Juvenile gametophyte development in the Blasiales. 3. Sporeling ontogeny of *Cavicularia densa*

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Abstract. Sporeling development in *Cavicularia densa* was determined using controlled culture and light microscopy techniques. Ontogeny is typified by endosporic germination followed by early protonematal development via the production of a terminal quadrant of four cells which ruptures the spore wall distally. Sporeling production is triggered by the delimitation of a cuneate apical cell in one of the quadrants. Regular apical cell segmentation produces derivatives and adult merophytes that ultimately result in a juvenile *Cavicularia* gametophyte. The fundamental sporeling pattern exhibited by *Cavicularia* is shared only with that of its sister genus *Blasia*. Moreover, the patterns of sporeling and gemma/gemmaling ontogeny in *Cavicularia* and *Blasia* share quadrant systems of precise uniformity, thus reinforcing the close relationship between the blasialean genera.

Keywords. *Cavicularia*, Blasiales, sporeling development.

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In 1966, Nehira investigated sporeling development in the monospecific genus *Cavicularia* as part of a comparative study aimed at identifying and classifying developmental types of jungermannialean sporelings. In this work, he described the sporeling pattern of *C. densa* as endosporic, with a 6–20-celled, massive globose protonema being formed within the stretched exospore, followed by the juvenile shoot differentiating outside of the spore wall. Based on these characters, Nehira (1966) deemed this sporeling pattern as unique among simple thalloid hepatics and named it the "*Cavicularia* type" of sporeling development.

Fulford (1975) reviewed the work of Nehira (1966) as part of her survey of young stages of gametophyte development in anacrogynous hepatics and concluded that the sporeling development of *Cavicularia* is fundamentally similar to that of *Pellia*, *Noteroclada*, *Blasia* and *Monoclea* in the production of a "globose, multicellular protonema developed within the exospores." Subsequently, Nehira (1983) agreed with Fulford's (1975) categorization of *Cavicularia*, and with the exclusion of *Monoclea*, whose sporeling development he classified within the *Pallavicinia* type, grouped *Cavicularia*, *Blasia*, *Pellia* and *Noteroclada* and renamed the category the *Pellia* sporeling type.

Although Nehira (1966, 1983) presented the foundational observations on sporeling ontogeny in *Cavicularia densa*, precise descriptions of division sequences, including those responsible for generative cell production were not reported. Thus, the complete pattern of *C. densa* sporeling development remains uncertain and as such, its usefulness in clarifying relationships among and within hepatic taxa is limited. This study provides the first full account of sporeling ontogeny in *C. densa*.

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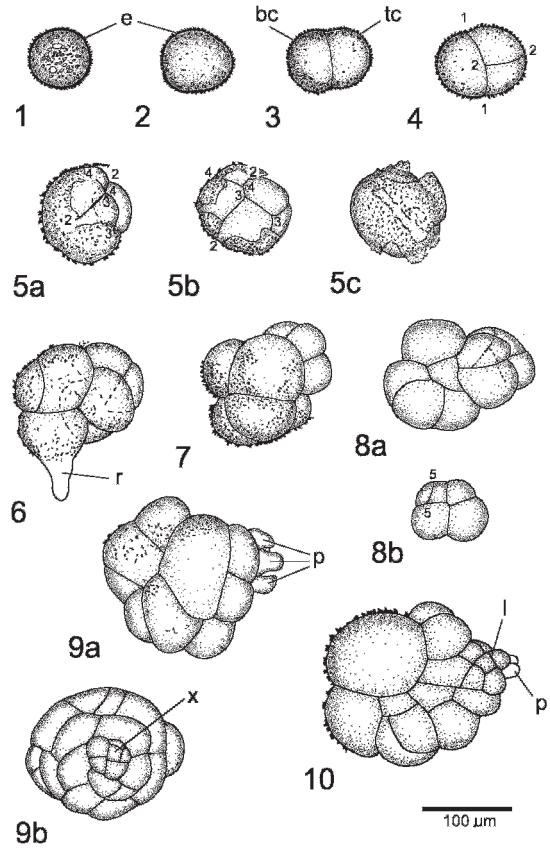
Materials and Methods

Live *Cavicularia densa* plants with mature sporophytes were obtained from Japan, Chiba Pref., Honshu, *Furuki s. n.*, 2002. Voucher specimens are housed in the William Darlington Herbarium at West Chester University (DWC).

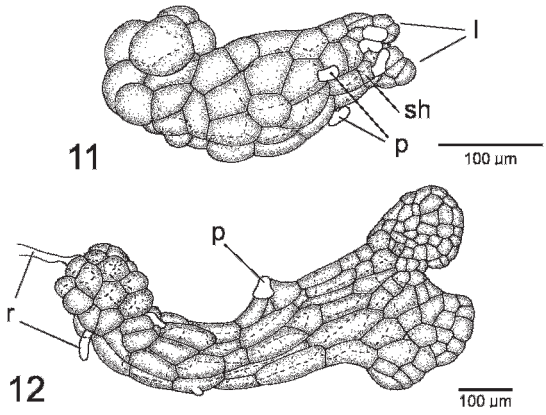
Mature spores were axenically cultured on Benecke (1903) macronutrient agar media supplemented with Hatcher micronutrients B and C (Hatcher 1965) according to the procedure outlined by Bartholomew (1985, 1986a). Cultures were maintained in a Lab-Line Biotronnette growth chamber equipped with Gro-lux and 25 W incandescent lights under a light intensity of 1050 lux with a 12 h day/night photoperiod at 18°C. Developing sporelings were sampled at 4–5-day intervals. Wet mounts of individual sporelings at numerous stages of maturation were utilized and multiple views of the same sporeling were obtained by manually manipulating the specimen under the coverslip of the wet mount. All observations and illustrations were made using an Olympus BX40 light microscope equipped with a drawing tube.

Results

The spores extracted from mature *Cavicularia densa* capsules are spherical with diameters of 47–60 μm, undivided, and bounded by thin, yellow-brown, granular-papillose exospores (**Fig. 1**). Upon germination, the spores become greener, imbibe water and ultimately swell to diameters of 57–70 μm (**Fig. 2**). Germination and early protonematal development is endosporic, i.e., the spore divides while still enclosed within the spore wall, but is not precocious. The initial division of the swollen spore bisects it to form a basal cell and a terminal cell (**Fig. 3**). The terminal cell is then cut into four quadrants by three successive divisions (**Figs. 4, 5**). First, a curved, oblique wall forms perpendicular to the initial division of the spore to bisect the terminal cell (**Fig. 4**, wall 2—2). Quadrant production is completed as each of the two resultant cells is traversed by a radial wall, each of which is oriented perpendicular to the first division of the terminal cell, but slightly offset from one another (**Figs. 5a, b**, walls 3—3 and 4—4). The physical pressures of combined cell expansion and quadrant building



Figures 1–10. Sporeling ontogeny in *Cavicularia densa* Steph. **1.** Ungerminated spore. **2.** Swollen spore. **3.** Transverse first division to form basal and terminal cells. **4, 5.** Construction of terminal quadrant of four cells. **4.** Oblique first division of terminal cell (wall 2—2). **5a–c.** Different views of same protonema bearing a terminal quadrant of four cells. Distal portions of quadrants have broken through the exospore. **5a.** Lateral view. **5b.** Nearly apical view. **5c.** Basal view. **6.** Lateral view of protonema bearing a terminal quadrant of four cells and a dividing basal region. **7.** Nearly basal view of protonema. Note divided basal region bearing spore wall remnants. **8a, b.** Different views of same protonema. **8a.** Near lateral view. **8b.** Apical view. Wall 5—5 is the first of two divisions of the terminal quadrant that complete adult apical cell construction. **9a, b.** Different views of same sporeling bearing an adult cuneate apical cell. **9a.** Lateral view. Papillae overarch the apical cell. **9b.** Apical view showing cuneate apical cell. **10.** Lateral view of sporeling showing derivatives and merophyte development. Key to labeling: bc = basal cell; e = exospore; l = developing lobe; p = slime papilla; r = rhizoid; tc = terminal cell; x = adult cuneate apical cell.



Figures 11, 12. Juvenile gametophyte development in *Cavicularia densa* Steph. **11.** Lateral view of sporeling bearing developing lobes and slime hairs. **12.** Juvenile gametophyte. Key to labeling: l = lobe; p = slime papilla; sh = slime hair; r = rhizoid.

stretch the spore wall until it tears irregularly, exposing the distal portions of the four quadrants (**Fig. 5**). Subsequent growth of the protonema continues to tear the stretched spore wall until only remote basal portions of the sporeling bear exospore remnants (**Figs. 6, 7, 9a, 10**).

Construction of the protonema is completed as division sequences cut both the basal cell and terminal quadrants. The basal cell is traversed by successive divisions that ultimately result in a sporeling with a relatively massive basal region (**Figs. 6–8a, 9a, 10–12**). The first rhizoid elongates from one of the early-formed cells of the basal region (**Fig. 6**), but the time of rhizoid origin is variable.

In the terminal portion of the protonema, a wedge-shaped apical cell is delimited in one of the apical quadrants by two successive oblique divisions (**Figs. 8b, 9b**). In surface view, this cuneate apical cell appears rectangular measuring 10–12 µm wide × 12–14 µm long (**Fig. 9b**). Slime cells that arise from the surrounding quadrants overarch and protect the newly formed apical cell (**Figs. 9a, 10**). As segmentation of this newly created apical cell forms merophytes that develop as in the adult plant, the posterior portion of the sporeling enlarges through cell divisions in the basal portion of each of the quadrants (**Figs. 10**, **11**). In this manner, a juvenile thallus is constructed that bears the structures that typify a *C. densa* gametophyte (**Fig. 12**).

Discussion

The fundamental pattern of sporeling ontogeny in *Cavicularia densa* is consistent and uniform. Early protonematal development is characterized by endosporic spore germination followed by the construction of a terminal quadrant of four cells that ruptures the spore wall distally. A massive basal region forms through divisions of the basal cell, while sporeling production is triggered by the delimitation of a cuneate apical cell in one of the terminal quadrants. Divisions in the basal portions of the terminal quadrants beneath the apical cell, along with regular segmentation from the wedge-shaped generative cell and development of the resulting merophytes ultimately complete the juvenile gametophyte of *C. densa.*

The pattern of sporeling development in *Cavicularia densa* as described in this study is substantiated by the work of Nehira (1966, 1983) which, to date, constituted the only published account of *C. densa* sporelings. Although Nehira (1966) summarized the ontogeny as endosporic, with a 6–20-celled globose, massive protonema produced within the stretched exospore, careful examination of his illustrations show the same pattern of early spore wall rupture as in this study. Lateral views of young protonemata (Nehira 1966, figs. D and E, respectively) show the endosporic, first transverse division of the spore, and an oblique, slightly curved radial division of the terminal cell (the latter wall being the first division in the sequence that builds the terminal quadrants). An apparent apical view (Nehira 1966, fig. C) shows a very young protonema bearing a quadrant of four cells with the distal portions of the quadrants no longer covered by the spore wall. Nehira's (1966) figs. H and J depict the early growth of the exposed sporeling; an indication that apical cell segmentation has begun. Although, in his 1966 work, Nehira provided no illustrations or written descriptions of the division sequence which produces the apical cell, he later inferred that the apical cell of the *Cavicularia* sporeling is cuneate (Nehira 1983).

The fundamental pattern of sporeling development in *Cavicularia* is like that described for its sister genus, *Blasia* (Bartholomew 1986b). Only taxon specific modifications are apparent, such as the

number of sequence repetitions that occur in building the massive basal region, and the exact time of spore wall rupture. In *Cavicularia*, the exospore is ruptured upon quadrant formation, while in *Blasia*, the spore wall is more flexible, stretching to encompass larger numbers of cells before finally being torn by the earliest apical cell derivatives. As mentioned by Bartholomew (1985), the exact time of spore wall rupture in hepatic spore germination and protonematal development is taxon specific and likely determined by the structural mechanics of spore wall architecture.

The fundamental pattern of sporeling development shared by *Blasia* and *Cavicularia* is quite different from the patterns that characterize the Pelliaceae (Bartholomew-Began 1996) and *Monoclea* (Bartholomew-Began & Crandall-Stotler 1994), and thus excludes blasialean sporelings from the *Pellia* sporeling type (Fulford 1975; Nehira 1983). Although both the Pelliaceae and Blasiales are characterized by endosporic development, only protonemata of the Pelliaceae are precocious. Protonematal development in the Pelliaceae is typified by the production of four tiers of cells, with the two central tiers built by a unique, regular internal division pattern. Sporeling and ultimately juvenile development involves the activity of a sporeling initial with two cutting faces which transitions into an adult hemidiscoid apical cell (Bartholomew-Began 1996). Sporeling ontogeny in *Monoclea* is fundamentally reminiscent of a marchantioid pattern, characterized by exosporic development and protonematal construction involving a quadrant system and early restricted division planes that generate a flattened, bilateral ''thalloid'' protonema. Sporeling and juvenile thallus production is through the activity of a wedge-shaped generative cell (Bartholomew-Began & Crandall-Stotler 1994).

When considered in conjunction with other morphogenetic and molecular data (Carothers 1973; Crandall-Stotler et al. 2005; Forrest & Crandall-Stotler 2005; Forrest et al. 2006; Garbary et al. 1993; Renzaglia 1982), sporeling ontogeny supports the close relationship between *Blasia* and *Cavicularia* and intimates the separation of the Blasiales from the simple thalloid hepatics and alliance with the complex thalloid lineage. The primary ontogenetic patterns that produce gametophytes from spores as well as gemmae in *Cavicularia* and *Blasia* share quadrant systems that develop with precise uniformity (Bartholomew 1985, 1986b; Bartholomew-Began & Jones 2005; Jones & Bartholomew-Began 2007). The fundamentality of a quadrant system in primary ontogenetic patterns is emerging as an important focal point for comparative study of young stages of gametophyte development. The retention of quadrant formation in sporeling ontogeny may well be a plesiomorphic trait (Crandall-Stotler 1993), given the apparent frequency of quadrant system involvement in the primary ontogenetic stages of some metzgerialean taxa (Bartholomew 1985; Bartholomew- Began 1991; Bartholomew-Began & Jones 2005; Crandall-Stotler 1993; Jones & Bartholomew-Began 2007; Renzaglia & Bartholomew 1985) and some complex thalloid taxa, particularly members of the Marchantiales and Sphaerocarpales (Bartholomew-Began & Crandall-Stotler 1994; Goebel 1905; Inoue 1960; Leitgeb 1879; Nehira 1966, 1983; Schuster 1966, 1992).

The shared, consistent and uniform expression of the quadrant system that clearly links the primary ontogenetic patterns of blasialean sporelings and gemma/gemmalings provides additional evidence for the phylogenetic relationship between *Blasia* and *Cavicularia*. In addition, it is likely that, considering the recently suggested close alliance of the Blasiales with the Marchantiopsida (Crandall-Stotler et al. 2005; Forrest & Crandall-Stotler 2005; Forrest et al. 2006; He-Nygrén et al. 2006; Heinrichs et al. 2005; Pass & Renzaglia 1995; Stech & Frey 2001) as opposed to the traditional blasialean alliance with the Metzgeriidae, future comprehensive, comparative information gained from detailed investigations of primary ontogenetic patterns, particularly those involving quadrant systems, will be useful in helping to clarify relationships among blasialean taxa, the Metzgeriidae and complex thalloid liverworts.

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