The moss calyptra: A maternal structure influencing offspring development

Jessica M. Budke[1]

Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, U.S.A.

ABSTRACT. The calyptra is a small, maternal gametophyte structure that covers the apex of the offspring sporophyte and is critical for the transition from seta formation to capsule differentiation in many moss taxa. It has been hypothesized to function by (1) providing a mechanical constraint that coordinates the development of the presumptive capsule, (2) secreting hormones that influence sporangium differentiation, (3) physically protecting the undifferentiated capsule region, (4) preventing water loss from the sporophyte apex, and/or (5) lowering the rate of sporophyte transpiration. This review explores the experiments that have tested these hypotheses and the evidence that has been found to support or refute them from scientific literature dating back to the late 1700's. Across mosses, calyptrae come in a wide array of shapes and sizes, which may correlate with aspects of morphology, habitat, and function. The overarching objective of this paper is to renew discussion about and research into this small, but important moss organ.

KEYWORDS. Anatomy, dehiscence, development, gametophyte, mosses, morphology, sporophyte, structure-function relationships, maternal effects, reproductive biology.

◆   ◆   ◆

Maternal plants can significantly influence offspring survival and development to reproductive maturity (Roach & Wulff 1987). In all embryophytes, the maternal gametophyte provides nourishment to the sporophyte during early development (i.e., matrotrophy; Graham & Wilcox 2000). Only in bryophytes does the sporophyte remain attached to, and nutritionally dependent upon, the maternal gametophyte throughout its lifespan (Ligrone et al. 1993). Moss gametophytes not only nourish the sporophyte, but they also influence sporophyte development through the calyptra, a cap of maternal gametophyte tissue that covers the sporophyte apex during early development (Bopp 1961; Budke et al. 2012; French & Paolillo 1975a).

Broadly applied to plants, a number of non-homologous hood-like structures are called calyptra (pl. calyptrae). In flowering plants, fused sepals, petals, and/or bracts can form the calyptra, which cover and protect interior parts of immature flowers (**Fig. 1A** from Endress 2003). Calyptra is also the term used for the exterior most cell layers of the root cap of monilophytes and lycophytes (**Fig. 1B** from Imaichi & Kato 1991; Bierhorst 1985). Additionally liverworts possess a true calyptra/shoot calyptra, both of which are composed of maternal gametophyte tissues that completely surround the sporophyte during early development (Crandall-Stotler et al. 2008; **Fig. 1C**), whereas, hornworts lack a calyptra. The moss calyptra is a detached cap of tissue formed from the maternal gametophyte (**Fig. 1D,E** from Budke et al. 2011) and is present in some form on all of the approximately 13,000 species of mosses.

Research focusing on moss calyptra function has a long history dating back to the experiments of Kölreuter (1777); however, over the past 200 plus years, research focusing on this structure has been sporadic (**Supplementary Table S1**). Additionally, from the time of these initial experiments to the present, the language used for scientific publication has shifted. Prior to World War II, mastery of English, French and German was often a doctoral dissertation requirement for both European and
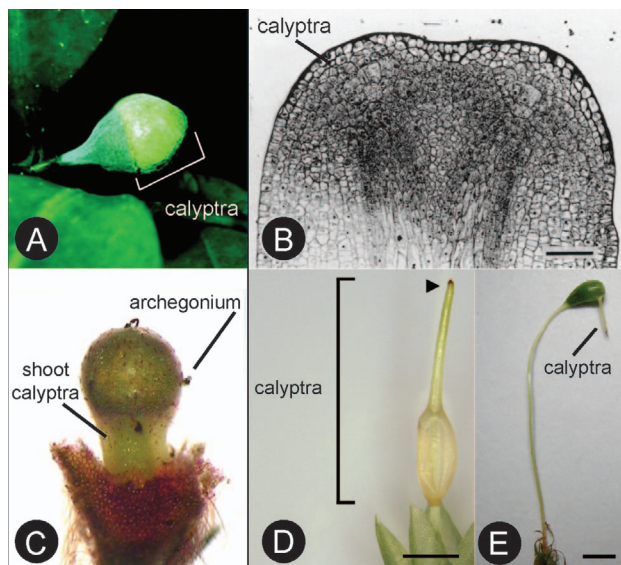
Figure 1. Examples of plant calyptrae. **A.** Calyptra covering an immature flower of *Eupomatia laurina* R.Br., figure 1C reprinted with permission from Endress (2003). **B.** Longitudinal section through the apex of a *Selaginella delicatula* (Desv. ex Poir.) Alston rhizophore. Thick-walled cells of the outer layers have been termed the calyptra; figure 24 reprinted with permission from Imaichi and Kato (1991). **C.** Sporophyte of the liverwort *Pellia epiphylla* (L.) Corda surrounded by a shoot calyptra, which is built from tissues of the archegonium and female gametophore and is distinguished by having unfertilized archegonia on the outer surface; image courtesy of Dr. Paul G. Davison. **D&E.** The moss *Funaria hygrometrica* from Budke et al. (2011) **D.** Maternal gametophyte calyptra covering an immature, unexpanded sporophyte. Arrow indicates sporophyte apex. **E.** Moss calyptra atop a mature sporophyte capsule. Scales in A, C = None associated with the images, B = 50 μm, D = 2 mm, E = 1 mm.

American scholars (Shigeru 1992). In contrast, current scientific training is most often English-centric, thus narrowing the abilities of researchers to independently access early scientific literature published in languages other than English. This shift may explain why over the past 50 years the majority of references to earlier moss calyptra research only reach back to the turn of the 20th century (e.g., references in Chopra & Kumra 1988; Crum 2001; French & Paolillo 1975a). This review provides a comprehensive discussion of moss calyptra development, morphology, function, and experimentation drawn from English, French, German, Latin and Swedish scientific literature dating back to the late 1700's. The objective of this paper is to renew discussion about and research into this small, but important moss organ, the calyptra.

Early Views on the Nature of the Calyptra

Kölreuter (1777) was the first botanist to publish his investigations on the role of the moss calyptra, based on experiments from several species, including *Bryum caespiticium* Hedw., *Hypnum serpens* Hedw. [= *Amblystegium serpens* (Hedw.) Schimp.], *Mnium hygrometricum* (Hedw.) With. (= *Funaria hygrometrica* Hedw.), and *Mnium punctatum* Hedw. [= *Rhizomnium punctatum* (Hedw.) T.J.Kop.]. Kölreuter demonstrated that complete removal of the calyptra typically led to malformed sporophytes that failed to develop a capsule, whereas removal and replacement of calyptrae onto the apex of spear-shaped sporophytes typically resulted in normal capsule formation. Kölreuter interpreted the moss sporophyte as the female portion of the plant, which upon fertilization formed a seed capsule, and the calyptra as the male portion. By removing the calyptra, fertilization was not achieved (calyptra removal = castration) and the 'seed capsule' did not develop.

Hedwig (1782) proposed an alternative interpretation and considered the moss calyptra to be a hood-shaped petal or corolla. This conclusion was based on similarities in function and dehiscence between flower petals and moss calyptra, wherein both of these organs protect underlying tissues and abscise after fertilization. Hedwig also argued that the effects of calyptra removal in mosses observed by Kölreuter (1777) were similar to his observations of corolla/calyx removal from developing fruits in flowering plants, thus supporting his hypothesis that these two structures are equivalent.

With his seminal publication on the alternation of generations, Hofmeister (1862) established that the leafy gametophyte in mosses is the vegetative part of the life cycle, which produces the sex organs, whereas the moss 'fruit' is the product of fertilization and is equivalent to the large, leafy plant body in ferns. He interpreted the moss calyptra as part of the vegetative plant that develops post-fertilization and covers the apex of the developing embryo (Hofmeister 1862). The maleness and fertilization abilities attributed to the calyptra and the femaleness of the 'fruiting body' proposed by Kölreuter (1777) were soon eschewed in favor of Hofmeister's views (e.g., Hy 1884; Lorentz 1867).

When the Term Calyptra Applies

The moss calyptra is established as an independent organ upon detachment of the upper portion
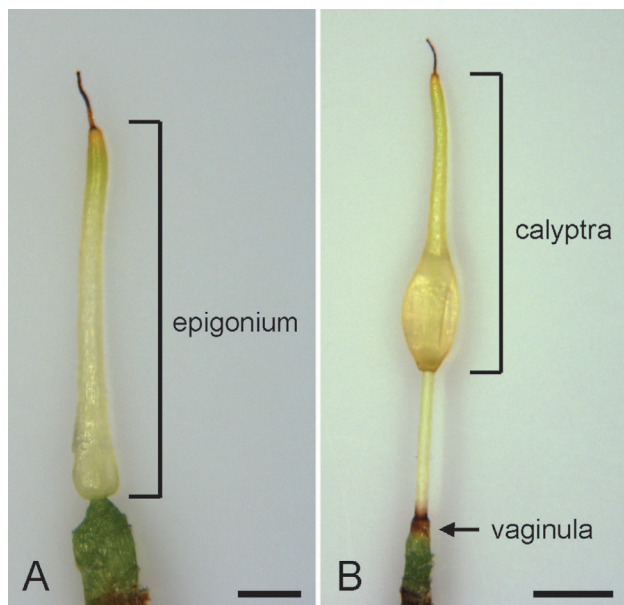
Figure 2. The moss *Funaria hygrometrica* with the leaves surrounding the base of the sporophyte removed. **A.** Tissues of the maternal gametophyte, epigonium, completely surround the offspring sporophyte. **B.** Apical tissues of the gametophyte are detached from the remainder of the maternal gametophyte. The calyptra covers the sporophyte apex above and the vaginula surrounds the sporophyte base below, exposing the sporophyte stalk in between. Scales in A = 0.5 mm, B = 1 mm.

of the gametophyte tissues that form the epigonium. The epigonium is derived from the venter and stalk of the fertilized archegonium, and sometimes, additional tissues of the subtending gametophyte (**Fig. 2A**). Post-detachment, the tissue surrounding the base of the sporophyte is termed the vaginula (Latin *vāgīna* "sheath, scabbard" + *-ula*, diminutive suffix [*Merriam-Webster.com* 2019]; **Fig. 2B**), and the cap of tissue covering the sporophyte apex is the calyptra (Greek *kalyptra* = veil [*Merriam-Webster.com* 2019]; **Fig. 2B**). The calyptra is derived exclusively from tissues of the epigonium, whereas the vaginula can be formed either from the epigonium, the archegonium stalk, the gametophyte apex or a combination of these tissues.

Consistent definitions for these terms have not always been applied. For example, the term epigonium was used interchangeably with the term calyptra by Lorch (1931). In an attempt to resolve this confusion, Roth (1969) proposed a new term to be used for the gametophyte tissues completely surrounding the sporophyte, 'Embryotheca' (embryo sac). However, this term was not adopted in

any subsequent discussions of calyptrae or epigonia. Alternative uses of similar terminology can make the interpretation of historical literature challenging. Fortunately the definitions outlined in the previous paragraph are now standard in the bryological literature (e.g., Magill 1990; Malcolm & Malcolm 2000) and thus these definitions will be used for the following discussion of the epigonium, vaginula and calyptra.

Epigonium Development, Prior to Calyptra Detachment

Early statements regarding the development of the epigonium, and thus the calyptra, include an array of contradictory ideas. Kölreuter (1777) proposed that the epigonium developed from the 'inner bark substance' (cortex), whereas the sporophyte/seta arose from the 'marrow' (inner core) of the leafy gametophyte stem. This description accurately represented the layered nature of the epigonium and sporophyte, but interprets both structures as developing from gametophyte tissues. Contrary to this interpretation, Schimper (1848) put forth that the epigonium and sporophyte were intimately connected and growing from the same terminal cell, with the epigonium representing the 'outer integument' of the 'embryo fruit'. Kölreuter (1777) and Schimper (1848) both interpreted the epigonium and sporophyte as developing from the same tissue, with the former proposing a gametophyte origin and the latter a sporophyte origin. Epigonium development and its relation to the sporophyte were later reinterpreted in light of Hofmeister's (1862) concept of the alternation of generations in plants. Thereafter, moss epigonia were defined as developing only from tissues of the maternal gametophyte, whereas the sporophyte develops exclusively from tissues resulting from fertilization (Goebel 1905; Hy 1884).

Remnant cells of the archegonial neck are often present on the epigonium apex, appearing to the naked eye as a small, brown filament (**Fig. 2**), and thus these cells do not contribute to epigonium formation. Cells that give rise to the epigonium all lie below the archegonial neck. Commentaries on epigonium development range from a general outline of the tissues involved (Goebel 1905), to detailed descriptions based on a specific taxon (e.g., *Orthotrichum schubartianum* Ltz. [Lorentz 1867]), to comparative studies of several taxa resulting in

the grouping of species into different ontogenetic types (Hy 1884; Janzen 1917; Rosander 1906). These types differ based on the tissues contributing to the epigonium (archegonium venter, archegonium stalk, and/or leafy gametophyte apex below the archegonia) and the direction of cell divisions (basipetal only or both basipetal and acropetal).

Portions of the calyptra developmental literature were previously summarized and analyzed by Janzen (1917), Lorch (1931) and Roth (1969). Of these, Roth (1969) carried out the most comprehensive and opinionated analysis. He proposed that Rosander's (1906) fourteen epigonial developmental categories could be condensed into eight, since the differences between the types were often minor. He also discussed the alternative perspective of Goebel (1905), who did not define epigonium developmental categories, but instead viewed the differences between taxa as a continuum. Roth (1969) interpreted Goebel's lack of defined categories for epigonium development as a distinct philosophical stance in opposition to previous concepts. However, based on my reading of the translated work, Goebel (1905) did not comment on or cite any previous epigonium research and thus his statements on epigonium development appear to be based on his personal observations alone. The emphasis Roth placed on describing calyptra developmental types as a continuum, opposed to categories, was not stressed in the earlier literature (Goebel 1905) and appears to solely reflect his strong opinions on the topic.

Roth (1969) also carried out original observations and analyses of epigonium development for an array of taxa, including members of the Andreaeidae, Bryidae, Buxbaumiidae, Polytrichidae and Sphagnidae. Two of the most often discussed taxa, *Andreaea* and *Sphagnum*, have morphologically simple calyptrae compared to other genera and are representatives of the earliest diverging lineages of mosses (Goffinet & Buck 2004; Liu et al. 2019). Roth observed that the *Sphagnum* epigonium develops from cells of the gametophyte stem, below the archegonium, confirming the observations of Hy (1884). In contrast, he questioned Hy's (1884) and Rosander's (1906) interpretations that the epigonium of *Andreaea* develops from the archegonium venter in both acropetal and basipetal directions, arguing instead for exclusively basipetal development from the archegonium stalk. These conflicting interpretations remain unresolved and warrant additional study (Goebel 1905; Hy 1884; Rosander 1906; Roth 1969). Understanding epigonium development in early diverging lineages of mosses may illuminate not only calyptra evolution, but also the unique role of the maternal calyptra in moss sporophyte evolution.

EPIGONIUM TO CALYPTRA TRANSITION

With the exception of *Bryobartramia,* which has a persistent epigonium (Stone 1977), epigonium development is terminated by detachment of the calyptra above from the vaginula below. This process occurs as an irregular rupturing during capsule expansion in mosses with very short sporophytes (e.g., *Andreaea, Archidium, Sphagnum*) (i.e., Duckett et al. 2009; Hy 1884; Renzaglia et al. 1997). This is similar to the rupturing of fern and liverwort sporophytes from their surrounding gametophyte tissues (Campbell 1918). In the majority of mosses, sporophyte capsules are elevated on a seta and the calyptra dehisces along a smooth, transverse line located around the epigonium base during an early spear stage of sporophyte development (Goebel 1905). However, there are a few species with a poorly developed dehiscence line that results in a jagged tearing at the base (e.g., *Tetraphis*; Janzen 1917). In taxa with a well-developed dehiscence line, these cells lack chlorophyll, have thinner cell walls, and are swollen compared to other cells of the epigonium (e.g., *Funaria*; Hy 1884; **Fig. 3** from Janzen 1917). These weakened cells then give way to mechanical forces generated by the sporophyte growing within. On occasion these cells do not properly detach and then the growing sporophyte can pierce through the calyptra (True 1906). The cellular features that facilitate moss calyptra dehiscence are similar to those present in abscission layers of angiosperm leaves (Esau 1977). Detachment of the maternal gametophyte calyptra in mosses could potentially represent one of the earliest forms of dehiscence to evolve in embryophytes.

MOSS SPOROPHYTE DEVELOPMENT

Moss sporophytes are unbranched and physically attached to the maternal gametophyte via the foot throughout their lifespan. Initial development begins with divisions of a single apical cell to produce a spear-shaped sporophyte that is completely sur-
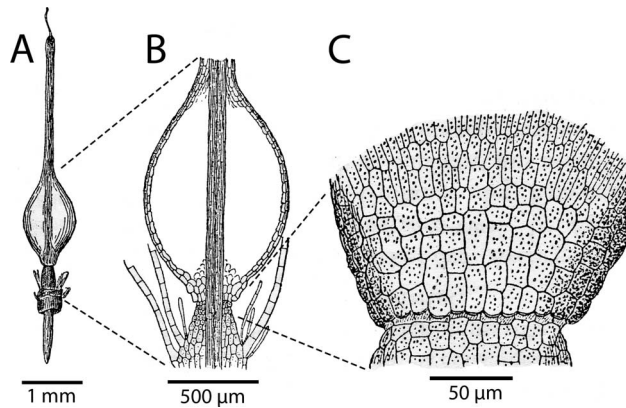
Figure 3. A–C. Illustrations of the calyptra dehiscence zone in *Funaria hygrometrica,* reprinted with permission from Janzen (1917; www. schweizerbart.de/journals/nova_hedwigia). **A.** Epigonium with sporophyte inside. **B.** Inflated base. **C.** Dehiscence zone where the calyptra will detach from the vaginula.

rounded by gametophyte tissues (**Fig. 4**; Crum 2001). The apical region of this small sporophyte later differentiates into the mature sporangium. Once the apical cell stops dividing and the presumptive capsule (apical region) is established, a second meristematic zone (seta meristem) initiates below the apical region, which divides to form the seta/stalk (French & Paolillo 1975b). The calyptra becomes detached from the reminder of the maternal gametophyte as this seta meristem begins to actively divide (**Fig. 4**). While the foot and lower part of the seta remain within the vaginula, both the apical region of the young sporophyte and the seta meristem are covered by the calyptra throughout seta development (French & Paolillo 1975b), which may be a short or extended period depending on the seta length of the species. Once the seta has formed, a major developmental transition occurs from increasing sporophyte height via seta growth to expansion and differentiation of the apical region, resulting in sporangium (capsule) formation (**Fig. 4**). The calyptra typically falls away once sporangium formation is complete. This sporophyte developmental sequence is unique to mosses and the presence of the maternal calyptra on the sporophyte apex during these critical developmental transitions may have been the initial observation stimulating research into calyptra function.

Calyptra Function

Removal experiments have repeatedly demonstrated that the calyptra is critical for moss sporophyte development (Bopp 1957; French &

Paolillo 1975a; Herzfelder 1923; Irmscher 1912; Kölreuter 1777; Zielinski 1910). These observations made over a span of 200 years have stimulated a number of questions regarding calyptra function, with the primary ones being: How are the calyptra and sporophyte interacting? What role/s does the calyptra play in sporophyte development? The proposed hypotheses are that the calyptra (1) provides a mechanical constraint that coordinates the development of the presumptive sporangium, (2) secretes hormones that influence sporangium differentiation, (3) physically protects the undifferentiated sporangial region, (4) prevents water loss from the sporophyte apex, and/or (5) lowers the rate of sporophyte transpiration. These putative functions are not mutually exclusive and the strength of the evidence supporting these hypotheses varies.

Mechanical constraint. Following experimental removal of the calyptra, the sporophyte apex expands, which is a developmental reaction initially interpreted as sporophytes undergoing a 'despair maturity', striving to achieve capsule formation quickly (Bopp 1954). However, this sporophyte expansion often results in incomplete or a lack of capsule formation with a small, undifferentiated region remaining at the sporophyte apex (**Fig. 5**; Bopp 1957; French & Paolillo 1975c), which contradicts Bopp's (1954) earlier interpretation of 'despair maturity'. The seta meristem often continues to divide indeterminately, producing an expanded, obconic-shaped stalk that is composed of wider, shorter, and more cells compared to the normally cylindrical seta (**Fig. 5D** from French & Paolillo 1976). In an earlier experiment by Herzfelder (1923) she removed the calyptra, cut off its apex, and then replaced the tube-shaped calyptra on the sporophyte, exposing the sporophyte apex above the cut end of the calyptra; this resulted in the sporophyte expanding both above and below the area covered by the calyptra (**Fig. 5E**, a reproduction of this experiment with the sporophyte expanding only above the cut calyptra). These experiments demonstrate that the calyptra acts as a straightjacket around the sporophyte constraining the actively dividing seta meristem, which influences the anatomy in terms of the cell shape and the rates of cell division, as well as the morphology of the sporophyte. Without the constraining force of the calyptra, the seta meristem does not produce a cylindrical seta and is unable to properly regulate its
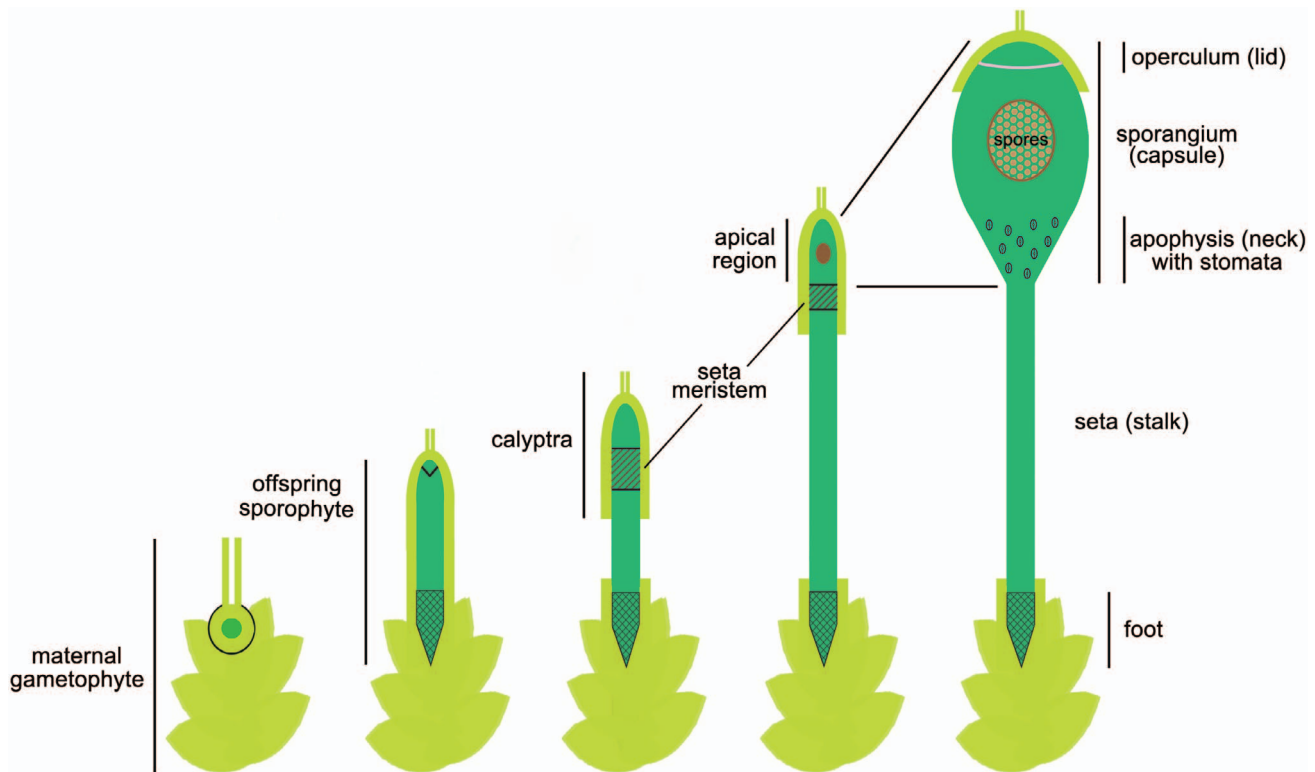
Figure 4. Diagram of moss sporophyte development. Sporophyte offspring begin development completely surrounded by the maternal gametophyte tissues. Initially the sporophyte grows by divisions of a single apical cell. Later a second meristematic region, the seta meristem, produces the seta/stalk, which elevates the undifferentiated apical region. This region will later differentiate into the capsule/sporangium. The capsule includes cells that will undergo meiosis to produce haploid spores, a lid/operculum that enables spore release, and below a neck/apophysis that is where stomata are located, if present. Throughout sporophyte development the apical region and seta meristem are covered by the maternal gametophyte calyptra. The calyptra is an integral structure for successful sporophyte development.

shape (**Fig. 5**). The physical presence of the calyptra also enables the transition from sporophyte growth via the seta meristem to differentiation of the apical region into a mature capsule. Without the calyptra this transition is permanently interrupted (Herzfelder 1923; French & Paolillo 1976).

These observations indicate that the control of sporophyte organ shape differs between mosses and vascular plants. The epidermal-growth-control theory proposes that the epidermis plays both a mechanical and physiological role in the control of plant organ shape (Kutschera & Niklas 2007). However, the vast majority of experimental evidence supporting this hypothesis is based on vascular plants (e.g., *Arabidopsis* in Gruel et al. 2016; Savaldi-Goldstein et al. 2007; and Vaseva et al. 2018; *Nicotiana* in Marcotrigiano 2010). The mechanical role includes an outer epidermal cell wall that is generally thicker, and stiffer, than the underlying cells and the cuticle, which can contribute to the ability of the outer epidermal cell walls to resist deformations (Kutschera & Niklas 2007). The immature moss sporophyte has thin epidermal cell walls that lack a well-developed cuticle, whereas the outer cell layer of the calyptra often has thick cell walls and a well-developed cuticle (Budke et al. 2012). Immature moss sporophytes without a calyptra produce aberrant morphologies and appear unable to control their shape (French & Paolillo 1976). The calyptra potentially represents an alternative and external mechanical system that controls the sporophyte shape and development in mosses.

Removing the mechanical constraint of the calyptra also impacts stomata development. Stomata on bryophyte sporophytes are typically located in the lower half or at the base of the capsule and are oriented parallel to the axis (**Fig. 4**; Paton & Pearce 1957). When calyptrae are removed prior to the formation of guard cell mother cells, the mature

stomata are disoriented; on average they are tilted at a 45° angle relative to the sporophyte axis, compared to an average angle of 82° in sporophytes with calyptrae (French & Paolillo 1975d). When calyptrae are removed later, when guard cell mother cells are actively dividing to produce guard cells, a majority of the guard cell mother cells remain undivided or produce abnormally shaped guard cells (French & Paolillo 1975d). The influence of the calyptra on stomata occurs late during sporophyte development compared to the other mechanical effects, demonstrating the influence and importance of the calyptra across multiple stages of sporophyte development.

Physiological influences. The calyptra may also have a physiological influence on sporophyte development via hormone secretion. Prior to the calyptra detaching (i.e., the epigonium stage), these gametophyte tissues are alive, and thus have the potential to secrete physiologically active compounds. At this stage a liquid fills the space between the sporophyte and the surrounding gametophyte tissues (Goebel 1905; Merced & Renzaglia 2016). This fluid is thought to contain nutritive compounds that may positively influence the sporophyte physiologically (Lorch 1931), but this hypothesis remains untested. In some taxa, inner cell layers of the calyptra break down, potentially releasing additional compounds into the fluid (Janzen 1917). Even after the calyptra has detached from the leafy gametophyte, the liquid can persist, especially in calyptrae with an inflated base that acts as a reservoir, as in *Funaria hygrometrica* (**Fig. 1D**; Janzen 1917; Roth 1969). Eventually the liquid beneath the calyptra evaporates and any subsequent physiological influences require cells of the moss calyptra to be living to actively produce and secrete compounds that are then transferred to the underlying sporophyte.

The calyptra can remain alive after detachment from the remainder of the maternal gametophyte (Bopp 1954; Oehlkers & Bopp 1957) and thus may have the potential to secrete physiologically active compounds that impact sporophyte development. However, the moss calyptra has no symplastic connections to the underlying sporophyte tissues and consequently any physiologically active compounds must be excreted from cells of the calyptra and taken up by the sporophyte through its epidermal cells. Sporophytes covered with calyptrae that were experimentally denatured via boiling in acetone, ethanol, and water to remove any physiologically active compounds resulted in normal sporophyte development (Bopp 1957; French & Paolillo 1975a). Attempts to chemically detect hormones present in the calyptra also yielded negative results (Bopp 1957). Thus any hormones or physiologically active compounds that the calyptra may produce have yet to be detected and may not be required for later stages of sporophyte development.

Despite the lack of evidence for calyptra-produced hormones, the sporophyte is responsive to hormonal treatments. When indole-3-acetic acid (IAA) was externally applied to sporophytes without calyptrae, both seta swelling and apical curling were accelerated compared to controls where calyptrae were removed but no additional hormones were applied (Bopp 1957). Applying either maleic hydrazide (a plant growth regulator) or an extract of ground calyptrae to sporophytes that had their calyptrae removed, resulted in individuals that completed capsule formation normally (Bopp 1957). In these experiments it was essential that the solution with the regulator or extract was applied to the region of the seta meristem. Sporophyte response to a calyptra extract contradicts the other observations and experiments that concluded the calyptra lacks any physiologically active compounds. That being said, many of these experiments were performed over 50 years ago and this idea warrants additional testing.

There is no direct evidence that the calyptra is actively secreting hormones that affect sporophyte development. Nonetheless, the calyptra may be influencing the internal hormonal patterns of the moss sporophyte (French & Paolillo 1975a). The major developmental transition that is disrupted by calyptra removal is the shift from divisions of the seta meristem that produces the stalk early during development, to the differentiation of the apical region into the capsule (**Fig. 4**). The transition between these developmental stages is most likely mediated by the plant hormone auxin. Auxin movement within moss sporophytes is basipetal during the early developmental stage of seta elongation, whereas later during capsule differentiation there is auxin transport both basipetally and acropetally (Poli et al. 2003). Calyptra removal during early development may disrupt the auxin flow, resulting in a lack of capsule differentiation

(**Fig. 5A–D**). The calyptra has been described as coordinating sporophyte development (Bopp 1961). Whether an exclusively mechanical constraint carries out this coordination alone or a hormonal influence is also involved remains to be determined.

Physical protection. Another function of the moss calyptra is protection of the undifferentiated apical region of the sporophyte. This includes protection from biotic factors, such as browsing by snails and slugs, physical damage caused by animals passing by (Janzen 1917), and pathogens. More importantly may be protection from abiotic factors, including protection against both high and low temperatures, excess light, and rotting due to high humidity (Firtsch 1883; Hedwig 1782; Janzen 1917). The protective nature of the calyptra is regularly mentioned in the literature (e.g., Frahm 2001; Lorch 1920; Schofield & Hebant 1984); however, experimental and/or ecological studies examining the role of the calyptra in sporophyte protection are currently lacking.

Dehydration protection. Of the protective functions of the moss calyptra, protection from dehydration is the most well-studied (Budke et al. 2013; Grebe 1912; Haberlandt 1886; Lorch 1920; Zielinski 1910). Experiments examining sporophytes of five moss species dried both with and without calyptrae resulted in all individuals without calyptrae dying, whereas greater than 60% of the individuals with calyptrae survived (Irmscher 1912). Calyptra removal experiments are typically carried out inside chambers in order to keep sporophyte apices without calyptrae hydrated and to prevent death from dehydration (Bopp & Stehle 1957; Herzfelder 1923).

This dehydration protection ability was attributed to a waxy cuticle covering the calyptra (Goebel 1905; Hy 1884; Janzen 1917). In an early experimental test, calyptrae were removed from young sporophytes prior to seta elongation and placed in sulfuric acid, resulting in the distal half of the calyptra dissolving last (Janzen 1917). This observation was used to support the hypothesis that the calyptra, and in particular the narrow apical rostrum, is covered by a cuticle early in development (Janzen 1917). Acid resistance in bryophytes has been attributed to phenolic compounds that can be present in either the cell wall or cuticle (Kroken et al. 1996; Kodner & Graham 2001), thus Janzen's

observations of acid resistance cannot be definitively attributed to only a cuticle and may indicate that the cell walls and/or cuticle of the calyptra rostrum contain higher levels of phenolic compounds. Following these experiments, the advent of electron microscopy has enabled direct observation of thin bryophyte cuticles (Budke et al. 2011; Cook & Graham 1998; Koch & Frahm 2007; Proctor 1979; Sack & Paolillo 1983). Structural and developmental studies, using electron microscopy, have demonstrated that the calyptra cuticle of *Funaria hygrometrica* is significantly thicker than the leafy gametophyte cuticle (Budke et al. 2011) and the calyptra cuticle develops precociously relative to the sporophyte cuticle (Budke et al. 2012), with the potential to prevent dehydration early during sporophyte development. Chemistry analyses have also determined that the cuticular waxes are thicker per unit area on the calyptra compared to the rest of the maternal gametophyte (Busta et al. 2016). Experimental removal of the calyptra cuticle under low humidity has been shown to negatively impact sporophyte survival, development, and fitness, demonstrating the importance of the calyptra cuticle for dehydration protection (Budke et al. 2013).

Hairs on the calyptra may also aid in dehydration protection (Lorch 1920). Hairy calyptrae (e.g., *Polytrichum*), were originally equated to a thatched roof in terms of their protective abilities (e.g., Firtsch 1883; Goebel 1905; Haberlandt 1886; Janzen 1917). Plant hairs can decrease water loss by increasing light reflectance (e.g., Billings & Morris 1951; Ehleringer & Mooney 1978) and increasing boundary layer thickness (Wuenscher 1970). Hairy calyptrae may represent an alternative structural adaptation helping to prevent water loss from the sporophyte. If hairs do decrease moss sporophyte water loss, a trade-off between structural investment in hairs and the cuticle may be observed. Species with hairy calyptrae may have a thinner, less complex cuticle, whereas species with a smooth calyptrae have a thicker, more complex cuticle. Despite hairy calyptrae often being mentioned as functioning to prevent water loss, no explicit experiments have been undertaken to test this hypothesis.

Calyptrae with an inflated base and the liquid that fills it early in development may also function in dehydration protection of the sporophyte apex (e.g., Goebel 1905; Janzen 1917; Roth 1969). The
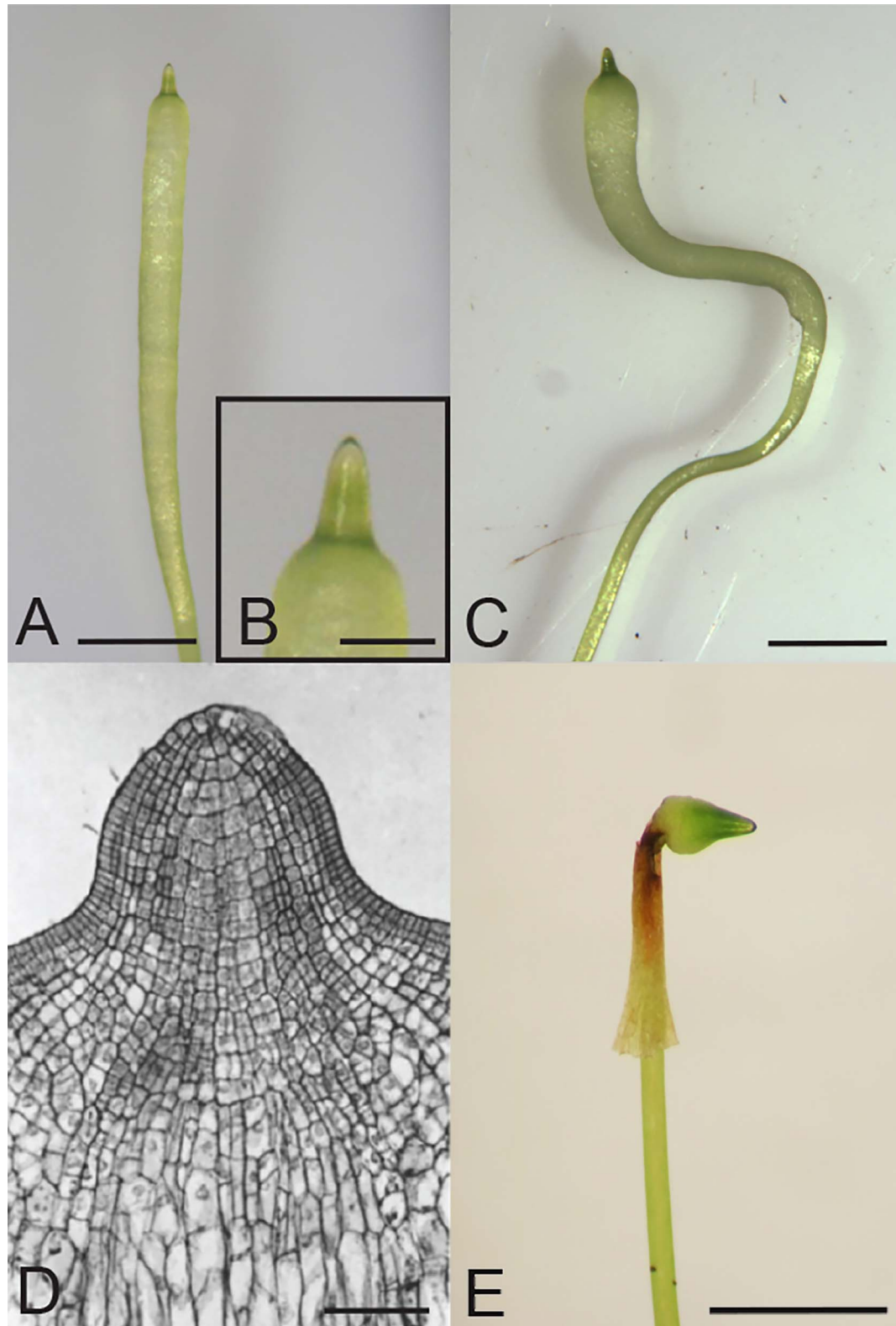
Figure 5. *Funaria hygrometrica* sporophytes. **A–D.** Calyptra removed from sporophyte apex in a high humidity chamber. Sporophyte continues to grow via the activity of the seta meristem with undifferentiated apical region (**B**) remaining at the top after calyptra removal, which never forms a mature capsule. **D.** Median longitudinal section of actively growing sporophyte, after removal of maternal calyptra, figure 2 reprinted with permission from French & Paolillo (1976). **E.** Calyptra experimentally removed, the apex cut off, and then the remaining calyptra lacking the apex was replaced on the unexpanded sporophyte. Swelling above the calyptra due to the actively dividing seta meristem is shown, in contrast to swelling both above and below the calyptra as observed by Herzfelder (1923). Scales in A, C, D, E = 1 mm, B = 0.25 mm.

liquid beneath the inflated base can remain long after the calyptra has detached from the remainder of the maternal gametophyte (Merced & Renzaglia 2016) and thus may aid in decreasing water loss from the moss sporophyte apex. When exposed to a dehydration event this liquid evaporates first (pers. obs.), providing a buffer for the sporophyte apex and delaying its exposure to dehydration. The reservoir of liquid in the calyptra inflated base may play an important role in preventing dehydration of the sporophyte apex early during development.

Mucilage secreted by axillary hairs in mosses helps prevent young, expanding leaves from drying (Ligrone 1986). Additionally the apical cells of hornwort gametophytes are covered by a desiccation-retardant mucilage that is secreted by the epidermal cells (Renzaglia et al. 2008). Though the composition of the liquid beneath the calyptra has yet to be determined, the fact that they easily slide off the sporophyte apex in high humidity but are difficult to remove from the sporophyte apex at low humidity (pers. obs.; Herzfelder 1921) is suggestive of a pectin-rich gel. Pectin-rich gels in plants are known to have strong solidifying properties when dry, whereas when hydrated they allow cells to easily glide past each other (BeMiller 1986). The liquid beneath the calyptra may thus function to attach the calyptra more firmly to the sporophyte apex during stressful low humidity events, contributing to the dehydration protection abilities of the calyptra.

Decreasing sporophyte transpiration. An additional interpretation for the prevention of water loss from the sporophyte apex by the calyptra involves lowering the rate of sporophyte transpiration, which can potentially decrease the resources extracted from the maternal gametophyte by the sporophyte offspring. In this parent-offspring conflict, maternal gametophytes are predicted to possess adaptations for regulating resource transfer to sporophytes, whereas the sporophytes are predicted to consume or attempt to consume resources beyond the point that is optimal for maternal fitness (Haig 2012). The gametophyte calyptra has been shown to decrease water transport rates in the sporophyte via an experiment where a dye solution ascended sporophytes without calyptrae 1.3× faster compared to control sporophytes with their calyptrae in place (Bopp & Stehle 1957).

The sporophytes in many moss lineages form stomata at the sterile base of the sporangium and these structures may also play a role in transpiration (**Fig. 4**; Merced & Renzaglia 2017). However, the stomata develop beneath the calyptra and are most likely unable to function in transpiration until the calyptra has fallen away. Similarly, the pseudostomata in *Sphagnum* are covered by the epigonium throughout sporophyte ontogeny, blocking their potential for functioning in gas exchange (Duckett & Ligrone 2004). They are not exposed to the surrounding environment until the pseudopodium fully elongates and the capsule begins to dry out, which is when the thin epigonium ruptures and withers away (Duckett et al. 2009; Schimper 1858). Thus the functionality of the *Sphagnum* pseudostomata in either transpiration or capsule dehiscence is blocked prior to the epigonium and calyptra falling away.

DEVELOPMENTAL TIMING

The functional importance of the calyptra is not static across sporophyte development, but has been shown to vary depending on the developmental stage. During early sporophyte development of *Funaria hygrometrica*, calyptra removal results in sporophytes that continue to grow via the seta meristem, but they rarely (approximately 10%) produce a mature capsule (**Fig. 5A–D**; Bopp 1954; French & Paolillo 1975a). Later in development, sporophytes without a calyptra are more likely to form a capsule (>27%; French & Paolillo 1975a). As sporophytes approach maturity, calyptra removal results in sporophytes that stop their growth in height and transition to capsule expansion two days earlier than individuals that retained their calyptra (French & Paolillo 1975a). This early transition to capsule differentiation was also accompanied by a 40% increase in dry weight of the mature capsules compared to the controls (French & Paolillo 1975a). Capsule weight correlates with the number of spores per capsule and thus can be used as a measure of sporophyte fitness (Budke et al. 2013). This indicates a tradeoff in resource allocation between increasing in height versus producing a larger capsule containing more spores. These observations in *Funaria hygrometrica* contrast sharply with observations of mosses in the genus *Mnium*, where capsules are formed at significantly higher rates independent of the calyptra. When the calyptra was

removed from *M. punctatum* sporophytes capsules were always formed, independent of developmental stage (Kölreuter 1777), whereas in *M. cuspidatum* Hedw. [= *Plagiomnium cuspidatum* (Hedw.) T.J.Kop.] up to 30% of the sporophytes in an experiment continued to elongate and produced capsules. Thus some moss species may be more dependent on the calyptra for coordination of sporophyte growth and successful capsule production than others.

Sporophyte susceptibility to desiccation also varies depending on the developmental stage. Early during sporophyte development, calyptra removal under low humidity results in sporophyte death. Thus, calyptra removal experiments on young sporophytes are carried out under high humidity conditions (e.g., Bopp & Stehle 1957; Herzfelder 1923). Later in development, sporophytes are able to survive without calyptrae at lower humidity levels (Dalmer 1891). This transition from susceptible to desiccation resistant is most likely due to the development of a thicker sporophyte cuticle later in development (Budke et al. 2012), a hypothesis for cuticle development proposed close to 100 years prior (Watson 1914). Moss sporophyte development is a dynamic process that must be thoughtfully considered when examining the functional importance of the calyptra.

The sporophyte and its dependence on the calyptra transitions as the sporophyte develops, whereas the influential abilities of the calyptra develop early and are relatively static across sporophyte development. Originally, Schimper (1848) hypothesized that the calyptra remains alive and continues to grow late into sporophyte development. However, the calyptra of *Funaria hygrometrica* achieves its mature size and shape early in development and upon detachment from the remainder of the leafy gametophyte it does not grow further (Budke et al. 2012). Calyptrae also develop a relatively thick cuticle early in development that is maintained, but not elaborated during sporophyte development (Budke et al. 2012). In contrast, one observation of calyptra growth post-detachment was in the moss *Campylopus*, where hairs on the bottom edge of the calyptra were observed growing after detachment from the leafy gametophyte (Hy 1884). With the exception of growing hairs in this genus, the overall size and shape of the calyptra appears to be established early

during development and does not change following dehiscence, though few species have been analyzed in depth.

The moss calyptra can remain alive post-detachment from the leafy gametophyte, at least in laboratory settings (Bopp 1954; Budke et al. 2012; Meyer 1942; Wynne & Budke 2012). These studies have either observed live calyptra cells using microscopy (Bopp 1954; Budke et al. 2012) or protonema production from the cut edge of a wounded calyptra (Meyer 1942) or the lower edge of an entire calyptra (Wynne & Budke 2012). These observations point toward the potential for the moss calyptra to function as a dispersal unit; calyptrae are relatively small, ultimately fall away from the sporophyte apex, and have the ability to produce protonema. The length of time the calyptra remains alive may vary depending on the stresses experienced during its lifespan and by the species examined. Observations of live calyptra cells and experiments testing protonema production have yet to be carried out for species growing in the wild and would be an important test to determine the capacity for calyptrae to function as a dispersal unit in nature.

Apogamous moss sporophytes are a unique case wherein sporophyte development occurs completely independently of the calyptra. Sporophytes formed via apogamy develop directly from the vegetative tissues of a diploid or polyploid leafy gametophyte without fertilization (Springer 1935). Their development is initiated on the surface of the gametophyte body, rather than within the archegonium, thus they lack an epigonium and the ability to form a calyptra. Apogamous sporophytes of some species can produce viable spores, despite lacking a calyptra (literature reviewed in Cvetic et al. 2005). However, other species that develop apogamous sporophytes never undergo sporogenesis. To test whether calyptra presence could stimulate sporogenesis, Hughes (1969) experimentally applied calyptrae from sporophytes produced via fertilization to apogamously produced sporophytes of *Phascum cuspidatum* Hedw. In this species, apogamous sporophytes were not responsive to calyptra presence and sporogenesis was not induced (Hughes 1969). Comparing apogamous sporophytes to sporophytes produced by fertilization may provide insights into the developmental role of the calyptra; however, the ploidy level differences between these
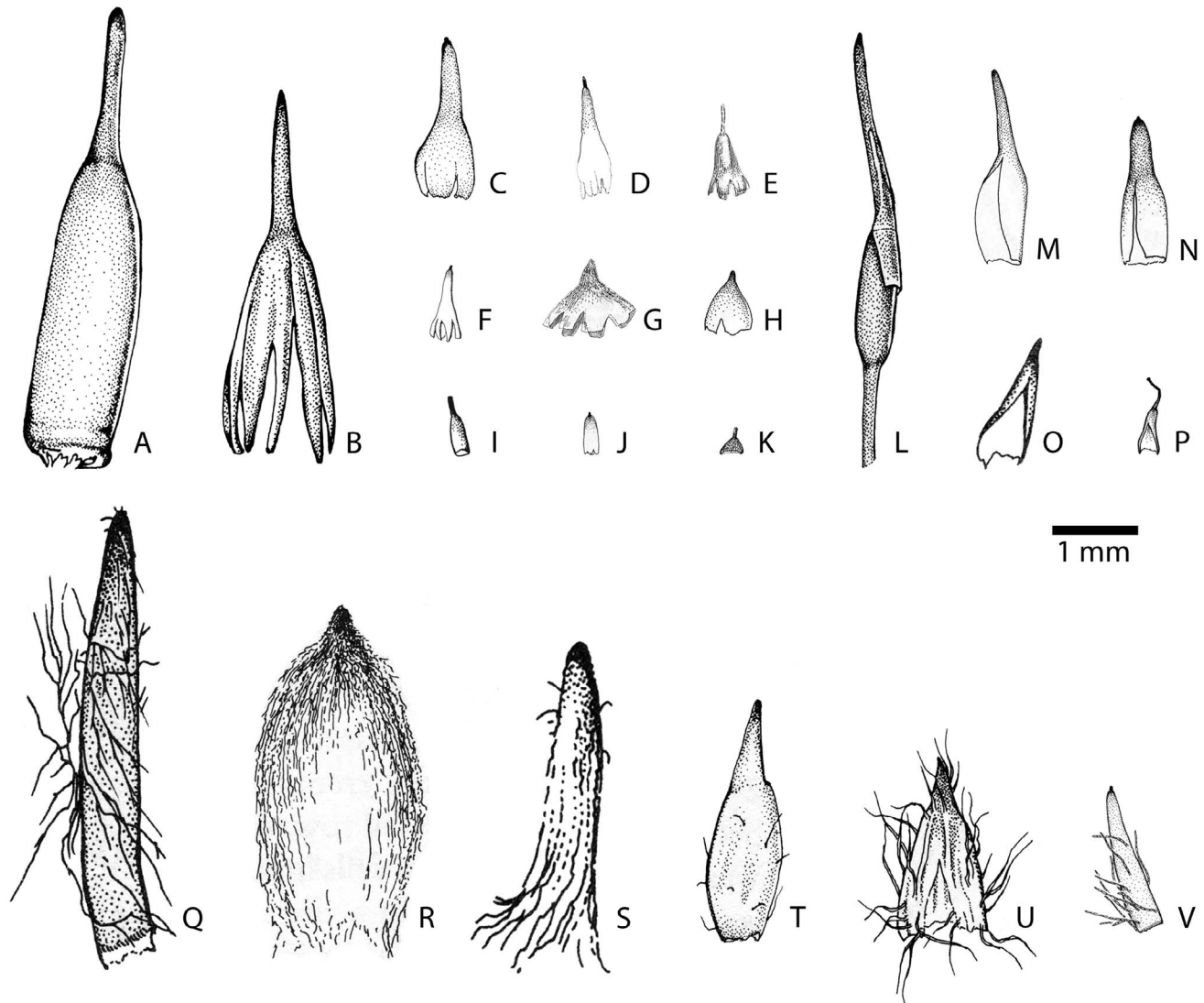
Figure 6. Calyptra morphological diversity. **A.** *Encalypta texana* Magill. **B.** *Ptychomitrium gardneri* Lesq. **C.** *Bruchia bolanderi* Lesq. **D.** *Callicostella pallida* (Hornsch.) Ångstr. **E.** *Physcomitrella patens* (Hedw.) Bruch & Schimp. **F.** *Campylostelium saxicola* (F.Weber & D.Mohr) Bruch & Schimp. **G.** *Bruchia ravenelii* Wilson ex Sull. **H.** *Pterygoneurum kozlovii* Laz. **I.** *Lorentziella imbricata* (Mitt.) Broth. **J.** *Brachydontium olympicum* (E.Britton) T.T.McIntosh & J.R.Spence. **K.** *Ephemerum crassinervium* (Schwägr.) Hampe. **L.** *Didymodon bistratosus* Hébr. & R.B.Pierrot **M.** *Trematodon ambiguus* (Hedw.) Hornsch. **N.** *Tetraplodon mnioides* (Hedw.) Bruch & Schimp. **O.** *Bartramiopsis lescurii* (James) Kindb. **P.** *Weissia muhlenbergiana* (Sw.) W.D.Reese & B.A.E.Lemmon. **Q.** *Meiotrichum lyallii* (Mitt.) G.L.Merr. **R.** *Polytrichum hyperboreum* R.Br. **S.** *Pogonatum urnigerum* (Hedw.) P.Beauv. **T.** *Orthotrichum laevigatum* J.E.Zetterst. **U.** *Orthotrichum lyellii* Hook. & Taylor. **V.** *Homalotheciella subcapillata* (Hedw.) Broth. A–C, F, H–M, O, P–S. Illustrations reprinted with permission from Flora of North American Editorial Committee (2007). E&G. Illustrations reprinted with permission from Crum and Anderson (1981). D, G, N, T–V. Illustrations reprinted with permission from Flora of North American Editorial Committee (2014).

two types of sporophytes could potentially confound the interpretations.

MORPHOLOGICAL AND ANATOMICAL VARIATION

Functional explorations of moss calyptrae have focused on few taxa (i.e., *Funaria* and *Polytrichum*), which represent a small range of the morphological and anatomical diversity in calyptrae observed across mosses (Janzen 1917). Calyptrae come in a wide range of sizes from very small (e.g., 200–300 µm in *Ephemerum* and *Physcomitrella*; Flora of North American Editorial Committee 2007; **Fig. 6**), to quite large (e.g., 4–10 mm in *Encalypta*; Flora of North American Editorial Committee 2007; **Fig. 6**). They also vary in shape, ranging from an awl-shaped, narrow tube to cone-shaped to bell-shaped,
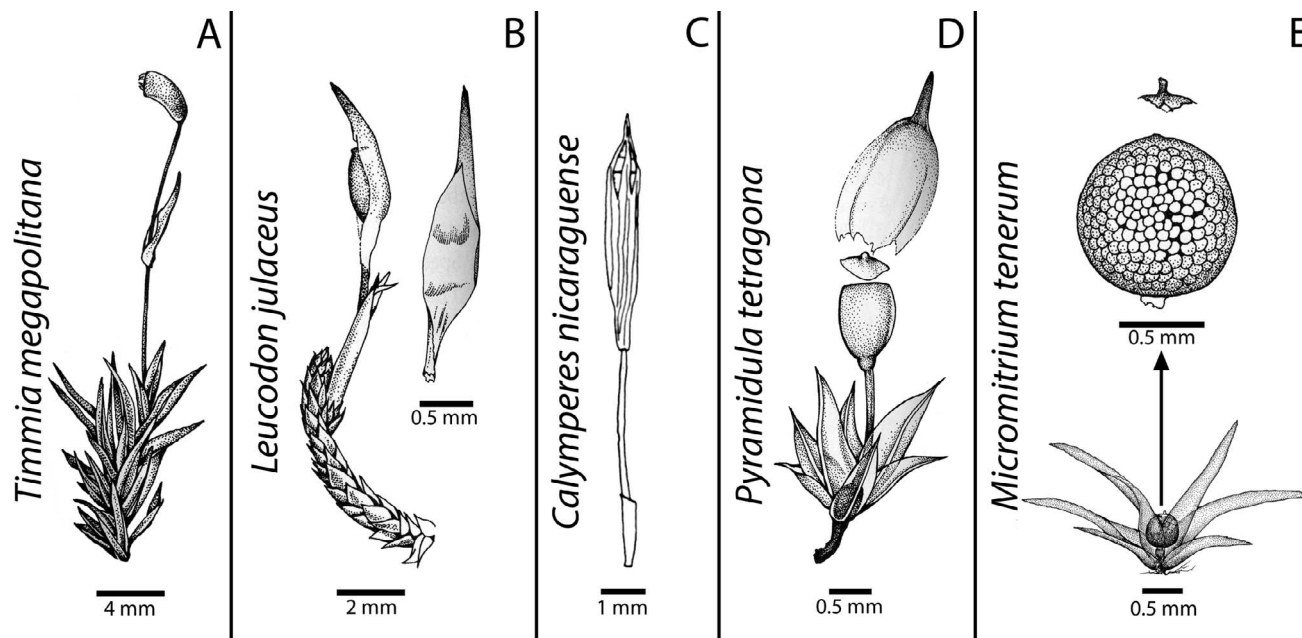
Figure 7. Moss sporophytes that retain their calyptra at maturity. **A&B.** The calyptra remains attached via a clasping base **A.** *Timmia megapolitana* Hedw. **B.** *Leucodon julaceus* (Hedw.) Sull. illustration reprinted with permission from Flora of North American Editorial Committee (2014). **C.** *Calymperes nicaraguense* Renauld & Cardot lacks peristome teeth to control spore release. Illustration modified from Reese (1961). The calyptra grips the operculum and under dry conditions lifts it away from the mouth of the capsule, releasing the spores. **D.** *Pyramidula tetragona* (Brid.) Brid. has a relatively large calyptra that covers the entire capsule at maturity. **E.** *Micromitrium tenerum* (Bruch & Schimp.) Crosby has a very small calyptra that remains atop the capsule apex at maturity. A, D & E. Illustrations reprinted with permission from Flora of North American Editorial Committee (2007).

or having two main regions, a narrow rostrum at the apex and an inflated base below that may be constricted at the bottom around the seta (**Fig. 6**). Calyptrae can also be hairy throughout, fringed at the bottom edge, toothed, papillose, plicate, or smooth (**Fig. 6**). Some calyptrae can even be covered in unfertilized archegonia (i.e., *Fontinalis;* Glime 1983). Calyptrae can be either persistent, remaining atop the sporophyte at capsule maturity (e.g., *Pyramidula*), deciduous, falling off just prior to or at maturity (e.g., *Funaria*), or ephemeral, falling off long before capsule maturity (e.g., *Andreaea*). Calyptra morphological variation may indicate differences in calyptra function across taxa, with species having small, ephemeral calyptrae lacking a significant role in sporophyte development; however, this hypothesis remains untested.

In the vast majority of moss taxa, calyptrae are ultimately lost from the sporophyte apex prior to spore dispersal. The manner in which they come off the apex varies based on both the calyptra and sporophyte morphologies. Calyptrae can be either cucullate (hood-like), with a single vertical slit up the side (**Fig. 6L–P**) or mitrate (cap-like), with or without multiple slits (**Fig. 6A–K**). These slits are a secondary modification of the calyptrae that occurs during capsule expansion and inclination. Pre-formed slits or weakened regions in the calyptra have not been observed. The number of slits per calyptra and thus the categorization as either cucullate or mitrate is dependent on and thus potentially correlated with sporophyte capsule shape.

Several moss species are an exception to this rule and retain their calyptrae at maturity. In the genera *Leucodon* and *Timmia,* the calyptra remains attached via a clasping base just below the sporophyte capsule with the expanding capsule emerging through a slit in the side of the calyptra (**Fig. 7A,B**). In the genus *Calymperes*, which lacks peristome teeth to control spore release, the apex of the calyptra grips the operculum by its rostrum and under dry conditions lifts the operculum away from the mouth of the capsule, allowing the spores to escape through openings in the calyptra, whereas when the sporophyte is wet, the operculum seals the capsule and the calyptra openings close (**Fig. 7C** modified from Reese 1961). *Pyramidula tetragona* and members of

the Encalyptaceae also have relatively large and persistent calyptrae that cover the entire capsule at maturity (**Fig. 7D**). Spore dispersal occurs through the longitudinal slits that run the length of the clasping base in *Pyramidula* and in the Encalyptaceae, since the calyptra lack any slits, spores must escape via the space between the capsule and base of the calyptra. Members of the genus *Micromitrium* also have a persistent calyptra that is minute, consisting of merely the archegonial neck and distal portion of the venter, which adheres to the capsule apex and does not impact spore dispersal (**Fig. 7E**).

The internal anatomy of the calyptra is highly variable and differences between taxa may be functionally significant. In transverse section, calyptrae can be either heterogeneous, with thick walled cells composing the outermost layer/s and thinner walled cells composing the inner layer/s, or homogeneous with uniform cell wall thicknesses across all layers (**Figs. 8&9**; Janzen 1917). The cell walls of the exterior-most cell layer of the calyptra can also vary in thickness. When this occurs, typically the outer periclinal cell walls are thickest with the anticlinal and inner periclinal cell walls being thinner (e.g., *Orthotrichum* and *Racomitrium* in Janzen 1917; *Funaria* in Budke et al. 2011). Thick calyptra cell walls may be functionally important for increasing the protective abilities of the calyptra or its ability to mechanically coordinate sporophyte development.

Cell wall thickness can also vary across the length of the calyptra. Calyptra cells typically have thicker walls toward the distal apex and thinner walls toward the proximal end (Janzen 1917; Roth 1969). In *Funaria hygrometrica* the cells with the thickest walls are located at the calyptra apex, including cells of the archegonium neck and those immediately below (pers. obs.). The transition from thick to thin walled cells may occur continuously along the longitudinal length of the calyptra (e.g., *Leucobryum glaucum* and *Timmia megapolitana*, **Fig. 8D,F**; Janzen 1917) or as an abrupt transition with distinctly different cell types occurring in morphologically distinct regions, such as between the rostrum and inflated base in *F. hygrometrica* (*Encalypta ciliata* and *Funaria hygrometrica,* **Fig. 8G,H;** Janzen 1917).

Overall calyptra thickness is not only influenced by the thickness of the cell walls, but also by the number of cell layers that compose the calyptra. The number of calyptra cell layers can vary both between different regions of the calyptra and across taxa. Calyptra are most commonly multiple cell layers thick toward the apex with the number of layers decreasing toward the base (Janzen 1917; Roth 1969). Calyptra are typically 1–3 cell layers thick (**Figs. 8&9**; Janzen 1917), but can be up to a dozen cell layers thick, as has been noted in *Dicnemon,* (Crum 2001). Differences in calyptra thickness may have functional significance related to the mechanical constraint the calyptra can provide to the sporophyte in early development.

Plicate calyptrae are present in several moss lineages and morphologically can appear quite similar; however, these calyptrae can be built by different mechanisms. In the upper region of the calyptrae in *Brachysteleum, Orthotrichum,* and *Tetraphis,* variation in the number of cell layers that comprise the calyptra around the circumference can create the plications in these taxa (**Fig. 9A,B,E**). Whereas in the lower region of the calyptra in *Coscinodon* and *Funaria* the calyptra has a uniform number of cell layers around the circumference and the plications are the result of a physical folding of the loose/inflated parts of the calyptra (**Figs. 8H, 9C**).

THE CALYPTRA AS A SYSTEMATIC CHARACTER

In the seemingly morphologically austere mosses (Medina et al. 2012), calyptra morphology has been used as a source of systematic characters. Janzen (1917) created a key to 73 moss genera from an array of families using only features of the calyptra. Due to the choice of taxa this key resulted in seemingly little homoplasy across the species, with many taxa having unique calyptra morphologies that were not repeated across the dataset. When calyptra characters are considered more broadly across the 13,000 species of mosses, many character states appear homoplasious. For example, an array of distantly related genera have hairy calyptrae (i.e., *Ctenidium, Homalothecium, Orthotrichum, Polytrichum*), and thus their hairy calyptrae are not likely to have been inherited from a common ancestor and are more likely the result of convergent evolution.

That being said, calyptra morphology can be a useful synapomorphy for distinguishing taxa. The level of calyptra hairiness can be helpful for distinguishing species in the Orthotrichaceae (Medina et al. 2012) and calyptra size/shape has been
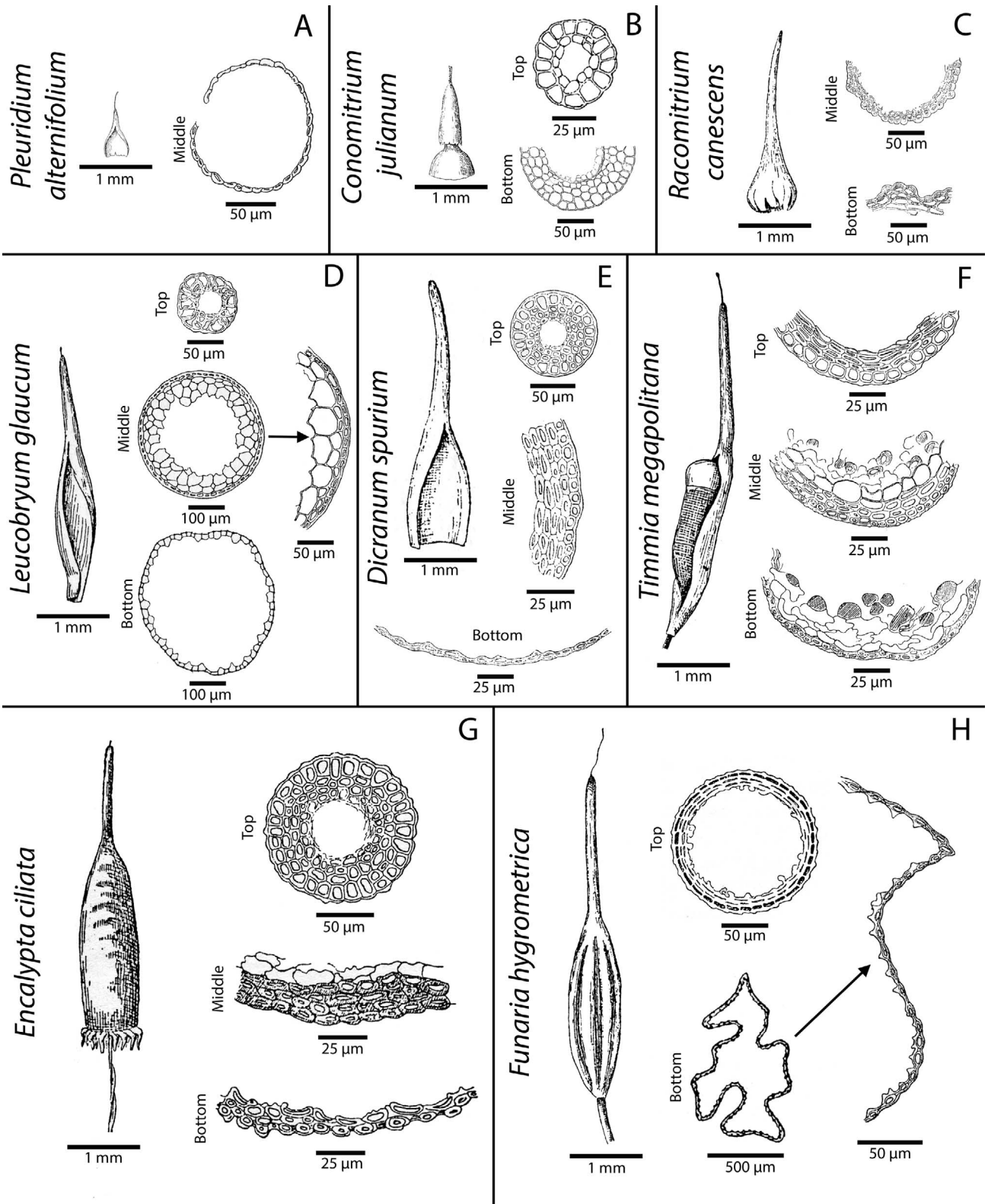
Figure 8. Calyptra morphology and anatomy illustrations reprinted with permission from Janzen (1917; www.schweizerbart.de/journals/nova_hedwigia). Calyptra morphologies are illustrated on the far left of each panel. Drawings of transverse anatomical sections through the top, middle, and/or bottom regions of mature calyptra are to the right. Illustrations displaying further magnification of the cell anatomy are indicated with arrows. **A.** *Pleuridium alternifolium* (Dicks. ex Hedw.) Brid. **B.** *Conomitrium julianum* (Savi ex DC.) Mont. **C.** *Racomitrium canescens* (Hedw.) Brid. **D.** *Leucobryum glaucum* (Hedw.) Ångstr. **E.** *Dicranum spurium* Hedw. **F.** *Timmia megapolitana* Hedw. **G.** *Encalypta ciliata* Hedw. **H.** *Funaria hygrometrica*.
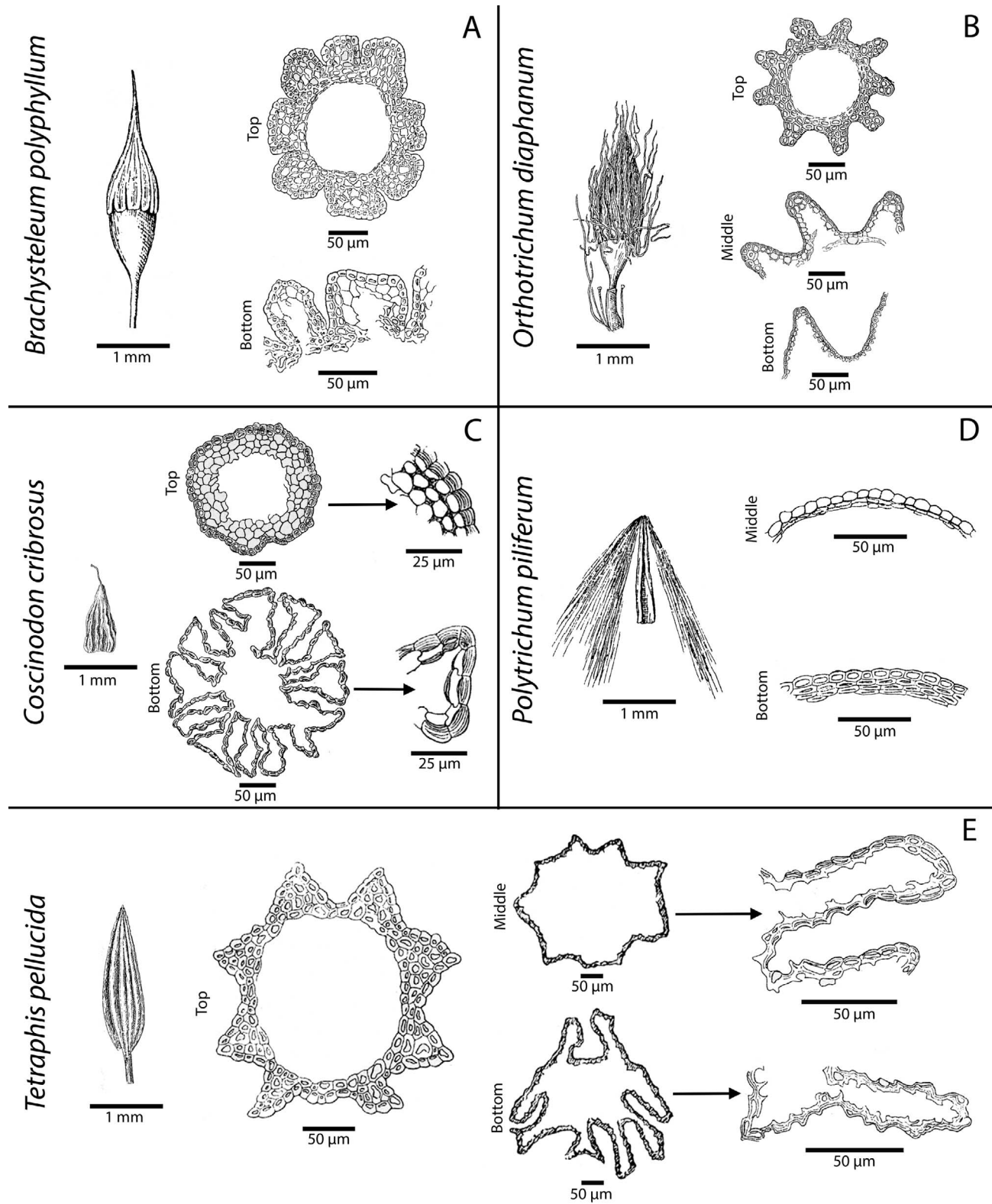
Figure 9. Calyptra morphology and anatomy illustrations reprinted with permission from Janzen (1917; www.schweizerbart.de/journals/nova_hedwigia). Calyptra morphologies are illustrated on the far left of each panel. Drawings of transverse anatomical sections through the top, middle, and/or bottom regions of mature calyptra are to the right. Illustrations displaying further magnification of the cell anatomy are indicated with arrows. **A.** *Brachysteleum polyphyllum* (Sw.) Hornsch. **B.** *Orthotrichum diaphanum* Brid. **C.** *Coscinodon cribrosus* (Hedw.) Spruce. **D.** *Polytrichum piliferum* Hedw. **E.** *Tetraphis pellucida* Hedw.

used to define genera in the Funariaceae (Fife 1985; Liu et al. 2012). As with many morphological features, the amount of homoplasy and utility of the characters for systematics is often rank dependent. Within species, calyptra shape is relatively uniform; species-level variation typically includes variation in calyptra length, hairiness and papillosity (Schimper 1848; Schofield & Hébant 1984). Overall the species-level variation reported for calyptrae appears to be smaller than the variation observed at the genus or family levels (Crum & Anderson 1981). Examining calyptrae in a broad phylogenetic comparative context will enable us to explore the patterns of calyptra evolution, their usefulness as a systematic character, and their potential correlations with other morphological features of the gametophyte and sporophyte (Schofield 1981; Vitt 1981).

Ecological Adaptations of the Calyptra

Calyptrae with different morphologies and/or anatomies may be correlated with the environment or habitat where those species live. This relationship was proposed by Schofield (1981) based on the calyptra morphological and anatomical categories defined by Janzen (1917). Schofield discussed the potential relationship between hairy/bristly calyptrae and their role in protection, as well as a potential connection between species with minute calyptrae and wet sites. In contrast, species with large calyptrae have been proposed to correspond with xerophytic taxa due to the increased protection offered by these larger calyptrae (Vitt 1981). Despite these intellectually engaging discussions of the potential relationships between calyptra morphology and habitat, as well as Schofield's (1981) statement that "…there appear to be no features of habitat that correlate with any specific calyptra morphology," these ideas have yet to be tested. Large datasets and phylogenetic comparative methods, as used by Rose et al. (2016) and Coudert et al. (2017), will enable us to explicitly test relationships between calyptra morphology and ecology.

Calyptra Evolution – Evolutionary Significance

The evolution and elaboration of the moss calyptra may have enabled the seta elongation phase to be extended and capsule differentiation delayed. Calyptrae are present in all of the approximately 13,000 species of mosses. In the earliest diverging moss lineages, calyptrae are relatively simple; they are either very thin, enveloping the entire capsule until it bursts through at maturity (i.e., *Andreaea, Sphagnum*) or small, only covering the very apex of the capsule (i.e., *Takakia*). Sporophytes of both *Andreaea* and *Sphagnum* lack a seta/stalk and the entire sporophyte is elevated via a pseudopodium, a stalk of maternal gametophyte tissue. This elevation via a maternal stalk enables the fully enclosing epigonium to remain intact until late in development, delaying the exposure to dehydration stresses from beneath the maternal tissues until the sporophyte is mature. In *Takakia*, the influence of the calyptra on sporophyte development is assumed to be minimal (Murray 1988; Renzaglia et al. 1997), though calyptra removal experiments have yet to be undertaken.

Beyond these early diverging lineages, the vast majority of mosses have a tall sporophyte with a capsule elevated on a seta topped by a comparatively large calyptra. Larger calyptrae have the potential for a superior capacity to protect both the immature apical region and the seta meristem from desiccation. Even a small increase in capsule height that elevates these immature regions above the laminar boundary layer can expose these plants to increased stresses (Proctor 1980, 1982; Rice et al. 2001; Rice & Schneider 2004). At the same time increases in sporophyte height also have the potential to increase spore dispersal distance. Thus the calyptra may have been a key innovation enabling the evolution of taller sporophyte setae as well as the evolution of complex structures such as the peristome teeth.

That being said, large calyptrae are not a consistent feature across mosses and many species have undergone evolutionary reductions resulting in the loss of features (Medina et al. 2018). Reductions in calyptra size, potentially from ancestors with large calyptrae, appears to correlate with reductions in seta length in some lineages (Vitt 1981), which would decrease dehydration stresses, by nestling the sporophyte within the protection of the surrounding perichaetial leaves and placing the developing capsule within the protective boundary layer of the substrate.

Thus far, no studies have examined the morphological evolution of the calyptra across moss diversity. With the resolution of the backbone phylogeny of mosses and the relationships between many of the major lineages solidified (e.g., Goffinet

& Buck 2004; Liu et al. 2019), the time is ripe to examine calyptra character evolution using phylogenetic comparative methods (e.g., Coudert et al. 2017; Rose et al. 2016).

CONCLUSIONS

The moss calyptra is a small and relatively thin organ. Despite its size, the calyptra is not a vestigial structure, but is a specialized organ that has been elaborated morphologically in many moss taxa. Based on observations and experiments over the last 250 years, the calyptra has been found to be critical for moss sporophyte development, especially during the early developmental stages of seta formation prior to capsule differentiation. The calyptra influences sporophyte development by both coordinating and providing desiccation protection during early sporophyte development. It is particularly critical for the developmental transition from seta formation to apical region differentiation (French & Paolillo 1975c) and thus has a major influence on sporophyte reproductive fitness.

These developmental observations point to the influence of the calyptra on both the underlying hormone and gene expression patterns of the sporophyte. Molecular genetic tools have been established in the model organism moss *Physcomitrella patens* that can be used to examine hormone and gene expression patterns (e.g., Fujita et al. 2008; Quatrano et al. 2007; Sakakibara et al. 2013). Unfortunately, calyptra manipulation experiments during the early, spear-shaped stages of sporophyte development are not possible in *P. patens*. At this early developmental stage the sporophyte foot is not firmly attached to the maternal gametophyte and attempts at calyptra removal result in detachment of the sporophyte from the leafy gametophyte (pers. obs.). Even if these manipulations could be performed, *P. patens* has a very short seta and thus the length of developmental time devoted to seta formation is reduced relative to the development of the entire sporophyte. In order to study the underlying effects on sporophyte development, a taxon with a larger sporophyte and longer seta, such as *Funaria hygrometrica*, must be used. With the advent of new molecular genetic tools, such as CRISPR, which has been successfully applied to a small number of mosses [i.e., *P. patens*, Collonnier et al. 2017a,b, Lopez-Obando et al. 2016; Mara et al. 2019; Nomura et al. 2016; Yi et al. 2019; *Scopelophila*

cataractae, Nomura et al. 2016] the time is ripe for novel explorations of the genetic mechanisms underlying the interactions between the maternal gametophyte calyptra and offspring sporophyte in mosses.

LITERATURE CITED

BeMiller, J. N. 1986. An introduction to pectins: structure and properties. Pages 2–12. In: M. L. Fishman & J. J. Jen (eds.), Chemistry and Function of Pectins. American Chemical Society, Washington, DC.

Bierhorst, D. W. 1985. On rhizoclads in the oak fern, *Quercifilix zeilanica*. American Journal of Botany 72: 1159–1161.

Billings, W. D. & R. J. Morris. 1951. Reflection of visible and infrared radiation from leaves of different ecological groups. American Journal of Botany 38: 327–331.

Bopp, M. 1954. Untersuchungen über Wachstum und Kapselentwicklung normaler und isolierter Laubmoossporogone. Zeitschrift für Botanik 42: 331–352.

Bopp, M. 1956. Die Bedeutung der Kalyptra für die Entwicklung der Laubmoossporogone. Berichte der Deutschen Botanischen Gesellschaft 69: 455–468.

Bopp, M. 1957. Enwicklungsphysiologische Untersuchungen an Moosmutanten I. Zur Wirkung der Laubmooskalyptra. Zeitschrift für induktive Abstammungs- und Vererbungslehre 88: 600–607.

Bopp, M. 1961. Morphogenese der Laubmoose. Biological Reviews 36: 237–280.

Bopp, M. & E. Stehle. 1957. Zur Frage der Wasserleitung im Gametophyten und Sporophyten der Laubmoose. Zeitschrift für Botanik 45: 161–174.

Britton, E. G. 1902. *Octodiceras julianum*, its propagation, distribution and history. The Bryologist 5: 83–85.

Budke, J. M., B. Goffinet & C. S. Jones. 2011. A hundred-year-old question: is the moss calyptra covered by a cuticle? A case study of *Funaria hygrometrica*. Annals of Botany 107: 1279–1286.

Budke, J. M., B. Goffinet & C. S. Jones. 2012. The cuticle on the gametophyte calyptra matures before the sporophyte cuticle in the moss *Funaria hygrometrica* (Funariaceae). American Journal of Botany 99: 14–22.

Budke, J. M., B. Goffinet & C. S. Jones. 2013. Dehydration protection provided by a maternal cuticle improves offspring fitness in the moss *Funaria hygrometrica*. Annals of Botany 111: 781–789.

Budke, J. M. & B. Goffinet. 2016. Comparative cuticle development reveals taller sporophytes are covered by thicker calyptra cuticles in mosses. Frontiers in Plant Science 7: 832.

Busta, L., J. M. Budke & R. Jetter. 2016. The moss *Funaria hygrometrica* has cuticular wax similar to vascular plants, with distinct composition on leafy gametophyte, calyptra and sporophyte capsule surfaces. Annals of Botany 118: 511–522.

Campbell, D. H. 1918. The Structure and Development of Mosses and Ferns (3rd ed.). The Macmillan Company. London.

Chopra, R. N. & P. K. Kumra. 1988. Biology of Bryophytes. Wiley Eastern Limited, New Delhi.

Collonnier, C., A. Epert, K. Mara, F. Maclot, A. Guyon-Debast, F. Charlot, C. White, D. Schaefer & F. Nogué. 2017a. CRISPR-Cas9-mediated efficient directed mutagenesis and RAD51-dependent and RAD51-independent gene targeting in the moss *Physcomitrella patens*. Plant Biotechnology Journal 15: 122–131.

Collonnier, C., A. Guyon-Debast, F. Maclot, K. Mara, F. Charlot & F. Nogué. 2017b. Towards mastering CRISPR-induced gene knock-in in plants: survey of key features and focus on the model *Physcomitrella patens*. Methods 121–122: 103–117.

Cook, M. E. & L. E. Graham. 1998. Structural similarities between surface layers of selected Charophycean algae and bryophytes and the cuticles of vascular plants. International Journal of Plant Sciences 159: 780–787.

Coudert, Y., N. E. Bell, C. Edelin & C. J. Harrison. 2017. Multiple innovations underpinned branching form diversification in mosses. New Phytologist 215: 840–850.

Crandall-Stotler, B., R. E. Stotler & D. G. Long. 2008. Morphology and classification of the Marchantiophyta. Pages 1–54. In: A. J. Shaw & B. Goffinet (eds.), Bryophyte Biology 2nd edition. Cambridge University Press, Cambridge.

Crum, H. 2001. Structural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor.

Crum, H. & L. E. Anderson. 1981. Mosses of Eastern North America. Columbia University Press, New York.

Cvetić, T., M. Sabovljević, A. Sabovljević & D. Grubišić. 2005. In vitro culture and apogamy: Alternative pathway in the life cycle of the moss *Amblystegium serpens* (Amblystegiaceae). Archives of Biological Sciences 57: 267–272.

Dalmer, M. 1891. Über stärkereiche Chlorophyllkörper im Wassergewebe der Laubmoose. Flora 74: 460–465.

Duckett, J. G. & R. Ligrone. 2004. There are many ways of making water-conducting cells but what about stomata? Field Bryology 82: 32–33.

Duckett, J. G., S. Pressel, K. M. P'ng & K. S. Renzaglia. 2009. Exploding a myth: the capsule dehiscence mechanism and the function of pseudostomata in *Sphagnum*. New Phytologist, 183: 1053–1063.

Ehleringer, J. R. & Mooney, H. A. 1978. Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. Oecologia 37: 183–200.

Endress, P. K. 2003. Early floral development and nature of the calyptra in Eupomatiaceae (Magnoliales). International Journal of Plant Sciences 164: 489–503.

Esau, K. 1977. Anatomy of Seed Plants, 2nd edition. John Wiley & Sons, New York.

Fife, A. J. 1985. A generic revision of the Funariaceae (Bryophyta: Musci). Part I. Journal of the Hattori Botanical Laboratory 58: 149–196.

Firtsch, G. 1883. Über einige mechanische Einrichtungen im anatomischen Bau von *Polytrichum juniperinum*, Willd. Berichte der Deutschen Botanischen Gesellschaft 1: 83–97.

Flora of North American Editorial Committee (eds.). 2007. Flora of North America, Vol. 27, Bryophytes: Mosses, part 1. Oxford University Press, New York.

Flora of North American Editorial Committee (eds.). 2014. Flora of North America, Vol. 28, Bryophytes: Mosses, part 2. Oxford University Press, New York.

Frahm, J.-P. 2001. Bryophyta. Pages 89–90. In: Biologie der Moose. Springer, Berlin, Heidelberg.

French, J. C. & D. J. Paolillo. 1975a. On the role of the calyptra in permitting expansion of capsules in the moss *Funaria*. The Bryologist 78: 438–446.

French, J. C. & D. J. Paolillo. 1975b. Intercalary meristematic activity in the sporophyte of *Funaria* (Musci). American Journal of Botany 62: 86–96.

French, J. C. & D. J. Paolillo. 1975c. Effect of exogenously supplied growth regulators on intercalary meristematic activity and capsule expansion in *Funaria*. The Bryologist 78: 431–437.

French, J. C. & D. J. Paolillo. 1975d. The effect of the calyptra on the plane of guard cell mother cell division in *Funaria* and *Physcomitrium* capsules. Annals of Botany 39: 233–236.

French, J. C. & D. J. Paolillo. 1976. Effect of the calyptra on intercalary meristematic activity in the sporophyte of *Funaria* (Musci). American Journal of Botany 63: 492–498.

Fujita, T., H. Sakaguchi, Y. Hiwatashi, S. J. Wagstaff, M. Ito, H. Deguchi, T. Sato & M. Hasebe. 2008. Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots. Evolution & Development 10: 176–186.

Glime, J. M. 1983. Appendaged calyptra development in *Fontinalis*. Journal of Bryology 12: 567–570.

Goebel, K. 1905. Organography of Plants Especially of the Archegoniate and Spermatophyta, Part II. Special Organography. Clarendon Press, Oxford.

Goffinet, B. & W. R. Buck. 2004. Systematics of Bryophyta: from molecules to a revised classification. Monographs in Systematic Botany from the Missouri Botanical Garden 98: 270–289.

Graham, L. K. & L. W. Wilcox. 2000. The origin of alternation of generations in land plants: a focus on matrotrophy and hexose transport. Philosophical Transactions of the Royal Society of London, Series B 355: 757–766.

Grebe, K. 1912. Beobachtungen über die Schutzvorrichtungen xerophiler Laubmoose gegen Trocknis. Hedwigia 52: 1–20.

Gruel, J., B. Landrein, P. Tarr, C. Schuster, Y. Refahi, A. Sampathkumar, O. Hamant, E. Meyerowitz & H. Jönsson. 2016. An epidermis-driven mechanism positions and scales stem cell niches in plants. Science Advances 2(1): p.e1500989.

Haberlandt, G. 1886. Das Assimilationssystem der Laubmoos-Sporogonien. Flora 69: 45–47.

Haig, D. 2012. Filial mistletoes: the functional morphology of moss sporophytes. Annals of Botany 111: 337–345.

Hedwig, J. 1782. Fundamentum historiae naturalis muscorum frondosorum concernens eorum flores, fructus, seminalem, propagationem adiecta generum dispositione methodica iconibus illustratis. Apud S. L. Crusium, Lipsiae (Leipzig).

Herzfelder, H. 1921. Experimente an Sporophyten von *Funaria hygrometrica*. Flora 114: 385–393.

Herzfelder, H. 1923. Experimente an Sporophyten von *Funaria hygrometrica*. Flora 116: 476–490.

Hofmeister, W. F. B. 1862. On the Germination, Development, and Fructification of the Higher Cryptogamia, and on the Fructification of the Coniferae. Translated by F. Currey. Ray Society, London.

Hughes, J. G. 1969. Factors conditioning development of sexual and apogamous races of *Phascum cuspidatum* Hedw. New Phytologist 68: 883–900.

Hy, L. F. 1884. Recherches sur l'archéchone et le développement du fruit des muscinées. Librairie de l'académe de médecine, Paris.

Imaichi, R. & M. Kato. 1991. Developmental study of branched rhizophores in three *Selaginella* species. American Journal of Botany 78: 1694–1703.

Irmscher, E. 1912. Über die Resistenz der Laubmoose gegen Austrocknung und Kälte. Jahrbücher für wissenschaftliche Botanik 50: 387–449.

Janzen, P. 1917. Die Haube der Laubmoose. Hedwigia 58: 158–280.

Koch, K., J.-P. Frahm & R. Pollawatn. 2007. The cuticle of the *Buxbaumia viridis* sporophyte. Flora-Morphology, Distribution, Functional Ecology of Plants 204: 34–39.

Kodner, R. B. & L. E. Graham. 2001. High-temperature, acid-hydrolyzed remains of *Polytrichum* (Musci, Polytrichaceae) resemble enigmatic Silurian-Devonian tubular microfossils. American Journal of Botany 88: 462–466.

Kölreuter, J. G. 1777. Das entdeckte Geheimniss der Cryptogamie. Druckts und verlegts. Michael Maklot, Carlsruhe.

Kroken, S. B., L. E. Graham & M. E. Cook. 1996. Occurrence and evolutionary significance of resistant cell walls in charophytes and bryophytes. American Journal of Botany 83: 1241–1254.

Kutschera, U. & K. J. Niklas. 2007. The epidermal-growth-control theory of stem elongation: an old and a new perspective. Journal of Plant Physiology 164: 1395–1409.

Ligrone, R. 1986. Structure, development and cytochemistry of mucilage-secreting hairs in the moss *Timmiella barbuloides* (Brid.) Moenk. Annals of Botany 58: 859–868.

Ligrone, R., J. G. Duckett & K. S. Renzaglia. 1993. The gametophyte-sporophyte junction in land plants. Advances in Botanical Research 19: 231–318.

Liu, Y., J. M. Budke & B. Goffinet. 2012. Phylogenetic inference rejects sporophyte based classification of the Funariaceae (Bryophyta): rapid radiation suggests rampant homoplasy in sporophyte evolution. Molecular Phylogenetics and Evolution 62: 130–145.

Liu, Y., M. G. Johnson, C. J. Cox, R. Medina, N. Devos, A. Vanderpoorten, L. Hedenäs, N. Bell, J. Shevock, B. Aguero, D. Quandt, N. Wickett, J. Shaw & B. Goffinet. 2019. Resolution of the ordinal phylogeny of mosses using targeted exons from organellar and nuclear genomes. Nature Communications 10(1): 1485.

Lopez-Obando, M., B. Hoffmann, C. Géry, A. Guyon-Debast, E. Téoulé, C. Rameau, S. Bonhomme & F. Nogué. 2016. Simple and efficient targeting of multiple genes through CRISPR-Cas9 in *Physcomitrella patens*. G3-Genes|Genomes|Genetics 6: 3647–3653.

Lorch, W. 1920. Die Haube von *Polytrichum formosum* Hedw. Hedwigia 61: 346–347.

Lorch, W. 1931. Anatomie der Laubmoose. In: Handbuch der Pflanzenanatomie Linsbauer 7:1. Berlin, Germany.

Lorentz, P. G. 1867. Studien zur Naturgeschichte einiger Laub-moose: *Orthotrichum schubartianum* Ltz., *Campylopus mulleri* Ltz. und *Weissia zonata* Brid. Verhandlungen der Kaiserlich-Königlichen Zoologish-Botanischen Gesellschaft in Wien 17: 657–686 + Taf. XVII-XXII.

Magill, R. E. 1990. Glossarium Polyglottum Bryologiae. A Multilingual Glossary for Bryology. Missouri Botanical Garden Press, St. Louis.

Malcolm, B. & N. Malcolm. 2000. Mosses and Other Bryophytes: An Illustrated Glossary. Micro-Optics Press, Nelson, New Zealand.

Mara, K., F. Charlot, A. Guyon-Debast, D. G. Schaefer, C. Collonnier, M. Grelon & F. Nogué. 2019. POLQ plays a key role in the repair of CRISPR /Cas9-induced double-stranded breaks in the moss *Physcomitrella patens*. New Phytologist 222(3): 1380–1391.

Marcotrigiano, M. 2010. A role for leaf epidermis in the control of leaf size and the rate and extent of mesophyll cell division. American Journal of Botany 97: 224–233.

Medina, R., M. Johnson, Y. Liu, N. Wilding, T. A. Hedderson, N. Wickett & B. Goffinet. 2018. Evolutionary dynamism in bryophytes: phylogenomic inferences confirm rapid radiation in the moss family Funariaceae. Molecular Phylogenetics and Evolution 120: 240–247.

Medina, R., F. Lara, B. Goffinet, R. Garilleti & V. Mazimpaka. 2012. Integrative taxonomy successfully resolves the pseudo-cryptic complex of the disjunct epiphytic moss *Orthotrichum consimile* s.l. (Orthotrichaceae). Taxon 61: 1180–1198.

Merced, A. & K. S. Renzaglia. 2016. Patterning of stomata in the moss *Funaria*: a simple way to space guard cells. Annals of Botany 117: 985–994.

Merced, A. & K. S. Renzaglia. 2017. Structure, function and evolution of stomata from a bryological perspective. Bryophyte Diversity and Evolution 39: 7–20.

Merriam-Webster.com. 2019. https://www.merriam-webster.com [accessed 30 June 2019.]

Meyer, S. L. 1942. Physiological studies on mosses. IV. Regeneration in *Physcomitrium turbinatum*. Botanical Gazette 104: 128–132.

Murray, B. M. 1988. Systematics of the Andreaeopsida (Bryophyta): Two orders with links to *Takakia*. Beihefte Zur Nova Hedwigia 90: 289–336.

Nomura, T., T. Sakurai, Y. Osakabe, K. Osakabe, & H. Sakakibara. 2016. Efficient and heritable targeted mutagenesis in mosses using the CRISPR/Cas9 system. Plant and Cell Physiology 57: 2600–2610.

Oehlkers, F. & M. Bopp. 1957. Entwicklungsphysiologische Untersuchungen an Mossmutanten II. Die Korrelation Zwi-schen Sporogon und Kalyptra bei Mutanten von *Funaria* und *Physcomitrium*. Zeitschrift für induktive Abstammungs- und Vererbungslehre 88: 608–618.

Paolillo, D. J. 1968. The effect of the calyptra on capsule symmetry in *Polytrichum juniperinum* Hedw. The Bryologist 74: 327.

Paton, J. A. & J. V. Pearce. 1957. The occurrence, structure and functions of the stomata in British bryophytes. Transactions of the British Bryological Society 3: 228–259.

Poli, D., M. Jacobs & T. J. Cooke. 2003. Auxin regulation of axial growth in bryophyte sporophytes: its potential significance for the evolution of early land plants. American Journal of Botany 90: 1405–1415.

Proctor, M. C. F. 1979. Surface wax on the leaves of some mosses. Journal of Bryology 10: 531–538.

Proctor, M. C. F. 1980. Diffusion resistance in bryophytes. Pages 219–229. In: J. Grace, E. D. Ford & P. G. Jarvis (eds.), Plants and Their Atmospheric Environment. Blackwell Scientific, Oxford.

Proctor, M. C. F. 1982. Physiological ecology: water relations, light and temperature responses, carbon balance. Pages 333–381. In: A. J. E. Smith (ed.), Bryophyte Ecology. Chapman and Hall, London.

Quatrano, R., S. McDaniel, A. Khandelwal, P. Perroud & D. Cove. 2007. *Physcomitrella patens*: mosses enter the genomic age. Current Opinion in Plant Biology 10: 182–189.

Reese, W. D. 1961. The genus *Calymperes* in the Americas. The Bryologist 64: 89–140.

Renzaglia, K., K. McFarland & D. Smith. 1997. Anatomy and ultrastructure of the sporophyte of *Takakia ceratophylla* (Bryophyta). American Journal of Botany 84: 1337–1350.

Renzaglia, K. S., J. C. Villarreal & R. J. Duff. 2008. New insights into morphology, anatomy, and systematics of hornworts. Pages 139–172. In: A. J. Shaw & B. Goffinet (eds.), Bryophyte Biology 2nd edition. Cambridge University Press, Cambridge.

Rice, S. K., D. Collins & A. M. Anderson. 2001. Functional significance of variation in bryophyte canopy structure. American Journal of Botany 88: 1568–1576.

Rice, S. K. & N. Schneider. 2004. Cushion size, surface roughness, and the control of water balance and carbon flux in the cushion moss *Leucobryum glaucum* (Leucobryaceae). American Journal of Botany 91: 1164–1172.

Roach, D. A. & R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology and Systematics 18: 209–235.

Rosander, H. A. 1906. Studier öfver bladmossornas organisation: mössa, vaginula och sporogon. Wretmans Boktryckeri, Uppsala, Sweden.

Rose, J. P., R. Kriebel & K. J. Sytsma. 2016. Shape analysis of moss (Bryophyta) sporophytes: insights into land plant evolution. American Journal of Botany 103: 652–662.

Roth, D. 1969. Embryo und Embryotheca bei den Laubmoosen. Eine histogenetische und morphologische Untersuchung. Bibliotheca Botanica 129: 1–49.

Sack, F. D. & D. J. Paolillo. 1983. Stomatal pore and cuticle formation in *Funaria*. Protoplasma 116: 1–13.

Sakakibara, K., S. Ando, H. K. Yip, Y. Tamada, Y. Hiwatashi, T. Murata, H. Deguchi, M. Hasebe & J. L. Bowman. 2013. KNOX2 Genes regulate the haploid-to-diploid morphological transition in land plants. Science 339: 1067–1070.

Savaldi-Goldstein, S., C. Peto, & J. Chory. 2007. The epidermis both drives and restricts plant shoot growth. Nature 446: 199–202.

Schimper, W. 1848. Recherches anatomiques et morphologiques sur les mousses. Mémoires de la Société du Muséum d'Historie Naturelle de Strasbourg, Strasbourg, France.

Schimper, W. 1858. Versuch einer Entwicklungs-geschichte der Torfmoose (*Sphagnum*) und einer Monographie der in Europa verkommenden Arten dieser Gattung. E. Schweitzerbart's Verlagshandlung, Stuttgart.

Schofield, W. B. 1981. Ecological significance of morphological characters in the moss gametophyte. The Bryologist 84: 149–165.

Schofield, W. B. & C. Hébant. 1984. The morphology and anatomy of the moss gametophore. Pages 627–657. In: R. Schuster (ed.), New Manual of Bryology. The Hattori Botanical Laboratory, Nichinan.

Shigeru, N. 1992. "Science et développement: une politique scientifique peut-elle tirer un enseignement de l'histoire des sciences?" Pages 375–379. In: P. Petitjean, C. Jami & A. M. Moulin (eds.), Science and Empires: Historical Studies about Scientific Development and European Expansion. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Springer, E. 1935. Über apogame (vegetativ entstandene) Sporogone und der bivalenten Rasse des Laubmooses *Phascum cuspidatum*. Zeitschrift für induktive Abstammungs- und Vererbungslehre 69: 249–262.

Stone, I. G. 1977. Some morphological and anatomical features of the monotypic genus *Bryobartramia* Sainsbury (Musci). Australian Journal of Botany 25: 141–157.

True, R. H. 1906. Notes on the physiology of the sporophyte of *Funaria* and of *Mnium*. Beihefte zum botanischen Centralblatt 19: 34–44.

Vaseva, I. I., E. Qudeimat, T. Potuschak, Y. Du, P. Genschik, F. Vandenbussche & D. Van Der Straeten. 2018. The plant hormone ethylene restricts *Arabidopsis* growth via the epidermis. Proceedings of the National Academy of Sciences of the United States of America 115: E4130–E4139.

Vitt, D. H. 1981. Adaptive modes of the moss sporophyte. The Bryologist 84: 166–186.

Watson, W. 1914. Xerophytic adaptations of bryophytes in relation to habitat. New Phytologist 13: 149–169.

Wuenscher, J. 1970. The effect of leaf hairs of *Verbascum thapsus* on leaf energy exchange. New Phytologist 69: 65–73.

Wynne, M. A. & J. M. Budke. 2012. Examining the ability of calyptrae to produce protonema in *Funaria hygrometrica*. Evansia 29: 61–64.

Yi, P. & G. Goshima. 2019. Fast, efficient, and precise gene editing in the moss *Physcomitrella patens*. bioRxiv 643692.

Zielinski, F. 1910. Beiträge zur Biologie des Archegoniums und der Haube der Laubmoose. Flora 100: 1–36.

Supplementary documents online:

Supplementary Table S1. Significant contributions to the knowledge of calyptra function, development and morphology.