Review of Palaeobotany and Palynology 216 (2015) 1–17

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

# Spores of relictual bryophytes: Diverse adaptations to life on land

R.C. Brown [a], B.E. Lemmon [a], M. Shimamura [b], J.C. Villarreal [c], K.S. Renzaglia [d,*]

[a] Department of Biology, University of Louisiana, Lafayette, Lafayette, LA 70504, USA
[b] Department of Biological Science, Graduate School of Science, Hiroshima University, Kagami-yama, Higashi Hiroshima, Hiroshima 739-8526, Japan
[c] Systematic Botany and Mycology, University of Munich (LMU), Menzinger Strasse 67, Munich, Germany
[d] Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901, USA

ARTICLE INFO

*Article history:*
Received 13 October 2014
Received in revised form 2 January 2015
Accepted 3 January 2015
Available online 28 January 2015

*Keywords:*
Bryophyte
Cryptospore
Early land plant
Spore
Tripartite lamella
Trilete spore

ABSTRACT

Sporogenesis and the evolution of thick-walled meiospores were of paramount importance in the establishment of the first life on land nearly half a billion years ago. Indeed, the first evidence of plant life on land is the sudden appearance of spores (palynomorphs or cryptospores) about 470 million years ago, predating the first megafossils by some forty million years. Affinities of these fossil spores with living embryophytes are often difficult to assess due to significant gaps in knowledge of spore diversity in critical extant taxa. The bryophytes (liverworts, mosses and hornworts) are the most ancient living plant lineages. To fill in data and provide a framework in which to evaluate spore evolution, we conducted ultrastructural and developmental studies of eight early-divergent or relictual bryophytes, as identified by cladistic/molecular and morphological studies. Spore wall data are presented for the liverworts *Apotreubia*, *Haplomitrium*, and *Blasia*; the mosses *Takakia*, *Sphagnum*, *Andreaea*, and *Oedipodium*; and the hornwort *Leiosporoceros*. Wall ontogeny is based on tripartite lamellae (TPL) in all taxa except *Andreaea*, which has a unique spongy exine. While a single foundation TPL is typical of bryopsid mosses, *Takakia* has a multilamellate layer (MLL) composed of numerous appressed segments of TPL. Spores of *Sphagnum*, liverworts, and the hornwort *Leiosporoceros* have a MLL layer composed of continuous, not segmented, TPL. We conclude that TPL and multilamellate layers are plesiomorphic in sporoderm development in land plants. Proximal wall modifications evolved into distinct apertures in mosses and hornworts but not in liverworts. Analysis of relictual bryophyte species indicates that the ancestral spore types would be tetrahedral with trifacial proximal surfaces having less ornamentation than the distal surfaces. Identification of features specific to relictual liverwort, moss and hornwort spores provides a foundation for determining fossil spore affinities and interpreting spore evolution and diversification in the earliest land plants.

## 1. Introduction

The innovation of sporogenesis at the algal/plant transition was critical to the establishment of life on land. Haploid meiospores enclosed in sporopollenin walls (sporoderm) provided secure storage of DNA during adverse periods, facilitated dispersal by air/wind, and allowed for dissemination of genetically different gametophytes. Spore fossils predate plant megafossils by 40 million years and afford the limited clues to the pioneering events in land colonization some 470 million years before present (Wellman, 2004; Edwards et al., 2014; Strother et al., 2015). The diverse, widespread and prolonged existence of only fossil spores supports the notion that spores evolved prior to the appearance of sporophytes (Brown and Lemmon, 2011). Affinities of the earliest fossil spores with living groups are often speculated in spite of significant gaps in knowledge of spore diversity in phylogenetically important extant taxa (Wellman et al., 2003).

Spores are protected by some of the most elaborate and resistant walls produced by plants. All contain the highly durable substance sporopollenin (Wallace et al., 2011). In spite of the importance of spores in plant evolution and survival, the molecular biology and biochemistry of spore wall components and synthesis are little known (Quilichini et al., 2014). One outstanding developmental characteristic of spore walls obvious from comparative study of fossil spores as well as those of extant bryophytes and tracheophytes is the occurrence of distinct tripartite lamellae (TPL) in exospore development (Brown and Lemmon, 1990a; Wellman et al., 2003; Wellman, 2004; Taylor and Strother, 2008). As spore walls mature, the lamellae are cemented with sporopollenin and obscured, but a distinctive multilamellate layer (MLL) may remain as an inner exine, and in some liverworts, layers of MLL with sporopollenin form the entire outer wall.

The status of bryophytes (liverworts, mosses and hornworts) as the oldest living land plants is widely recognized (Mishler and Churchill,

---

[*] Corresponding author.

http://dx.doi.org/10.1016/j.revpalbo.2015.01.004
0034-6667/© 2015 Elsevier B.V. All rights reserved.

1984; Shaw and Renzaglia, 2004; Renzaglia et al., 2007). The diagnostic character of one sporophyte/one sporangium per fertilization event has led to thumb nailing these plants as monosporangiates. They differ from all other extant plants (polysporangiates) in the terminal position of a solitary sporangium on a sporophyte that is nourished by the gametophyte through a placenta. Since the gametophyte is the persistent vegetative generation that often reproduces asexually, sporophytes are not always obtainable in the right developmental stages to examine spore ultrastructure and development. Thus, spore characteristics in key, often elusive, early divergent bryophytes have not been described. To fill this scientific lacuna and to clarify misconceptions relating to variability in spore wall architecture in bryophytes, we present developmental summaries and ultrastructural data on eight early-divergent bryophyte genera, as identified by molecular and morphological phylogenies. We refer to these taxa as relictual bryophytes or living fossils because they are genetically isolated and they exhibit peculiar morphological features not seen in other plants within their major clades (i.e., liverworts, mosses or hornworts) (Renzaglia et al., 1994; Carafa et al., 2003; Renzaglia et al., 2007).

The premise of this study is that this basal grade of bryophytes has retained reproductive and structural traits that provide insight on early radiation into terrestrial habitats. Internal and external spore wall features, including development of wall ornamentation, number and nature of wall layers, and aperture existence and features, are documented for three liverworts – *Apotreubia* (Haplomitriopsida), *Haplomitrium* (Haplomitriopsida), and *Blasia* (Marchantiopsida) –, four mosses – *Takakia* (Takakiopsida), *Sphagnum* (Sphagnopsida), *Andreaea* (Andreaeopsida), and *Oedipodium* (Oedipodiopsida) –, and one hornwort — *Leiosporoceros* (Leiosporocerotopsida).

Although the spores of these relictual bryophytes are highly diverse when compared with each other, fundamental characters within each of the three bryophyte groups are evident. The information presented here extends the known characteristics in spore wall architecture in bryophytes and provides important comparisons with extinct and extant spores. We consider fossil spores and spores from more derived and better known bryophyte taxa to evaluate spore evolution within the three groups. Given that spores are the only evidence of the earliest land plants, this information is essential to assess more completely the initial stages of life on land.

### 1.1. Taxon selection

Generally viewed as sister to all other land plants (Qiu et al., 2006), the liverworts (Marchantiophyta) are key in assessing the hypothesis that sporogenesis and spores originated before the sporophyte through heterochrony and the transfer of specialized wall from zygotes in green alga to meiospores in plants (Brown and Lemmon, 2011). Therefore, the ephemeral liverwort sporophyte and spores contained within may be living evidence of the pioneering land plants that evolved following the "spores first" era of terrestrialization.

As a result of the Liverwort Tree of Life initiative and intensive molecular analyses over the past decade, phylogenetic relationships among Marchantiophyta largely have been solidified (Forrest et al., 2006). The phylum comprises three major lineages: Haplomitriopsida, Marchantiopsida (complex thalloids), and Jungermanniopsida (simple thalloids and leafy liverworts) with approximately 5000 species in some 400 genera (Crandall-Stotler et al., 2009a,b). Among these, the Haplomitriopsida, including Treubiales with two genera and the monotypic Haplomitriales, is sister to all other liverworts, while the Blasiales with two genera is sister to the remaining complex thalloids. As representative relictual taxa, spores of *Apotreubia*, and *Blasia* are described herein. Gametophytes of these three orders are morphologically distinct from other liverworts, including members of the diverse Jungermanniopsida that contains simple thalloid and leafy liverworts with over 90% of liverwort species. Because aspects of sporogenesis, especially patterns and behavior of plastids, microtubules and centrosomes during meiosis, are exceptionally variable across liverworts (Brown and Lemmon, 2013), it is reasonable to expect similar diversity of spore wall architecture.

The mosses (Bryophyta) are the largest group of bryophytes consisting of some 10,000 species in three or four distinct clades usually recognized formally as classes (Goffinet et al., 2009; Shaw et al., 2011). The morphology of both life history phases is distinctive and features of the sporophyte and sporogenesis are remarkably uniform throughout most taxa in this specialized and successful group. All mosses investigated to date undergo monoplastidic meiosis and most produce hilate spores (Brown and Lemmon, 1990b, 2013). Unlike liverwort spores that are inaperturate, spores of mosses may have well-developed apertures, which are localized modifications of the proximal pole involving all wall layers and functioning in germination. Although the order of diversification is controversial, it is clear that the Sphagnopsida with four genera and the monogeneric Takakiopsida are sister to all other mosses, followed by the Andreaeopsida with two genera and then the remainder of advanced orders (Renzaglia et al., 2007; Cox et al., 2010). Representatives from these three early divergent classes as described in this study exhibit peculiarities in patterns of spore wall deposition and organization. Together with the enigmatic *Oedipodium*, the taxon emerging as sister to peristomate mosses, *Takakia* and *Sphagnum* have distinct trilete apertures that distinguish them from the more derived hilate apertures of other mosses (Renzaglia et al., 1997; Shimamura and Deguchi, 2008; Brown and Lemmon, 2013).

Hornworts (Anthocerophyta) are a small (about 200 species in 12 genera) but evolutionarily important group. Like the mosses, sporocyte quadripolarity is reflected consistently in cytoplasmic lobing and monoplastidic meiosis. Most current phylogenies find hornworts to be the closest extant relatives of the tracheophytes (Qiu et al., 2006; Renzaglia et al., 2009; Chang and Graham, 2011), but this widely accepted hypothesis has been challenged recently by rigorous comprehensive analyses (Cox et al., 2014; Wickett et al., 2014). Within group classifications align the monospecific *Leiosporoceros* as sister to all other hornworts and in a separate class Leiosporocerotopsida (Renzaglia et al., 2009; Villarreal et al., 2010). A clade containing traditional members of *Anthoceros*, *Sphaerosporoceros* and *Folioceros* is the next to diverge. Here we describe spores of *Leiosporoceros*.

## 2. Materials and methods

### 2.1. Microscopy

Specimens were fixed in our laboratories over several decades using standard TEM and SEM procedures similar to those in Brown and Lemmon (1986) and Renzaglia et al. (1997). Developing capsules were cut in half and fixed in 4% glutaraldehyde pH 6.9–7.2 in 0.05 or 0.1 M phosphate or Pipes buffer for at least 30 min, postfixed in 1% osmium tetroxide for 15 min to 1 h, and washed overnight in d-$H_20$ (all at room temperatures). The materials were dehydrated in a graded acetone series and for SEM critical point dried, mounted on stubs and viewed. For light and transmission electron microscopy, acetone was replaced by propylene oxide and specimens were gradually infiltrated with Spurr's resin over several days. Semi-thin sections (0.5–1.0 μm) were collected on glass slides, stained with 1.0% toluidine blue containing sodium borate, monitored for mature or nearly mature spores and photographed on a light microscope. Ultrathin sections were stained sequentially with uranyl acetate and lead citrate, and viewed in a transmission electron microscope.

## 3. Results

### 3.1. Developmental patterns

In *Haplomitrium* and *Apotreubia*, and retained in some core taxa, patterned precursors that define spore wall ornamentation are present on