

PHYLOGENETIC STUDIES OF THE SPIRIDENTACEAE (MUSCI): OBSERVATIONS OF THREE MORPHOLOGICAL CHARACTERS ASSOCIATED WITH PLEUROCARPY

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RESUMEN

El análisis cladístico de los datos de la secuencia *rbcL* sugiere que las Spiridentaceae pertenecen a un linaje pleurocárpico claro que es filogenéticamente independiente del de otros musgos pleurocárpicos. Como los datos *rbcL* sugieren un juego nuevo de taxa relacionados con las Spiridentaceae, es importante reexaminar tres caracteres que se han usado para diferenciar linajes pleurocárpicos. Éstos a menudo han sido mal usados como parte de la definición de pleurocarpia en lugar de las condiciones correlacionadas. Con base en nuestros resultados, en los pleurocárpicos relacionados con las Spiridentaceae, los primordios de ramas ya no se pueden tipificar como exclusivamente axilares y desnudos y los pseudoparafilios no siempre están ausentes. Por ello, estos caracteres no sirven para distinguir el linaje pleurocárpico de las Spiridentaceae del de otros linajes pleurocárpicos.

Palabras clave: musgos, Spiridentaceae, análisis cladístico, filogenia, secuencia *rbcL*.

ABSTRACT

Cladistic analysis of *rbcL* sequence data suggests that the Spiridentaceae belong to a distinct pleurocarpous lineage that is phylogenetically independent of other pleurocarpous mosses. As the *rbcL* data suggest a novel set of taxa allied with the Spiridentaceae, it is important to re-examine three characters that have been used to differentiate pleurocarpous lineages. These characters have often been misleadingly regarded as part of the definition of pleurocarpy itself, rather than correlated conditions. According to the present results, in pleurocarps related to the Spiridentaceae, branch primordia can no longer be typified as exclusively axillary and naked, and pseudoparaphyllia are not always absent.

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Therefore, these characters no longer serve to distinguish a pleurocarpous lineage containing the Spiridentaceae from other pleurocarpous lineages.

Key words: mosses, Spiridentaceae, cladistic analysis, phylogeny, *rbcL* sequence.

INTRODUCTION

The Spiridentaceae is composed of two genera of mosses, *Franciella* and *Spiridens*, that grow epiphytically (primarily on tree ferns) in montane forests of islands in the Pacific Basin. The Spiridentaceae have traditionally been considered a unique group of pleurocarps of uncertain taxonomic affiliation (Brotherus, 1909; Buck and Vitt, 1986; Crosby, 1980; Dixon, 1932; Fleischer, 1920; Jaeger and Sauerbeck, 1872; Robinson, 1971; Vitt, 1984).

As part of a study of the higher-level phylogenetic relationships of the Spiridentaceae, the evolution of pleurocarpy was also examined. Results from *rbcL* sequence data indicated that there were at least two independent evolutionary origins of pleurocarpy among the study taxa (Withey, unpublished data; Fig. 1). According to this hypothesis of relationships, the order Bryales (including the acrocarps *Leptobryum*, *Bryum*, *Bartramia*, *Philonotis*, and *Plagiomnium*) is paraphyletic, with different members included in both Clade A and Clade B. The remainder of Clade B is composed of various representatives of the orders Leucodontales (*Leucodon*), Hypnales (*Thuidium*), and Hookeriales (*Hookeria* and *Hypopterygium*). Apart from the basal acrocarpous genera *Bartramia*, *Philonotis*, *Plagiomnium*, and *Cryptopodium*, the rest of Clade A is composed of pleurocarps of various ordinal affiliation, all of which have been allied with the Spiridentaceae at one time or another (Brotherus, 1909; Buck and Vitt, 1986; Crosby, 1980; Dixon, 1932; Fleischer, 1920; Jaeger and Sauerbeck, 1872; Robinson, 1971; Vitt, 1984).

Researchers have differed with regard to the definition of pleurocarpy. Meusel (1935) distinguished acrocarps and pleurocarps based on differences in the orientation of branches, the architecture and periodicity of the shoot system, and the presence or absence of rhizoidal shoots and permanent protonema. Schofield and Héban (1984) determined that pleurocarpous mosses have plagiotropic growth forms and lateral sporophytes. According to Buck and Vitt (1986), all pleurocarpous mosses share a characteristically lateral position of the archegonial bud, and different pleurocarpous lineages are distinguished by the presence or absence of pseudoparaphyllia and location of branch primordia. To Hedenäs (1994), pleurocarpy referred to one end of an evolutionary continuum. In his opinion, the transition from acrocarpy to pleurocarpy was a gradual process which may have occurred more than once and involved changes in many separate characters.

Because of these differences, it is not always clear what suite of characteristics is being referred to when the term "pleurocarp" is used. It is preferable to eliminate reference to secondary characteristics and define pleurocarpy only by the lo-

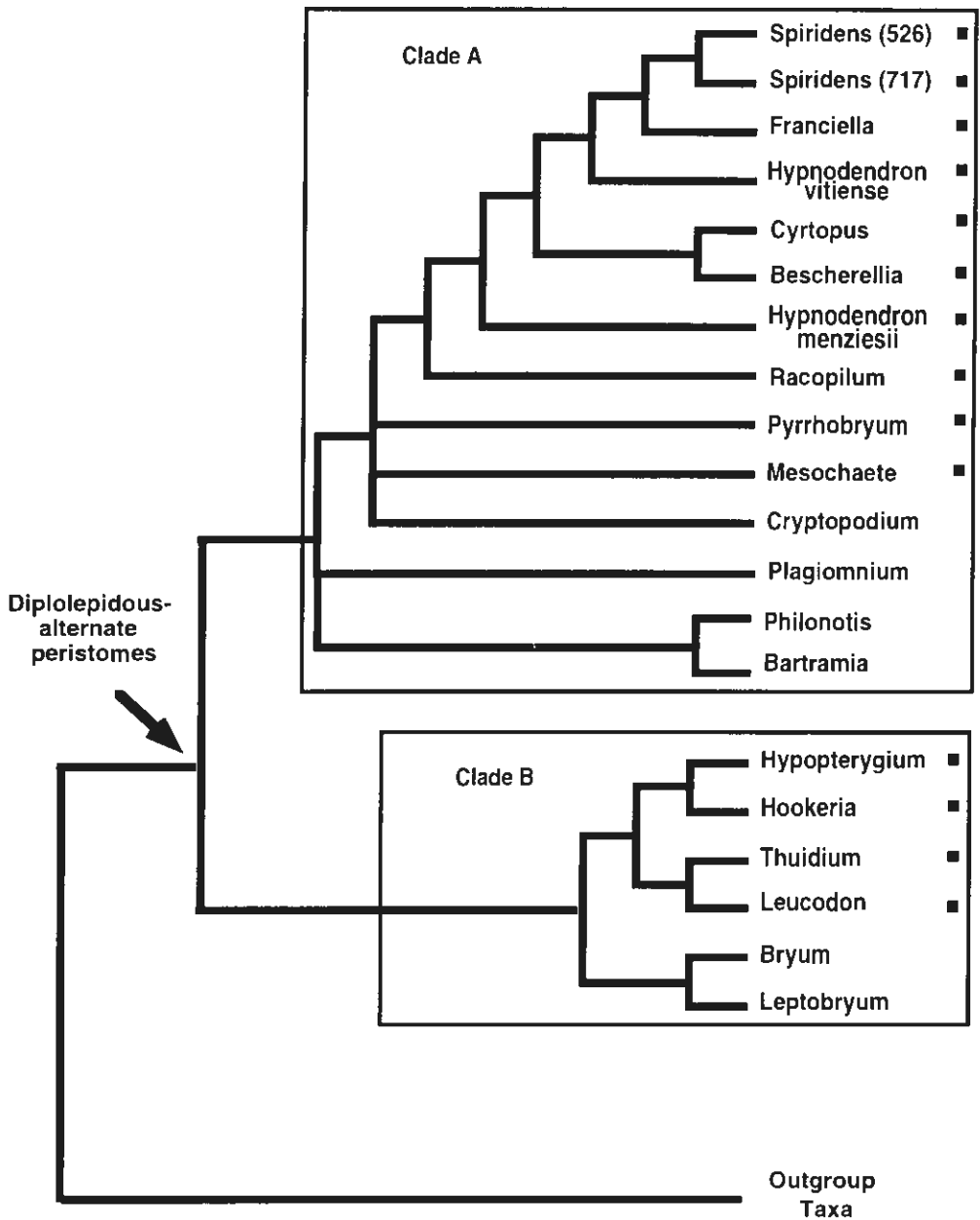


Fig. 1. Strict consensus of four most parsimonious trees constructed from *rbcl* sequence data. "■" indicates pleurocarps (Withey, unpubl. data).

cation of the archegonial bud. Strictly defined then, pleurocarps include all mosses that produce archegonia on lateral branches, as opposed to acrocarps which develop archegonia from the apical cell on the primary shoot (De Luna, 1990, 1992; Mishler and De Luna, 1991). With this definition, the cladocarpous condition could be interpreted as a special case of pleurocarpy, where archegonia terminate elongate lateral branches.

Separate pleurocarpous lineages may then be defined by the secondary characters associated with pleurocarpy. In the past, the lineage containing the Splachnaceae has been characterized as having no pseudoparaphyllia (Buck and Vitt, 1986) and naked branch primordia (Akiyama and Nishimura, 1993a) located in the axils of leaves (Buck and Vitt, 1986). These characters, considered central for understanding relationships among pleurocarps, are in need of further definition and clarification. In addition, a re-evaluation of these characters is needed to see if they show correlation with the novel pleurocarpous groupings in the *rbcL* phylogeny.

METHODS

Study taxa include representatives of all pleurocarps of Clade A (Withey, unpubl. data; Table 1). Observations were made from material examined with SEM, and microtome sections of paraffin-embedded material.

Sections of stems from herbarium specimens were soaked in a 2% aqueous solution of Contrad 70 (Schmid and Turner, 1977) to soften and expand the tissues. Specimens for SEM were then critical-point dried, placed on aluminum studs, and sputter coated. Observations and photographs were made with a JEOL T-20 Scanning Electron Microscope. Material for anatomical sectioning was gradually dehydrated through a tertiary butyl alcohol (TBA) series (Johansen 1940), embedded in paraffin, and sectioned transversely at a thickness of 8-10 μ m on a rotary microtome. Cross-sections were stained with safranin, fast green FCF, and Heidenhain's iron haematoxylin (Johansen, 1940) and permanently mounted.

CHARACTER INTERPRETATION AND RESULTS

Type of branch primordia

According to Akiyama (1990 a,b) and Akiyama and Nishimura (1993 a,b), there are two types of dormant branch primordia. One type involves branch primordia that produce juvenile leaves early and, therefore, form a small leafy bud prior to dormancy (bud or *Climacium* type). In the second type of branch initiation, the primordia do not produce juvenile leaves before dormancy and remain an undifferentiated group of cells at the time of dormancy (naked or *Bryum* type). Dormancy is easily assessed because all primordia along a piece of stem show the same

Table 1. Specimens examined with SEM and anatomically sectioned

Cyrtopodaceae*Bescherellia elegantissima* DubyPAPUA NEW GUINEA. Morobe Province: *Koponen 33971* (DUKE)*Cyrtopus setosus* (Hedw.) Hook.f.NEW ZEALAND. South Island: *Glenny 4827* (WELT);North Island: *Berggren 1756* (DUKE)**Hypnodendraceae***Hypnodendron menziesii* (Hook.) Par.NEW ZEALAND. Westland: *Visch 75* (DUKE)*Hypnodendron vitiense* Mitt. ssp. *australe* TouwAUSTRALIA. Victoria: *Streimann 36537* (DUKE);Bathurst: *Anderson 23,873* (DUKE)**Racopilaceae***Racopilum convolutaceum* (C.Müll.) Reichdt.NEW ZEALAND. South Island: *Glenny 4941* (WELT)**Rhizogoniaceae***Mesochaete undulata* Lindb.AUSTRALIA. New So Wales: *Streimann & Curnow 35768* (DUKE)*Pyrrhobryum mnioides* (Hook.) ManuelNEW ZEALAND. North Island: *Vitt 29831* (DUKE)**Spiridentaceae***Spiridens* sp. NeesNEW CALEDONIA. Mt. Panié: *Withey 717* (DUKE)SOCIETY ISLANDS. Tahiti: *Withey 526* (DUKE)*Franciella spiridentoides* Thér.NEW CALEDONIA. Mt. Panié: *Withey 753* (DUKE)

level of development. In Akiyama and Nishimura's (1993a) study, all members of the order Bryales as traditionally circumscribed (*i.e.*, including members of the Spiridentaceae, Rhizogoniaceae and the Hypnodendraceae) were reported to possess naked (*Bryum*) branch primordia, with the exception of the acrocarps Bartramiaceae and Timmiaceae.

Among the pleurocarpous taxa included in Clade A based on the *rbcl* data (Table 1), both types of branch primordia are found. *Hypnodendron* and *Pyrrhobryum* (Fig. 2) have the bud (*Climacium*) type of primordia (*contra* Akiyama and Nishimura, 1993a) as do *Bescherellia* and *Cyrtopus* (*contra* Akiyama, 1990a) and *Racopilum*. The other pleurocarps in Clade A, *Mesochaete*, *Franciella* (Fig. 3) and *Spiridens* have naked (*Bryum*) branch primordia. Therefore, the Clade A pleurocarps

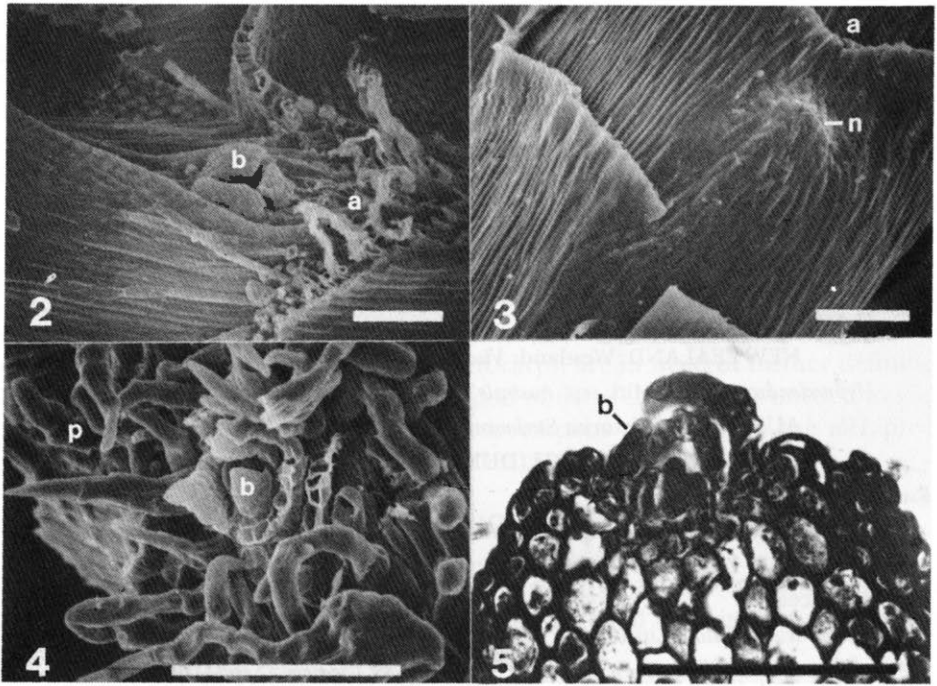


Fig. 2-5. Branch primordia. - 2. *Pyrrhobryum mnioides*, Vitt 29831; SEM of axillary bud primordium. - 3. *Franciella spiridentoides*, Withey 753; SEM of naked cauline primordium. - 4. *Cyrtopus setosus*, Glenny 4827; SEM of bud primordium with pseudoparaphyllia. - 5. *Racopilum convolutaceum*, Glenny 4941; cross-section of bud primordium. b = bud primordium, n = naked primordium, p = pseudoparaphyllia, a = leaf axil. All scale bars = 100 μ m.

are not characterized by a single type of dormant primordium. Both naked and bud types of primordia have also been recorded for the pleurocarpous mosses of the order Leucodontales (Akiyama, 1990a).

Location of branch primordia

The branch bud of mosses develops from the same segment or merophyte of the apical meristem as the leaf immediately above it (Koponen, 1968; Lorch, 1931; Mishler and De Luna, 1991). This developmental relationship is commonly obscured by later differential growth, and the branch primordium is displaced to a cauline position or to the axil of the older leaf below. A distinction can be made between plants that have branch primordia situated in the axils of subtending leaves, and those that have primordia in cauline positions between leaf insertions. Buck and Vitt (1986) suggested that the axillary placement of branch primordia distinguishes the pleurocarps of the order Bryales (Rhizogoniaceae, Spiriden-

taceae, Racopilaceae, Helicophyllaceae and Hypopterigiaceae, in their estimation) as an independently derived group. According to Hedenäs (1994), however, many other pleurocarps also have branch primordia in the axils of leaves. Although there is some variation in the degree to which a primordium can be interpreted as axillary, the primordia of all Clade A pleurocarps could arguably be deemed axillary (Fig. 2) with the exception of those of the Spiridentaceae. The mosses of the Spiridentaceae have branch primordia that are clearly not axillary, but located directly beneath the center of the line of insertion of the leaf immediately above (Fig. 3). Therefore, the pleurocarps of Clade A cannot be characterized by an axillary placement of branch primordia, and cannot be distinguished from other pleurocarpous lineages on the basis of this character.

Pseudoparaphyllia

Considerable literature has been devoted to defining and describing pseudoparaphyllia (Akiyama, 1990a,b; Akiyama and Nishimura, 1993a,b; Allen, 1987; Berthier, 1972; Buck, 1984; Ireland, 1971; Schofield and Héban, 1984; Schofield and Thompson, 1966). Pseudoparaphyllia, in contrast to paraphyllia, are small appendages that are only found surrounding branch primordia. According to some researchers, pseudoparaphyllia originate from cells of the main stem; *i.e.*, they are not derivatives of the apical cell of the developing branch primordium (Buck, 1984; Schofield and Héban, 1984; Schofield and Thompson, 1966). Therefore, unlike the juvenile leaves of a branch primordium, pseudoparaphyllia would not be expected to have axillary hairs. It has also been suggested that pseudoparaphyllia are homologous to the macronemata found around the branch primordia in some acrocarpous mosses (Berthier, 1972; Schofield and Héban, 1984). In contrast, Akiyama (1993b) suggested that regardless of their apparent position, both filamentous and foliose pseudoparaphyllia are actually derived from the apical cell of the branch primordium. Therefore, they could be considered juvenile leaves of the primordium. More extensive developmental studies of branch initiation patterns would help to clarify this issue. For purposes of this paper, I have chosen to define pseudoparaphyllia as appendages attached to the epidermal stem cells around a branch primordium. Therefore, when stems are viewed in cross-section, pseudoparaphyllia are not associated with the apparent derivatives of the apical cell of the branch primordium and have no axillary hairs. With this definition, foliose pseudoparaphyllia could be distinguished from the juvenile leaves of a dormant branch primordium (when it is the *Climacium* or bud type).

Of the pleurocarpous taxa of Clade A with bud type branch primordia (*Pyrrhobryum*, *Racopilum*, *Hypnodendron*, *Cyrtopus* and *Bescherellia*) only the Cyrtopodaceae were found to have pseudoparaphyllia (Fig. 4). In *Pyrrhobryum*, *Racopilum* (Fig. 5) and *Hypnodendron*, all of the leaf-like structures were associated with the obvious derivatives of the apical cell of the branch primordium and not the epidermal cells of the stem. According to Touw (1971), *Hypnodendron* has pseudoparaphyllia

that are inconspicuous, adpressed, and in some cases, missing. However, no pseudoparaphyllia were observed on the two species of *Hypnodendron* studied here.

As Buck and Vitt (1986) circumscribed the Bryales, including the Spiridentaceae, Rhizogoniaceae, and Racopilaceae, all taxa lacked pseudoparaphyllia. However, with the Cyrtopodaceae included among the taxa of Clade A, the absence of pseudoparaphyllia cannot be used to characterize the pleurocarps of that clade.

DISCUSSION

The characters in this study were not observed from a developmental perspective. In the future, a more thorough examination of the ontogeny of branch formation in mosses would be beneficial. Crandall-Stotler (1972) was able to identify eleven types of branch ontogeny patterns in leafy hepatics. Preliminary work on the pleurocarps of Clade A indicates the existence of at least three types of branch ontogeny patterns in this group alone (Fig. 2-4). Frey (1974; reviewed by Hedenäs, 1994) studied branch development in *Hypnodendron dendroides* and reported that the cell division process of the branch apical cell was more similar to that found in acrocarpous mosses than in other pleurocarpous mosses. This information may prove meaningful once it is combined with other comparative studies and put into a phylogenetic framework.

Because none of the characters associated with pleurocarpous branch primordia distinguish the Clade A pleurocarps from those of Clade B, the question remains whether there are any other characters that might do so. Researchers (Frey, 1974; Hedenäs, 1989, 1994; Kawai, 1968; Koponen, 1982) have suggested that pleurocarpous lineages may differ in the presence or absence of guide cells in their costae. All of the Clade A taxa do have costae with guide cells (Withey, unpubl. data), unlike a majority of other pleurocarps. If there are additional Clade A pleurocarps erroneously incorporated in other pleurocarpous orders (Leucodontales, Hypnales, Hookeriales) they might be identified initially by the presence of guide cells in their costae. Examples may, perhaps, include *Pterobryella*, *Rutenbergia*, *Trachypodopsis* and *Prionodon* (character states verified in Newton, 1993). The Clade A lineage of pleurocarps may also have a biogeographic component; all taxa in the clade (as presently constituted) are entirely or predominantly Australasian.

As the characters of the branch primordia discussed in this paper are variable among the Clade A taxa, they have been coded as cladistic characters and incorporated into a larger morphological analysis (Withey, unpubl. data). An independent morphological data set supporting the topology of the Clade A taxa (acrocarps as well as pleurocarps) would lend credence to the hypothesis of relationships resulting from cladistic analysis of the *rbcl* data.

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