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## Protonemal Development in the Hedwigiaceae (Musci), and its Systematic Significance

EFRAIN DE LUNA

Department of Botany, Duke University,  
Durham, North Carolina 27706

**ABSTRACT.** Spores from herbarium specimens of *Braunia secunda*, *Hedwigia ciliata*, *Hedwigidium integrifolium*, *Pseudobraunia californica*, and *Rhacocarpus purpurascens* were cultured on nutrient agar to observe germination and protonemal development. Germination in the five species was exosporic, and two protonemal types were observed. The protonema in *Rhacocarpus* consists of branched filaments composed of short oblong, or rectangular cells, and is, therefore, of the generalized *Macromitrium*-type. The protonema in *Braunia*, *Hedwigia*, *Hedwigidium*, and *Pseudobraunia* is globular, a type not known elsewhere in mosses. Two different developmental modes result in the globular protonema. *Hedwigia* and *Hedwigidium* illustrate one mode, in which the earliest cell divisions directly produce a spherical group of cells. The second mode is found in *Braunia* and *Pseudobraunia*, in which an early short filamentous germ tube later becomes globular. Differences in sporeling developmental patterns, as well as in other characters of the mature gametophytes and sporophytes, suggest that *Rhacocarpus* should be excluded from the Hedwigiaceae. The globular protonema is interpreted as a synapomorphy that helps to define the Hedwigiaceae as a monophyletic group, including only *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia*.

The application of developmental data to systematic and phylogenetic problems is not free of controversy. Most of the controversy is focused on whether ontogeny, under certain circumstances, can help in assessing evolutionary polarity of characters (Humphries 1988; Kluge and Strauss 1985; Lundberg 1973; Nelson 1978, 1985). Nevertheless, there is agreement that developmental studies can be of value in understanding character homology and transformation series (Patterson 1982; Roth 1984, 1988; Stevens 1984). Furthermore, there is a consensus that comparative descriptions of stages of development may reveal new, taxonomically useful characters (Kaplan 1971; Kluge 1985; Sachs 1982).

In bryophytes, several stages of the life cycle have been studied for taxonomic purposes, namely: sporogenesis (Neidhart 1979), patterns of spore germination and protonema development (Nehira 1983), leaf development (Frey 1974), ontogeny of leafy shoots (Mishler 1988), and patterns of peristome development (Shaw et al. 1987). These authors argue that developmental descriptions can be a source of useful evidence at different taxonomic levels. This paper reports observations of protonemal development in one species of each of the five genera commonly classified in the moss family Hedwigiaceae.

The Hedwigiaceae was first described to in-

clude *Hedwigia*, P. Beauv., *Hedwigidium* B. S. G., and *Braunia* B. S. G. (Bruch et al. 1855). The concept of the family was expanded by Broth. (1925) when he added *Pseudobraunia* (Lesq. & Jam.) Broth. (based on *Braunia californica* Lesq.), *Rhacocarpus* Lindb., and *Cleistostoma* Brid. (= *Bryowijkia* Noguchi). Later the genus *Bryowijkia* was transferred to the Trachypodaceae (Vitt and Buck 1984). There has been debate over whether *Rhacocarpus* belongs to the Hedwigiaceae (Barthlott and Schultze-Motel 1981; Koponen and Norris 1986), or whether it deserves its own familial status (Buck and Vitt 1986). The Hedwigiaceae has been characterized by eperistomate capsules, ecostate leaves, leaf cells irregularly short oblong, and several types of leaf papillae. A unique type of protonemal development was described for *Hedwigia* (Nehira 1983). Therefore, in view of the uncertainty of the circumscription of the Hedwigiaceae and the doubtful familial relationships of *Rhacocarpus*, it was thought that studies of protonemal developmental patterns in each genus might help to clarify the taxonomic understanding of the family.

Spore germination and protonemal development are two different but continuous phenomena, as indicated by biochemical, cytological, and physiological differences between germination and protonemal growth (Bopp 1983; Knoop 1984). Most authors, like Bopp

(1983), Knoop (1984), and Valanne (1966), divide spore germination into two main morphological stages, spore swelling and protrusion of a germ tube. The later stage is used as a practical diagnostic feature of actual spore germination (Hartman and Jenkins 1984). The transition from spore germination to protonemal development is usually defined as the occurrence of the first mitosis (Fulford 1956; Inoue 1960; Nehira 1983). According to these authors, protonemal development starts with the first cell division of the spore, continues with the differentiation of chloronema, caulonema, rhizoids, and "concludes" with the formation of buds.

Protonemal types known in mosses are differentiated on the basis of the overall morphology of the mature protonema, whether the germination is endosporic or exosporic, and the shape of chloronemal cells, particularly those at the earliest stages of protonemal development (Nehira 1983; Nishida 1978). These protonemal features exhibit patterns of variation that are useful at different taxonomic levels. Allsop and Mitra (1958), Fulford (1956), Mishler and Churchill (1984), Nehira (1983), Nishida (1978), and Sussman (1965) discussed the phylogenetic significance of patterns of protonemal development at the division level. Also, Nehira (1971, 1974) provided a review of the relationships between protonemal patterns and the main groups of liverworts. Similarly, within the mosses, according to Nehira (1983) and Nishida (1978), protonemal patterns are correlated with major phylogenetic groups at the ordinal level. Few systematic studies have considered patterns of protonemal development in making taxonomic decisions at familial and lower taxonomic levels. Non-filamentous sporeling types have been used in the familial classification of hepatics (Bartholomew 1986; Schuster 1983). In mosses, spore germination patterns were used in the circumscription of *Sphaerotheciella* Fleisch. (Manuel 1977, 1982), in *Muelleriella* Dusen (Vitt 1976), in *Drummondia* Hook. ex Drumm. (Allen 1987a) and in the Dicnemoniaceae (Allen 1987b).

In the Hedwigiaceae, previous studies of protonemal development were carried out by Nehira (1976), Nishida (1972), and Noguchi and Mizuno (1959), who described the protonema of *Hedwigia ciliata* (Hedw.) Ehrh. ex P. Beauv. as consisting of a "massive protonema." There have been no studies of the protonemal development of *Braunia*, *Hedwigidium*, *Pseudobraunia*, or *Rhaco-*

*carpus*. Therefore, my goal was to test whether the "massive" protonema is unique to *Hedwigia* or whether it occurs also in closely related genera.

#### MATERIALS AND METHODS

*Hedwigia*, *Hedwigidium*, and *Pseudobraunia* are monotypic; *Braunia* as well as *Rhacocarpus* include about 22 species each. One species for each genus was chosen based on availability of relatively fresh capsules containing spores. The spores used for germination were taken from specimens in DUKE:

*Braunia secunda* (Hook.) B. S. G.: México, Zcatecas, Laguna Grande, 27 Aug. 1983, *Cárdenas* 3034.

*Hedwigia ciliata* (Hedw.) Ehrh. ex P. Beauv.: United States, Louisiana, Sicily Island, 15 Mar. 1986, *Reese* 16971.

*Hedwigidium integrifolium* (P. Beauv.) Dix.: México, Hidalgo, Cerro Xihuingo, 16 Feb. 1984, *Cárdenas* 3429.

*Pseudobraunia californica* (Lesq.) Broth.: United States, California, Siskiyou Co., 10 May 1985, *Schofield* 82811.

*Rhacocarpus purpurascens* (Brid.) Par.: Australia, Tasmania, north coast, 8 Dec. 1981, *Vitt* 29355.

For each species, spores were collected from three to six mature, deoperculate capsules and placed in 2.4 ml of distilled water. Approximately 0.8 ml of water containing spores of a single species was then distributed on an agar surface in a petri dish. Three dishes were inoculated for each species. Agar and culture medium were prepared using a modified Hoagland's Solution, as in Mishler (1985). All fifteen dishes were placed in a culture room illuminated by two 40 W Agro-Lite fluorescent lamps, suspended at 35 cm above the surface of the petri dishes, giving about 70 microEinsteins  $m^{-2} sec^{-1}$ . The light period was 12 hours per day, and the room temperature was maintained at 18°C. Two additional dishes for each species prepared as above were placed in a window-sill oriented towards the NW, under a room temperature of 23–25°C.

All cultures were started the same day (21 Jan 1987). During four months, regular observations were made with a compound microscope and recorded by camera lucida drawings and

photographs. Samples of germinating spores and different developmental phases of the protonema were mounted on permanent slides with Hoyer's Solution.

#### RESULTS

Hydrated spores in all cultures became swollen and green in two to four days. By the end of the first week after hydration, germination occurred in some spores of all species except *Braunia secunda*; in this species the spores germinated after three weeks of culture. Spores of all five species studied here showed exosporic germination. However, the protrusion of the germ tube was not synchronous in any of the culture dishes. Spores continued to germinate during the first eight weeks of culture.

Two protonemal types were observed, regardless of the location of the culture dishes in the growth room or on the window-sill. In *Rhacocarpus purpurascens*, as in most Bryidae, the fully developed protonema was filamentous (figs. 1-3). But in the other four species, the mature protonema was globular ("massive" sensu Nehira 1983) (figs. 4-8). Besides these major differences in the morphology of the mature protonema, there were further differences in the sequence of development. Comparative descriptions are given below.

***Rhacocarpus purpurascens.*** After the expansion of the spore and the rupture of the spore coat, the first septum was formed. The initial cell divisions produced a single or bifurcate filamentous germ tube, which consisted of two to three oblong-elongate cells (figs. 9, 10). The resulting germ tube underwent several cell divisions and differentiated filamentous chloronema (figs. 11-13). The cells at these early stages contained abundant chloroplasts, had colorless cell walls, and the cross-walls were oriented at right angles to the lateral walls. In *Rhacocarpus*, like most Bryidae, these chloronemal filaments grew along the surface of the agar. Later in development, differentiation of caulonema from chloronema occurred in older filaments (fig. 14). Caulonemal cells had colored walls, oblique cross-walls, and a reduced number of chloroplasts. The cells with oblique cross-walls were formed at the apical parts of the filaments. Some caulonemal cells produced rhizoidal filaments that had reddish cell walls and contained no chloroplasts. Buds developed from middle portions of caulonemal filaments.

***Hedwigia ciliata.*** After rehydration, the spore expanded and protrusion occurred without the formation of new cells. The first septum was formed outside the spore coat (fig. 15). Additional cell divisions occurred in several planes, producing a massive protonema of irregular shape, mainly composed of rounded cells (figs. 16, 17).

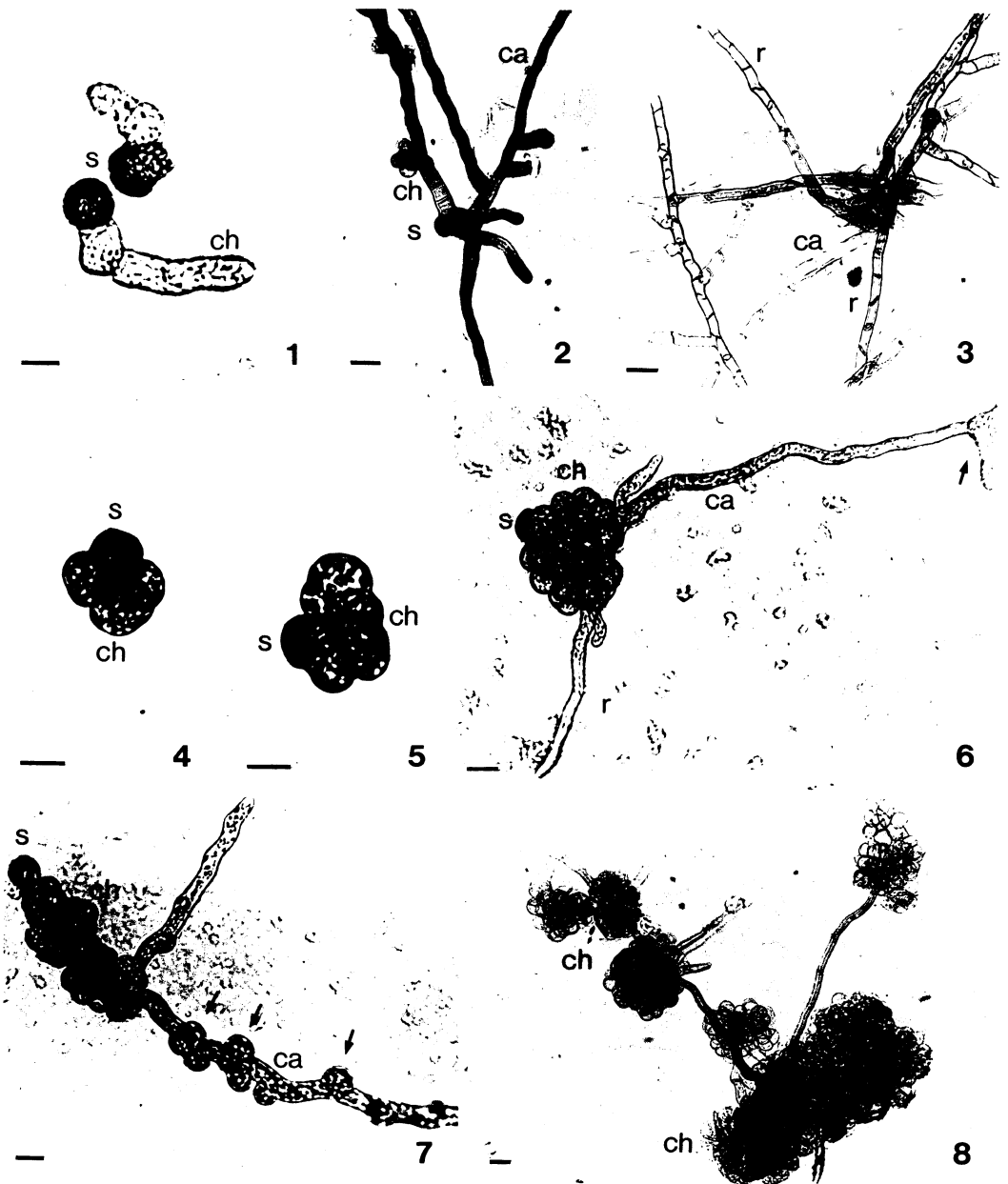
Further cell divisions resulted in the formation of 10 to 12 cells in a group of globular shape (fig. 18). These cells were similar to the chloronemal cells in the filamentous protonemal types. They had numerous chloroplasts and the cell walls were hyaline. Two or three of these cells became elongated and each one subsequently developed into an unbranched filament with oblique cross-walls and fewer chloroplasts (fig. 19). These were like the caulonemal cells in filamentous protonemal types (e.g., fig. 14).

As development progressed, the globular (or "massive") chloronema continued growth, producing up to 18-24 small rounded cells (figs. 19, 20). The caulonemata also continued growth, producing very elongated and unbranched filaments composed of several cylindrical cells with oblique walls. At this stage of development, rhizoids were formed from middle portions of the caulonemal filaments and directly from cells of the globular group (fig. 20). Bud development occurred only from cells of the globular chloronema.

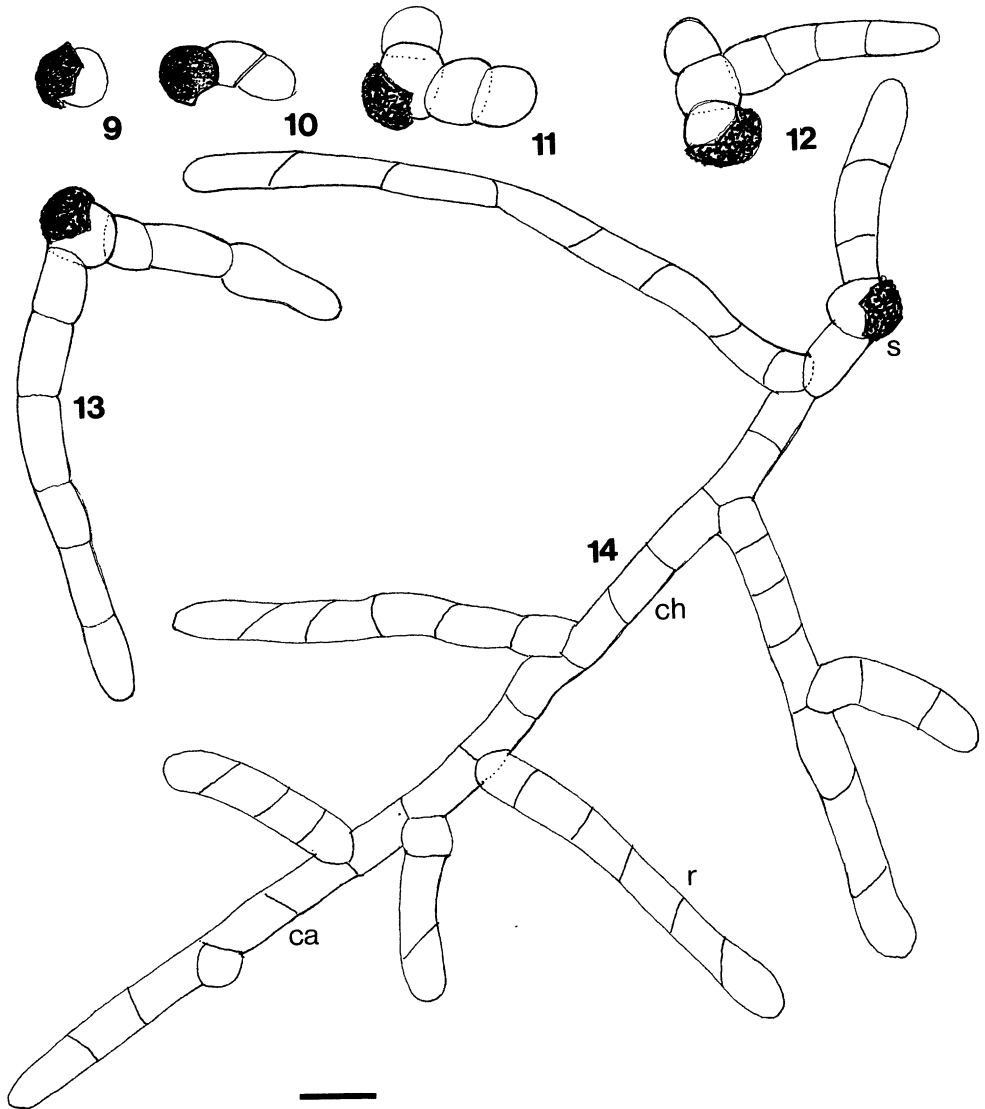
***Hedwigidium integrifolium.*** Protonemal development was very similar to the pattern observed in *Hedwigia ciliata*. Early cell divisions also produced a globular protonema (figs. 21-23). Subsequently, several unbranched caulonemal filaments differentiated from the globular chloronema (figs. 24, 25). Finally, rhizoids developed from older caulonemal filaments or from cells of the globular chloronema (fig. 26).

***Braunia secunda.*** Following cell expansion, a short germ tube protruded, elongated, and the first cross-wall was formed at right angles. A second right angle septum was formed, and the resulting cells in this short germ tube were like those of *Rhacocarpus* (see fig. 10): rounded to short oblong, containing abundant chloroplasts, and with hyaline cell walls (figs. 27, 28).

Further cell divisions were not in one plane, as in *Rhacocarpus purpurascens* and most other Bryidae. Rather the subsequent cell divisions of the initial germ tube were oriented in three

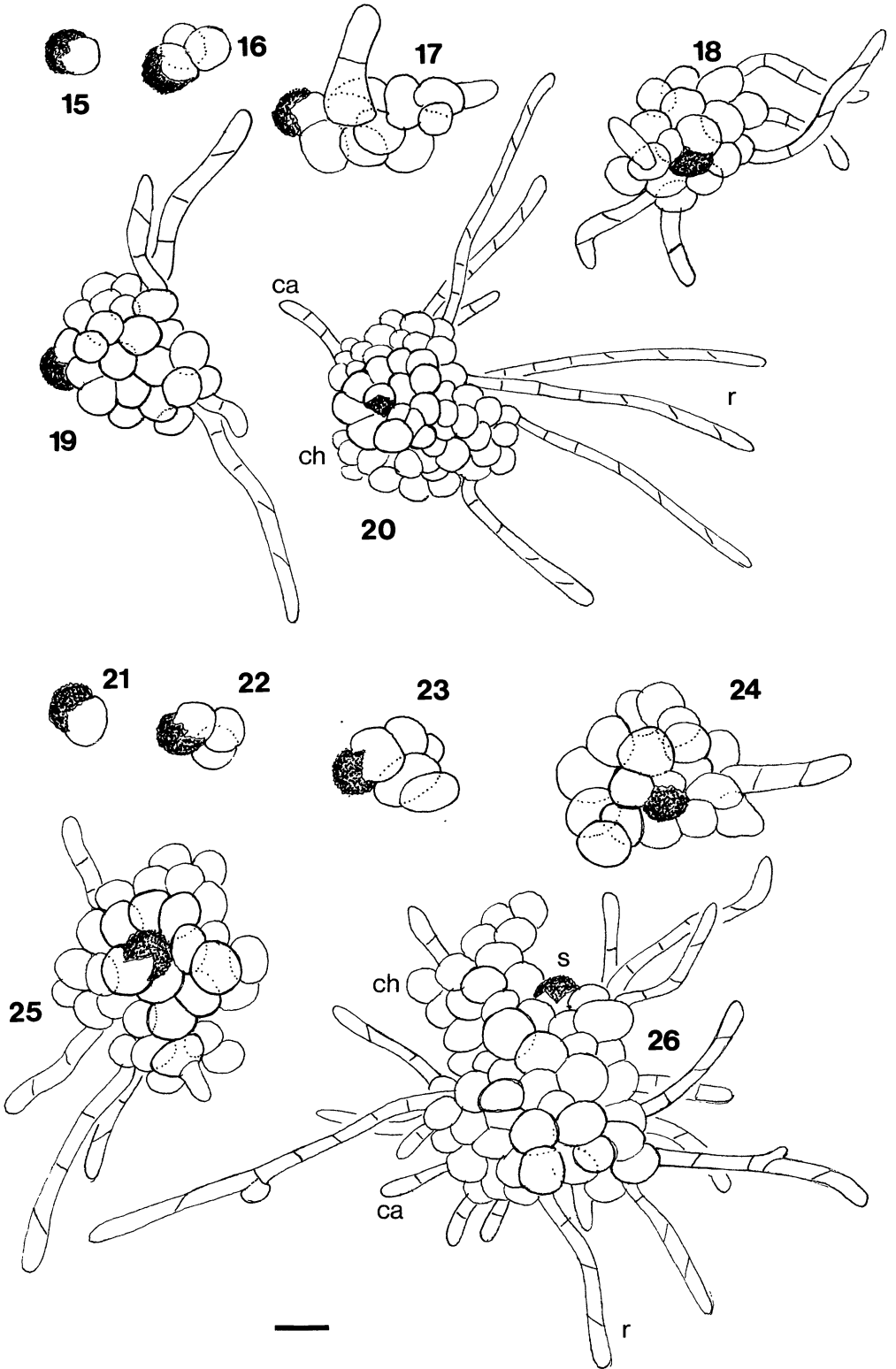


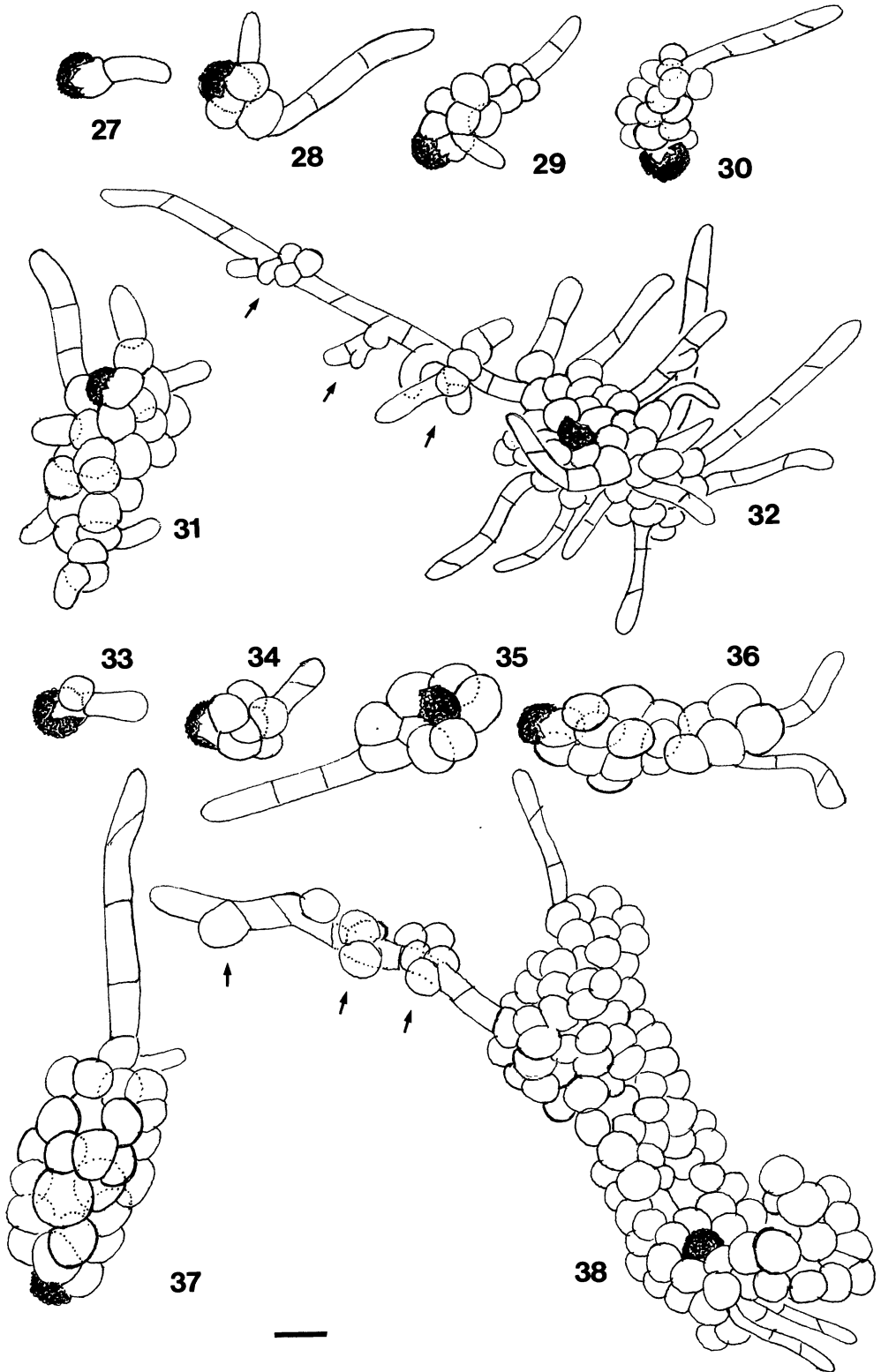
FIGS. 1-8. Patterns of protonemal development in the Hedwigiaceae s.l. 1-3. *Rhacocarpus purpurascens*. 1. Initiation of filamentous chloronemal phase. 2. Branching of filamentous chloronema and differentiation of caulonemal phase. 3. Portion of the fully developed filamentous protonema. 4-6. *Hedwigium integrifolium*. 4, 5. Initial chloronemal phase with cells in a globular pattern. Note that cells are not covered by the spore coat. 6. Differentiation of unbranched filamentous caulonema. 7, 8. *Pseudobraunia californica*. 7. Intermediate developmental stage with chloronemal cells in a globular arrangement and unbranched caulonemal filaments. 8. Fully developed globular protonema with additional globular chloronemal cells differentiated along caulonemal filaments. In these and subsequent figures, s: spore; ch: chloronema; ca: caulonema; r: rhizoids. Arrows indicate sites along caulonemal filaments where additional globular protonemal cells develop. Scale bars = 25  $\mu$ m.



FIGS. 9-14. Protonemal development in *Rhacocarpus purpurascens*. 9. Germinating spore; note rupture of the spore coat and protrusion of a germ tube. 10. Initial protonemal development following elongation of the germ tube and the formation of new transverse cell walls. 11-13. Filamentous chloronemal phase. The filament is often branched at the base. 14. Differentiation of caulonema in the apical part of older filaments. Scale bar = 25  $\mu$ m.

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 FIGS. 15-26. Protonemal development in *Hedwigia ciliata* (15-20) and *Hedwigidium integrifolium* (21-26). 15, 16. Initial protonemal development with cells in a globular arrangement. 17, 18. Differentiation of caulonemal filaments. 19, 20. Mature globular protonema with peripheral caulonemal and rhizoidal filaments. 21-23. Early protonemal development. 24, 25. Differentiation of caulonemal filaments from the globular cells. 26. Mature globular protonema with caulonemal filaments and few rhizoids. Scale bar = 25  $\mu$ m.





orthogonal planes. The result was a globular cell arrangement, as in *Hedwigia* and *Hedwigidium*. The globule was usually composed of eight to twelve rounded cells, which contained abundant chloroplasts and had hyaline cell walls (figs. 29, 30).

Differentiation of caulonema began when one or two cells in the periphery of the globular group elongated and produced successive cells in one plane resulting in a filament (figs. 31, 32). The cross-walls in these filaments were oblique and the cells contained fewer chloroplasts. The caulonemal filaments occurred all around the periphery of the globular mass of cells.

Once caulonemal filaments had been formed, rhizoids were differentiated either directly from the cells of the globular group, or from cells of older caulonemal filaments (fig. 32). At this stage of the development, the protonema consisted of a globular ("massive") chloronema, several unbranched caulonemal filaments, and rhizoids, similar to the mature protonema of *Hedwigia* (fig. 20). In the subsequent development of this initial protonemal "unit," new globular groups of cells formed on medial portions of very elongated caulonemal filaments.

The formation of new "protonemal units" started when an intermediate cell of the caulonemal filament divided in a radial pattern. Figure 32 shows the initiation of new globular cells in three points along the caulonemal filament in *Braunia* (see fig. 7). The shape, chloroplast content, and globular arrangement of the resulting cells were similar to the cells produced immediately after germination. Further differentiation of caulonema and rhizoids from new globular groups of cells proceeded in the same sequence as in the formation of the first "protonemal unit." As a result of this repetition process, several interconnected "protonemal units" were produced from a single spore (see fig. 8). Bud differentiation was observed only

on the massive cells and not on the filaments of the caulonema.

***Pseudobraunia californica*.** Protonemal development was very similar to that observed in *Braunia secunda*. During germination, a short germ tube was formed (figs. 33, 34) and further cell divisions produced a globular protonema, as in *Hedwigia*, *Hedwigidium*, and *Braunia* (figs. 35, 36). Caulonemal filaments differentiated from cells in the globular chloronema. However, unlike *Braunia*, only two to four caulonemal filaments were differentiated (see figs. 32, 38). Eventually, rhizoids were developed either from globular or caulonemal cells (figs. 37, 38). Finally, new "protonemal units" were differentiated from medial cells of caulonemal filaments. As in *Braunia secunda*, these protonemal units consisted of globular chloronema, caulonemal unbranched filaments, and rhizoids (figs. 7, 8).

#### DISCUSSION

Protonemal development did not proceed through the same pattern in all five species surveyed in this study, even though all spores were cultured together under the same growth conditions. In *Rhacocarpus purpurascens*, the development of protonema follows the basic filamentous pattern (figs. 1-3, 9-14) described for a large number of moss species and classified as the *Macromitrium*-type by Nehira (1983). The *Macromitrium*-type is similar to the filamentous *Funaria*- and *Bryum*-types and it has been observed in several other pleurocarpous mosses, namely the Leucodontaceae, Pterobryaceae, Neckeraceae, Leskeaceae, and some species in the Thuidiaceae (Nishida 1978). In *Hedwigia ciliata*, *Hedwigidium integrifolium*, *Braunia secunda*, and *Pseudobraunia californica* the development of protonema is of the *Hedwigia*-type described by Nehira (1983). This pattern has not been observed in any other moss and it is characterized

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FIGS. 27-38. Protonemal development in *Braunia secunda* (27-32) and *Pseudobraunia californica* (33-38). 27. Protrusion and elongation of the germ tube. 28, 29. Differentiation of the globular chloronemal phase as a result of mitosis in the basal cells. 30, 31. Differentiation of caulonemal filaments. 32. Mature globular protonema with peripheral caulonemal and rhizoidal filaments. 33. Elongation of the germ tube during initial filamentous stage. 34, 35. Further mitosis in the basal cells initiate the globular phase. 36, 37. Differentiation of caulonemal filaments from the globular cells. 38. Mature globular protonema with filamentous caulonema and rhizoids. Scale bar = 25  $\mu$ m.

by a globular arrangement of cells in the mature protonema.

In the past there has been confusion about whether the germination in *Hedwigia ciliata* was endosporic or exosporic. Nishida (1978) described spore germination in this species as taking place inside the stretched spore wall (i.e., as endosporic). Later, Nehira (1983) interpreted it as being exosporic. My observations show clearly that the cells of the developing protonema are not covered by the spore coat, and that the spore coat is not broken in several pieces, as happens during endosporic germination. Therefore, my observations confirm Nehira's conclusion that the germination in *Hedwigia ciliata* is exosporic.

It is possible to argue that the spores of *Hedwigia ciliata* may germinate either endosporically or exosporically, depending on growth conditions. However, such a possibility is not supported by the results of this and previous studies using several different growth conditions. Noguchi and Mizuno (1959), Nishida (1972, 1978), and the present study, together have used at least seven different combinations of nutrients, pH, light, and temperature for germination of spores of *Hedwigia ciliata*. Any expected differences between exosporic and endosporic germination are not evident when comparing my results with previously published illustrations. In fact, the drawings of spore germination and protonemal development in *Hedwigia ciliata* presented by Nehira (1976, 1983) and Nishida (1978, pl. 47), show basically the same cell patterns during germination and early protonema development as described in the present paper. Thus, it is likely that the different published accounts of spore germination in *Hedwigia ciliata* reflect the same exosporic germination pattern, but different interpretations and criteria for defining germination.

My observations indicate that there are two developmental modes that result in the globular protonema. One occurs in *Hedwigia* and *Hedwigidium*, in which the early cell divisions directly develop a massive group of cells (figs. 4–6, 15–26). The other developmental mode occurs in *Braunia* and *Pseudobraunia*, in which a short, two- or three-celled germ tube is produced first, which later becomes massive (figs. 27–38). The latter mode is reported here for the first time, and although the globular protonema is formed in a slightly different way, most of

the developmental sequence and the shape of the mature protonema is clearly like that found in *Hedwigia*.

Although only five species were studied, the results are representative of the patterns of protonemal development in the genera *Hedwigia*, *Hedwigidium*, *Pseudobraunia*, *Braunia*, and *Rhacocarpus*. The first three genera are monotypic and the later two consist of about 22 species each. However, there is no reason to expect heterogeneous protonemal patterns within each of these seemingly natural genera. Protonemal patterns are highly homogeneous within a genus. Surveys of more than 100 genera in diverse families include no record of different protonemal patterns within a genus (Kanda and Nehira 1974, 1976; Nehira 1976, 1983; Nishida 1978). Furthermore, as discussed above, no variation was seen in the protonemal development of four populations of *Hedwigia ciliata* studied previously (Nehira 1983; Nishida 1972, 1978; Noguchi and Mizuno 1959).

The differences seen between the filamentous protonema in *Rhacocarpus* and the globular protonema in *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia* could be attributed to a diversity of factors. Although spores used in the present study were obtained from herbarium specimens collected at different dates between 1981 and 1986, it is likely that differences in spore dormancy had no effect on the patterns of protonemal development. Differences in protonemal morphology based on the length of the dormant period have never been reported. Also, it is unlikely that any slight differences in culture conditions on the same shelf in a growth room could explain the greatly different protonemal patterns observed, particularly because the dishes were replicated within the growth room, and because the same differences were observed in cultures placed in two different locations.

The following arguments suggest that the contribution of phenotypic plasticity to differences observed in protonemal development between species is relatively low and that, therefore, protonemal patterns may often reflect genetic differences of systematic value.

Variation in protonemal development, as a morphogenetic system (Bopp 1984; Jaffe 1958; Sachs 1982), can be described as the combined result of at least three components. Part of the variation involves differences in symmetry given by the axial system of growth. Planes of cell

divisions follow a system of one or more axes, which presumably is an intrinsic property of any given morphogenetic process (Bloch 1965; Dormer 1965; Sachs 1984). Data on environmentally induced variability of non-filamentous protonemal types is limited (Nehira 1976; Nishida 1978). However, there is no indication that spores that normally develop a filamentous protonema (one axis of growth) could be induced to develop any other non-filamentous protonemal type (two or three axes of growth), like the *Sphagnum*-, *Hedwigia*-, or *Ptychomitrium*-types. Also, no indication exists that a spore normally developing a non-filamentous protonema could be induced to develop, instead, a filamentous protonema, like the *Funaria*-, *Bryum*-, or *Macromitrium*-types. Thus, the symmetry of mitotic divisions given by a system of axes during protonemal growth seems to be unaffected by environmental factors.

Other components in the variation of morphogenetic patterns are the polarity or direction of growth (Bloch 1965; Schnepf 1986) and the timing of development (Kauffman 1983). Shifts in polarity and changes in rates of protonemal development can be readily induced by environmental gradients. For example, the shape of protonemal filaments, the length and frequency of branches, and the rate of mitosis have been described as highly variable and easily changed by variation in environmental conditions (Berthier 1978; Bopp 1963, 1976, 1983; Cove and Ashton 1984; Hartmann and Jenkins 1984; Knoop 1984; Wettstein 1965). However, there is no evidence that environmental factors can induce changes in the relative timing of chloronema, caulonema, and rhizoids during protonemal development.

In *Rhacocarpus purpurascens*, cell divisions in the protonema have one axis of growth, so that added cells form a filament. Development of filamentous protonemata involves regular changes of the orientation of the growing axis. Branching points along the growing filament reflect shifts in the orientation of growth, but the number of axes remains constant for a given cell lineage because successive cell divisions in the branches continue to produce filaments.

In contrast, protonemal development in *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia* is characterized by initial cell divisions in a relatively stable sequence, oriented in more than one axis yielding a globular arrangement of cells.

This system of axes during cell proliferation from a germinated spore and the timing of events in development are likely the two most important differences between protonemal development in *Rhacocarpus* and that in the other four species. The axial system of growth in filamentous and non-filamentous protonema and the developmental sequence of protrusion, differentiation of chloronema and caulonema are generally not modified by environmental changes (Nehira 1983, Nishida 1978). Thus, differences in protonemal patterns seen in this study very likely reflect genetic differences between species.

The systematic significance of "massive" protonemal types has been questioned by several authors. For example, Nehira (1983), Nishida (1978), and Schuster (1983) have interpreted the different "massive" protonemal types known in a number of unrelated mosses, liverworts, and hornworts as ecological adaptations, and, therefore, argued that these are of limited phylogenetic value. For example, Allen (1987a) recently stated that "it is possible to view Nehira's *Ptychomitrium*-, *Hedwigia*-, and *Drummondia*-types of protonemata as a single type."

Likewise, the globular protonemata in *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia* could be interpreted as a result of ecological convergence, instead of an indication of common phylogenetic origin. The genera *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia* basically share a similar habitat. Most species in these genera colonize rocks in relatively xeric environments. However, a more plausible explanation is that similarities of protonemal pattern in the four genera are due to their derivation from a common ancestor, and, therefore, the globular protonema is homologous.

Similarity of features during their ontogeny and in their mature state (morphological, positional, or detailed structure), and character congruence are accepted tests of homology (Patterson 1982; Roth 1984, 1988; Stevens 1984). The morphological and developmental similarities described above suggest that the globular protonema is homologous among *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia*. This hypothesis is in agreement with Buck and Vitt's (1986) delimitation of the Hedwigiaceae. Furthermore, homology of the globular protonema has been corroborated by an initial evaluation of character congruence.

Preliminary cladistic analyses based on herbarium specimens (in prep.) suggest that the globular protonema is phylogenetically correlated with other characters of mature gametophytes and sporophytes (for example, branching patterns, leaf cell shape, papillae, and spore surface morphology). Thus, on the basis of similar morphology, common development, and character congruence, the globular protonema can be interpreted as homologous in the four genera.

Homology of the globular protonema provides a basis for reassessing the Hedwigiaceae. According to the studies of Nehira (1983) and Nishida (1978) the protonema is filamentous in most species within the Isobryales. This is also true at a more inclusive level in the subclass Bryidae sensu Schofield (1985). Therefore, character polarization by out-group comparison suggests a phylogenetic transformation from an ancestral filamentous protonema to a relatively derived globular one. The results from this survey suggest that the globular protonema can be interpreted as a synapomorphy that would define the Hedwigiaceae as a monophyletic group, including only *Hedwigia*, *Hedwigidium*, *Braunia*, and *Pseudobraunia*. This interpretation would corroborate the earlier suggestion of Buck and Vitt (1986) to exclude *Rhacocarpus* from the Hedwigiaceae. Furthermore, the two developmental modes that result in a globular protonema suggest a transformation series from a globular protonema with an initial filamentous germ tube, as in *Braunia* and *Pseudobraunia*, to a globular one without such germ tube, as in *Hedwigia* and *Hedwigidium*.

The hypothesis of homology of the globular protonema of the Hedwigiaceae is compatible with the interpretation of non-homology of the various "massive" protonemal types known in a number of unrelated mosses. One indication of non-homology between the various "massive" protonemal types is that the sequences of development are not similar (Nehira 1983). Another indication of homoplasy among "massive" protonemal types is their phylogenetic incongruence with other characters. None of the recent hypotheses of phylogenetic relationships of mosses indicate that the taxa with "massive" protonema share a unique recent common ancestor. Developmental differences as well as incongruence with other characters suggest that the "massive" protonemata can not be inter-

preted as homologous among *Andreaea*, *Drummondia*, *Ptychomitrium*, *Encalypta*, and the Hedwigiaceae. Also, it is very unlikely that "massive" protonema is the ancestral condition in mosses for a filamentous protonema is the most generalized condition. The most plausible interpretation is that "massive" protonemal types originated independently in several different lineages within the mosses.

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