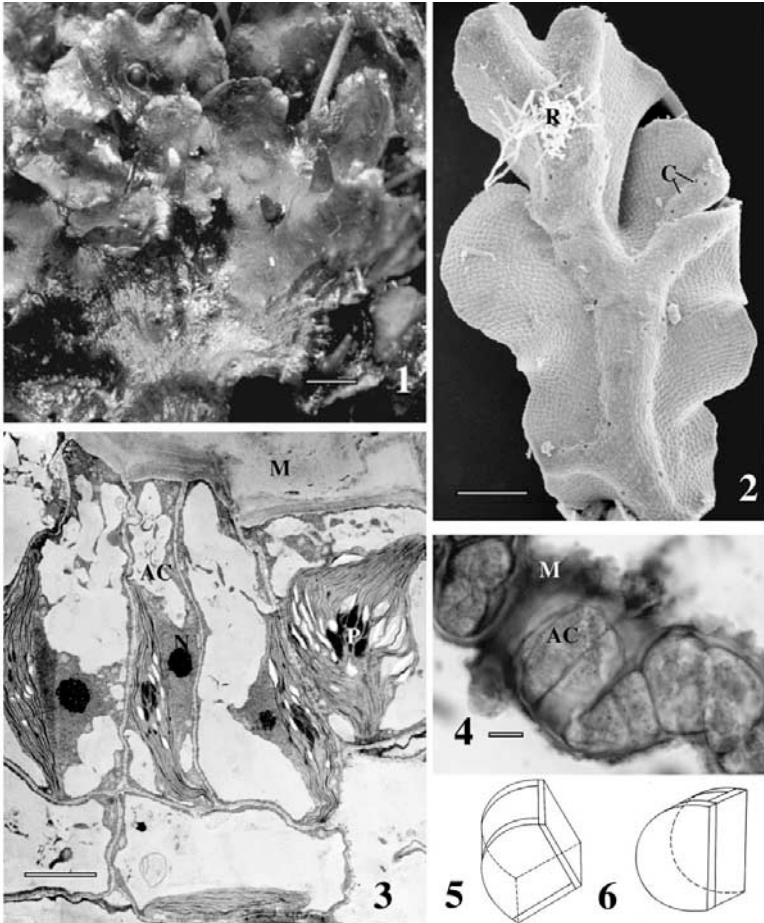


Fig. 1.1. *Phaeoceros laevis* subsp. *carolinianus* (Michx.) Prosk. Gametophyte with young sporophytes seen as bumps (upper thalli) and as horns breaking through protective involucre (right central thallus). Bar = 3.0 mm.

Fig. 1.2. *Dendroceros tubercularis* Hatt. SEM of ventral thallus showing swollen central midrib and monostromatic wings. Pore-like mucilage clefts (C) occur in two irregular rows on either side of the midrib and a tuft of rhizoids (R) is positioned below the terminal bifurcation. Bar = 0.25 mm.

Fig. 1.3. *Phaeoceros laevis* subsp. *carolinianus*. Transmission electron microscope (TEM) horizontal longitudinal section of growing notch overarched by mucilage (M). The rectangular apical cell (AC) and surrounded derivatives are highly vacuolated and contain a nucleus (N) in close association with a well-developed chloroplast (P). Bar = 4.0 μm .

Fig. 1.4. *Dendroceros japonicus* Steph. Light microscope LM cross-section of growing notch through hemidisoid apical cell (AC) and immediate derivative (indistinguishable from each other) surrounded by mucilage (M). This thallus may be in the process of branching. The single layered wing extends to either side. Bar = 10.0 μm .

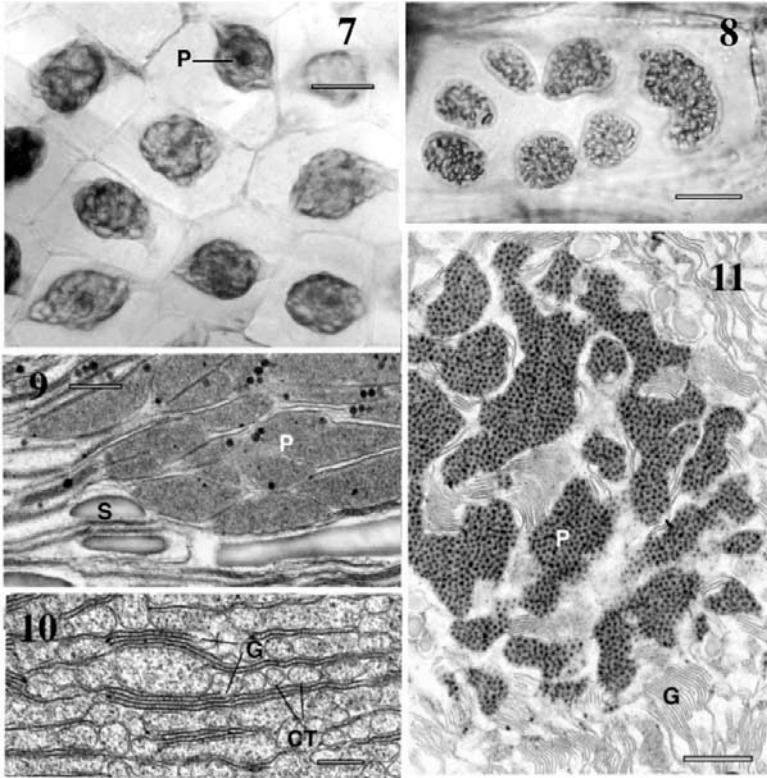
Among land plants, hornworts possess a bewildering array of structural features that are algal-like (chloroplast structure and biochemistry), liverwort-like (antheridial development and structure, apical cell architecture), moss-like (columella, stomates), and yet others that suggest affinities with seedless vascular plants (stomates, sunken gametangia, embryo development) (Campbell 1895, Bartlett 1928, Renzaglia 1978, Vaughn *et al.* 1992). These features will be examined at the cellular and tissue levels and the evolutionary significance of each will be considered.

The vegetative gametophyte of hornworts is a flattened thallus, with or without a thickened midrib (Figs. 1.1, 1.2). Growing regions that contain solitary apical cells and immediate derivatives typically are located in thallus notches and are covered by mucilage that is secreted by epidermal cells (Figs. 1.3, 1.4). Growth forms are correlated with apical cell geometry. The wedge-shaped apical cell of most taxa segments along four cutting faces: two lateral, one dorsal, and one ventral (Fig. 1.5). The resulting growth form tends to be orbicular and the thallus in cross-section gradually narrows from the center to lateral margins. In comparison, the hemidisoid apical cell (Figs. 1.4, 1.6) of *Dendroceros* cuts along two lateral and one basal face and is responsible for producing a ribbon-shaped thallus with an enlarged midrib (Fig. 1.2). Aside from anthocerototes, wedge-shaped and, less commonly, hemidisoid apical cells occur only in complex and simple thalloid liverworts (Crandall-Stotler 1980, Renzaglia 1982).

At the cellular level, hornworts typically contain solitary chloroplasts with central pyrenoids and channel thylakoids, features shared with algae but found in no other land plants (Duckett & Renzaglia 1988, Vaughn *et al.* 1992) (Figs. 1.7, 1.9, 1.10). Even the apical cell and immediate derivatives contain well-developed chloroplasts that are intimately associated with the nucleus (Fig. 1.3). Rubisco localizations in the pyrenoid and lack of grana end membranes (Figs. 1.10, 1.11) that characterize land plants may be viewed as plesiomorphies and further suggest ties with charophytes (Vaughn *et al.* 1990, 1992). Thylakoids traverse the pyrenoid and separate typically lens-shaped subunits, giving the appearance of

Fig. 1.5. Wedge-shaped apical cell characteristic of most hornworts. The two triangular lateral cutting faces, one rectangular dorsal cutting face, and one rectangular ventral cutting face produce a total of four derivatives in spiraled rotation. Modified from Renzaglia (1978).

Fig. 1.6. Hemidisoid apical cell of *Dendroceros* with two semicircular lateral cutting faces and a single rectangular basal cutting face. Modified from Renzaglia (1978).



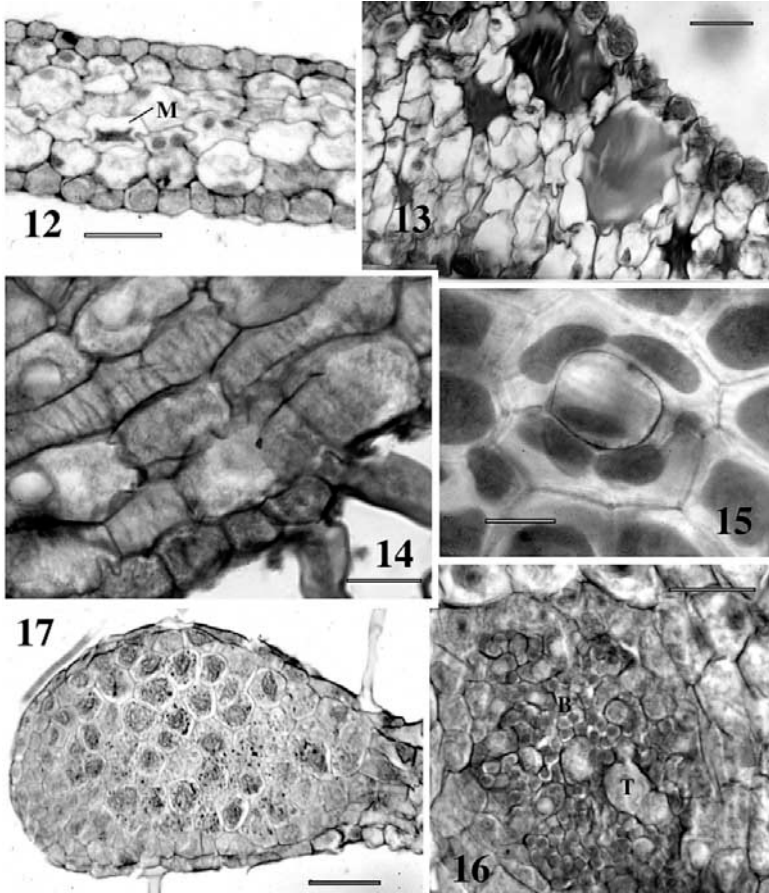
- Fig. 1.7.** *Phaeoceros laevis* subsp. *carolinianus*. Upper epidermal cells of gametophyte, each with single lens-shaped plastid containing abundant starch and central pyrenoid (P). Bar = 20.0 μm .
- Fig. 1.8.** *Megaceros aenigmaticus* Schust. Internal cell of thallus with seven starch-filled plastids that lack pyrenoids; the plastid on the right may be preparing for division. Bar = 10.0 μm .
- Fig. 1.9.** *Folioceros fuciformis* Bharadw. TEM of pyrenoid (P) consisting of lens-shaped subunits delimited by thylakoids and scattered pyrenoglobuli. Starch (S) surrounds the pyrenoid and narrow grana stacks traverse the plastid. Bar = 0.5 μm .
- Fig. 1.10.** *Folioceros appendiculatis* Haseg. TEM of plastid showing grana (G) that lack end membranes and associated channel thylakoids (CT). Bar = 0.2 μm .
- Fig. 1.11.** *Dendroceros tubercularis*. TEM of spherical pyrenoid (P) with irregularly shaped subunits containing uniform electron-dense inclusions. Thylakoids, including grana, interrupt the pyrenoid and stroma grana (G) lack end membranes. Bar = 0.5 μm .

“multiple pyrenoids” (Fig. 1.9). The shape of pyrenoid subunits and the existence/location of pyrenoid inclusions are taxonomically informative features of the chloroplast. For example, chloroplast structure in *Dendroceros* deviates from that of the “typical” hornwort in that the pyrenoid is spherical and contains irregularly-shaped subunits with regularly spaced electron-opaque inclusions (Fig. 1.11). Chloroplasts of *Megaceros* further diverge from the anthocerotite norm in that they occur in multiples and lack a pyrenoid (Fig. 1.8). Chloroplasts in this genus may number as many as 14 per internal thallus cell (Burr 1969). As in other land plants, Rubisco is scattered amongst starch grains in the chloroplast stroma of *Megaceros*.

Cell division in all hornworts is monoplastidic and involves plastid division and morphogenetic migration that is tightly linked with nuclear division (Brown & Lemmon 1990, 1993). Spindle microtubules originate from an aggregation of electron-dense material at the poles, suggesting the vestige of algal-like centriolar centrosomes (Vaughn & Harper 1998). Further investigations into cell cycle and cytoskeletal proteins are required to specify homologies of this structure to the polar bodies of liverworts and to centrosomes of other eukaryotes.

The thickened thallus of the hornwort gametophyte lacks internal differentiation (Fig. 1.12), except for the occurrence of rather extensive schizogenous mucilage canals in species of *Anthoceros* (Fig. 1.13) and *Apoceiros* (Schuster 1987). In some taxa, especially *Megaceros*, the epidermal cells are smaller than internal parenchyma cells (Fig. 1.12). Unlike in the sporophyte, all epidermal cells of the gametophyte contain chloroplasts (Fig. 1.15). Mucilage-filled cells are abundant and scattered amongst photosynthetic parenchyma in most taxa (Fig. 1.12). Band-like wall thickenings (Fig. 1.14) and primary pit fields may occur in thallus cells subtending archegonia and later the sporophyte foot (Leitgeb 1879, Proskauer 1960, Renzaglia 1978). Ultrastructural observations of these cells will enable an evaluation of their potential role in food transport. Vesicular–arbuscular endomycorrhizae are common in internal thallus cells of most taxa (Renzaglia 1978, Ligrone 1988). Rhizoids are unicellular, smooth and may have branched tips (Hasegawa 1983). They are typically ventral in position and occasionally they develop from the outer cell derived from a periclinal division of an epidermal cell (Fig. 1.14).

A distinctive feature of anthocerotes is the occurrence of apically-derived mucilage clefts (Figs. 1.2, 1.15) on the ventral thallus through which *Nostoc* enters the plant and becomes established as a colonial endosymbiont. Two cells that resemble guard cells surround the opening



- Fig. 1.12.** *Megaceros aenigmaticus*. LM cross-section of undifferentiated, simple thallus. Epidermal cells are smaller than internal cells of which one is mucilage-filled (M). Bar = 25.0 μm .
- Fig. 1.13.** *Anthoceros punctatus* L. Numerous mucilage-containing schizogenous cavities below the upper epidermis of the gametophyte. Bar = 25.0 μm .
- Fig. 1.14.** *Dendroceros* sp. LM cross-section of the gametophyte thallus showing several internal cells with band-like wall thickenings which often subtend archegonia and later sporophytes. Rhizoids are unicellular and smooth and may originate from a surface cell after periclinal division of the epidermal cell (left rhizoid). Bar = 25.0 μm .
- Fig. 1.15.** *Megaceros aenigmaticus*. Surface view of mucilage cleft in ventral epidermis of gametophyte. Both cells contain recently divided plastid. Bar = 10.0 μm .
- Fig. 1.16.** *Phaeoceros laevis* subsp. *carolinianus*. LM section through a *Nostoc* colony. Cells of the hornwort thallus (T) penetrate the colony and are interspersed amongst the small, spherical cells of the cyanobacterium (B). Bar = 20.0 μm .
- Fig. 1.17.** *Anthoceros punctatus*. LM longitudinal section of tuber. This swollen thallus protuberance contains abundant food reserve. Bar = 50.0 μm .

which, once formed, lacks the ability to open and close. Although considered by some authors (e.g., Schuster 1992) homologous to the stomates in the sporophyte of some anthocerotales, this interpretation is likely inaccurate due to the function and ventral location of these clefts. As the *Nostoc* colony increases in size, the internal chamber in which the alga is housed increases in size and fills with mucilage secreted by the hornwort. Thallus outgrowths penetrate the algal colony (Fig. 1.16).

As in most bryophytes, asexual reproduction is widespread in anthocerotales. Indeed, taxa such as *Megaceros aenigmaticus* in which the male and female plants are geographically separated into different watersheds rely entirely on vegetative reproduction for dissemination and propagation (Renzaglia & McFarland 1999). Fragmentation, regenerant formation, and gemmae production have been reported in various taxa. Under adverse environmental conditions, some species of hornworts produce nutrient-filled tubers as perennating bodies (Goebel 1905, Parihar 1961, Renzaglia 1978) (Fig. 1.17).

Gametangia are produced along the dorsal thallus midline. Archegonia are exogenous, i.e., they develop from surface cells, and ultimately they are sunken in thallus tissue (Fig. 1.18). In addition to the central cells of the archegonium, the archegonial initial gives rise to a one- to two-layered venter (Fig. 1.19) and six rows of neck cells that slightly protrude from the thallus surface and are overarched by a layer of mucilage (Figs. 1.20, 1.21). Two to four cover cells cap the canal until the egg reaches maturity at which time they are dislodged from the neck (Fig. 1.20). Venter cells are smaller than the surrounding parenchyma; they are less vacuolated and contain a prominent nucleus with nucleolus and an associated flattened plastid (Fig. 1.19). The central cells of the archegonium typically consist of four to six neck canal cells and a ventral canal cell and egg (Fig. 1.18). The ventral canal cell and egg are similar in ultrastructure; both contain dense cytoplasm including abundant lipid reserve and a single elongated undifferentiated plastid that encircles the nucleus (Fig. 1.19). The ventral canal cell persists beyond degradation of the neck canal cells and disintegrates when the egg reaches maturity. Both cells are surrounded by callose.

Antheridia are referred to as endogenous because they develop from subepidermal cells and ultimately are positioned within internal thallus chambers (Fig. 1.22). In other embryophytes, antheridia develop from single epidermal cells. The difference in hornworts is that the antheridial initial is located at the base of a schizogenous antheridial chamber, and

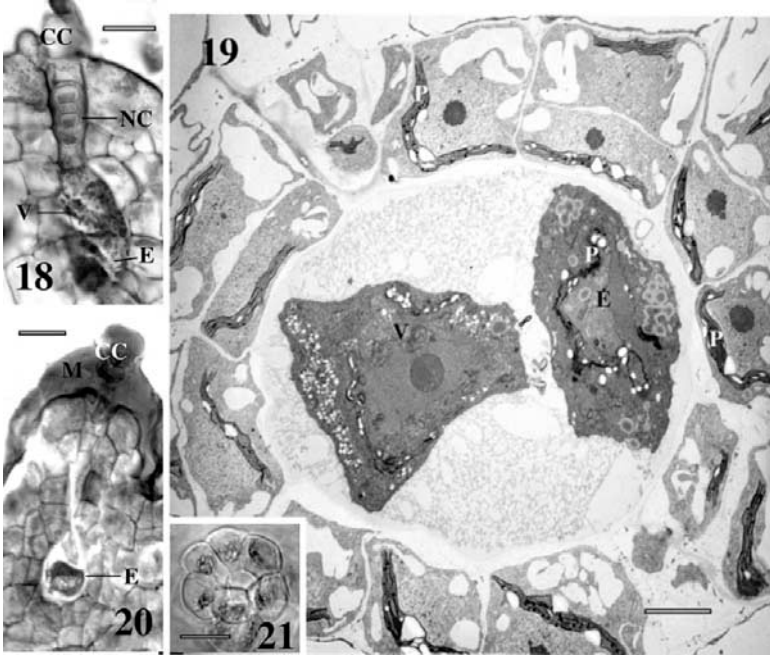


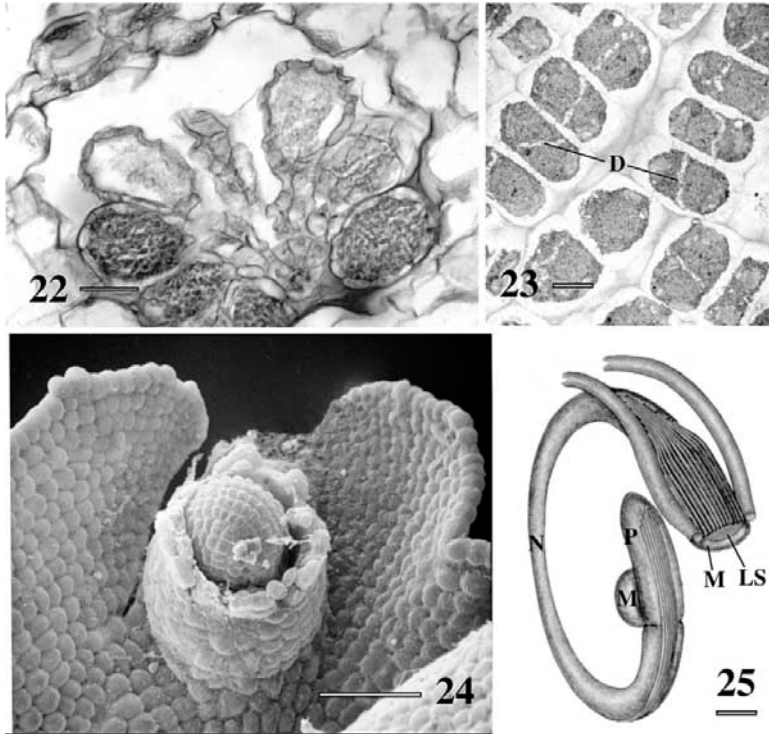
Fig. 1.18. *Phaeoceros laevis* subsp. *carolinianus*. Longitudinal section of an archegonium with two cover cells (CC), six neck canal cells (NC), ventral canal cell (V), and egg cell (E) containing nucleus. Bar = 20.0 μm .

Fig. 1.19. *Phaeoceros laevis* subsp. *carolinianus*. TEM oblique cross-section of venter of a nearly mature archegonium containing ventral canal cell (V) and egg cell (E); both are embedded in a callosic matrix and contain an elongated plastid near a large central nucleus (visible in ventral canal cell) and dense lipid-filled cytoplasm. The surrounding venter is one- or two-layered. Venter cells are small, each containing less-dense cytoplasm with small vacuoles and an elongated plastid (P) adjacent to the nucleus. Bar = 4.0 μm .

Fig. 1.20. *Dendroceros japonicus*. Longitudinal section of mature archegonium that projects from the dorsal thallus, is overarched by mucilage (M), and has discharged the cover cells (CC). The venter contains an egg cell (E). Bar = 20.0 μm .

Fig. 1.21. *Phaeoceros laevis* subsp. *carolinianus*. Surface view of mature archegonium containing six rows of neck cells each with a single prominent chloroplast. Bar = 20.0 μm .

not at the thallus surface. During hornwort evolution, there has been an apparent shift in developmental potential from epidermal (layer surrounding the external surface) to epithelial (layer surrounding an internal space) cells, both of which are surface cells that enclose tissue. However, development of the antheridium proper in hornworts resembles that of



- Fig. 1.22.** *Anthoceros punctatus*. LM of antheridial chamber showing eight stalked antheridia. Bar = 20.0 μm .
- Fig. 1.23.** *Notothylas orbicularis* (Schwein.) Sull. TEM showing diagonal final mitotic division (D) that produces pairs of polygonal spermatids. Bar = 3.0 μm .
- Fig. 1.24.** *Dendroceros tubercularis*. SEM of dorsal thallus with ruptured, projecting chamber containing a single antheridium. Bar = 0.1 mm.
- Fig. 1.25.** *Phaeoceros laevis* subsp. *carolinianus*. Diagrammatic illustration of biflagellated sperm cell (modified from Carothers & Duckett 1980). The locomotory apparatus consists of two flagella that are inserted symmetrically into the cell anterior over a spline of 12 microtubules and an underlying anterior mitochondrion (M). A rim of remnant lamellar strip (LS) lies directly anterior to the spline microtubules. The cylindrical nucleus (N) with central constriction occupies most of the cell length and a round posterior mitochondrion (M) is positioned on the plastid (P) that terminates the cell. Bar = 0.5 μm .

other bryophytes, especially complex thalloid liverworts, in that the antheridial initial elongates without apical cell involvement and four primary spermatogones with eight peripheral jacket initials are produced in the formative stages of organogenesis. Thus, the designation of hornwort antheridia as endogenous refers only to the location of development

and not to an inherently different developmental pathway from that in other bryophytes (Renzaglia *et al.* 2000).

Concomitant with antheridial morphogenesis is the growth and development of a two-layered chamber roof from the overlying epidermal initial. One to 25 antheridia (all derived from the same subepidermal cell) are ultimately enclosed in sunken chambers of the thallus (Figs. 1.22, 1.24). Thousands of minute spermatozoids are produced in each antheridium (Figs. 1.23, 1.25). When antheridia are mature, the plastids of the jacket layer typically have been converted to orange-colored chromoplasts (Duckett 1975). The roof of each antheridial chamber ruptures and the jacket cells dissociate, thus liberating the spermatozoids (Fig. 1.24).

Spermatogenesis provides clues to the phylogenetic history of hornworts (Renzaglia & Carothers 1986, Renzaglia & Duckett 1989, Garbary *et al.* 1993, Graham 1993, Vaughn & Renzaglia 1998). During spermiogenesis, pairs of bicentrioles arise *de novo* at the poles in the cell generation prior to the spermatid mother cell (Vaughn & Renzaglia 1998). Bicentrioles are diagnostic of archegoniates that produce biflagellated sperm cells but the timing of their origin in hornworts is earlier than in other taxa, where these organelles originate in the spermatid mother cell. Because green algal cells typically contain centrioles in all cell generations, this feature in hornworts is interpreted as a plesiomorphy (Vaughn & Harper 1998, Vaughn & Renzaglia 1998). As in *Coleochaete*, liverworts, and some pteridophytes, the final mitotic division in the spermatid mother cell is diagonal (Fig. 1.23). Spermatids develop in pairs and the mature spermatozoid is coiled, biflagellated, and symmetrical. Both flagella insert at the anterior extreme of the cell and are directed posteriorly. Spermatozoids are extremely small (approximately 3.0 μm in diameter) and only contain an anterior mitochondrion, a cylindrical nucleus with mid-constriction, and a posterior mitochondrion associated with a plastid containing one starch grain. Unlike spermatozoids of all other archegoniates which are sinistrally coiled, this cell exhibits a right-handed coil (Fig. 1.25). Because these cells are bilaterally symmetrical, as opposed to the bilateral asymmetry of motile sperm in other embryophytes, the direction of coiling may be inconsequential to swimming performance and thus was free to change during the extended evolutionary history of anthocerototes (Renzaglia *et al.* 2000).

The first division of the zygote is longitudinal and the endothecium of the embryo gives rise to a central columella in the sporophyte, if one