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Morphology and development of leaf papillae in the Pilotrichaceae

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ABSTRACT
Sporophyte morphology has been traditionally used in taxonomic studies of pleurocarpous mosses. However, the gametophyte morphology of the Pilotrichaceae still holds interesting and under-investigated characters. Here, we conducted an in-depth investigation of the morphology and development of Pilotrichaceae leaf papillae and assessed their usefulness in inferring the evolutionary history of the family. Specimens were observed under scanning electron microscopy, and we identified seven morphological types of papillae, as well as two different pathways of development. Our results suggest that papilla morphology and development are phylogenetically informative in the Pilotrichaceae.

Key words: Gametophyte, Hookeriales, pleurocarpous moss, outgrowths, scanning electron microscopy

Introduction

The Pilotrichaceae is the most diverse family within the Hookeriales, comprising 21 genera and approximately 420 species worldwide (Goffinet et al. 2009; Crosby et al. 2000). The family is distributed throughout the tropics but is particularly diverse in the neotropics, whereas there are approximately 200 species, representing all 21 genera, of which 15 are endemic (Gradstein et al. 2001).

Variation in sporophytic traits is central to most taxonomic concepts within the Pilotrichaceae (Brotherus 1925; Crosby 1974; Crum 1984; Buck & Goffinet 2000). Phylogenetic inferences (Buck et al. 2005), however, suggest that several genera, as circumscribed today, are polyphyletic: Brymela Crosby & B.H. Allen, Hypnella (Müll. Hal.) A. Jaeger; Lepidopilidium (Müll. Hal.) Broth.; Lepidopilum (Brid.) Brid.; Stenodictyon A. Jaeger ex M. Fleisch.; and Trachyxiphium W.R. Buck. The polyphyletic nature of those genera suggests that their diagnostic traits are homoplasious.

Sporophyte morphology has traditionally been used to delimitate certain taxa among the pleurocarpous mosses (Hedenäs 1996; Buck 1998; Buck et al. 2005), as observed in the Sematophyllaceae genera Paranapiacabaea W.R. Buck & D.M. Vital and Donnellia Austin, as well as in the Pilotrichaceae genera Lepidopilum (Brid.) Brid. and Lepidopilidium (Müll Hal.) Broth. However, sporophytes are highly susceptible to environmental alterations (Hedenäs 2007) and may not be as highly conserved as are gametophytes (Câmara & Buck 2012). In some groups, gametophyte morphology has been found to show better congruence with molecular data (Gardiner et al. 2005; Hedenäs 2007; Câmara & Kellogg 2010) and gametophyte morphology of pleurocarpous mosses remains a promising source of taxonomically distinctive features, such as leaf morphology (Buck 2007; Câmara & Kellogg 2010).

Papillae and mammillae are cell ornamentations present in many mosses. These protuberances are distinguished by their nature (Magill 1990): solid cell wall thickening (papillae) versus hollow projections without wall thickening (mammillae). The shape, location, development and number of papillae per cell are not easily visualized under light microscopy and may in fact often be misinterpreted as the C-shaped papillae observed in Syntrichia ruralis (Hedw.) F. Weber & D. Mohr, which reflects optical distortions in light microscopy (Robinson 1971; Cano 1994). Within the Pilotrichaceae, light microscopy reveals mostly whether papillae are present or absent, a trait used in distinguishing among various genera and species (Buck 1998; Vaz-Imbassahy et al. 2008). Other features such as shape, size and precise location of the papillae are virtually impossible to assess under light microscopy, and, to our knowledge, there have been no scanning electron microscopy (SEM) studies characterizing papillae diversity in the Pilotrichaceae.

Leaf papilla morphology in pleurocarpous mosses has been investigated under SEM only in the Sematophyllaceae (Câmara & Kellogg 2010). In acrocarpous mosses, such studies are more common, for example, in the Fissidentaceae (Belin et al. 1974) and Polytrichaceae (Atrichum P. Beauv. by Ireland (1991) and Pottiaceae (Werner et al. 2003). In such studies, SEM has revealed important taxonomic features, such as papillae shape, size and origin. Current observations of papillae within Pilotrichaceae are mostly
restricted to presence or absence, number per cell (unipapillose or pluripapillose) and location on the leaf (apex or base). In the present study, we use SEM to investigate further papillae development and morphology across genera in Pilotrichaceae and a published phylogeny (Buck et al. 2005) to determine the possibility of using it to infer the evolutionary history of the family.

**Material and methods**

**Taxon sampling**

We sampled at least two species of every papillose genus of the Pilotrichaceae. For Hypnella diversifolia (Mitt.) A. Jaeger, three specimens were sampled because the material is readily accessible in herbaria in Brazil. Two species of Cyclodictyon Mitt. (a non-papillose genus) were chosen at random among those studied by Buck et al. (2005) as a control to confirm papilla absence under SEM. We also investigated Hypnella pilifera (Hook. f. & Wilson) A. Jaeger, although it was not sampled in Buck et al. (2005), because it is a very common species with strongly papillose laminar cells. Overall, we studied the following species and specimens: Callicostella colombica R. S. Williams (Colombia, Churchill et al.; no. 17104 at the William and Lynda Steere Herbarium of the New York Botanical Garden [code, NY]); Callicostella pallida (Hornsch.) Ångstr. (Brazil, Soares; no. 544 at the Herbarium of the University of Brasilia [code, UB]); Callicostella pallida (Hornsch.) Ångstr. (Surinam, Allen; no. 25421 at NY); Callistostellopsis meridensis (Müll. Hal.) Broth. (Venezuela, Griffin & López; no. PV-89 at NY); Cyclodictyon albicans (Hedw.) Kuntze (Panama, Folsom; no. 4336 at NY); Cyclodictyon roridum (Hampe) Kuntze, (Ecuador, Crozet; no. 49416 at NY); Hypnella pallescens (Hook.) A. Jaeger (Puerto Rico, Reese; no. 14544 at NY); H. pilifera (Hook. f. & Wilson) A. Jaeger (Brazil, Vital & Buck; no. 19997 at NY); H. diversifolia (Mitt.) A. Jaeger (Ecuador, Steere; no. 27673 at NY); H. diversifolia (Mitt.) A. Jaeger (Colombia, Churchill et al.; no. 18335 at the Herbarium of the Botanical Institute of São Paulo [code, SP]); H. diversifolia (Mitt.) A. Jaeger, (Costa Rica, Crosby; no. 5914 at SP); H. diversifolia (Mitt.) A. Jaeger (Colombia, Churchill et al.; no. 14981 at SP); Pilotrachidium antillarum Besch. (Guadeloupe, Duss; no. 1045 at NY); and P. callistostatum (Müll. Hal.) A. Jaeger (Colombia, Ramos et al.; no. 1447 at NY).

**Preparation of material**

For each plant, 15-20 lateral and dorsal leaves were taken from branches. As in the pleurocarps, the branches are always generating new leaves, so they were removed from the tip (younger leaves) all the way to the base (older leaves). Samples were prepared according to Bozolla & Russel (1998) and Câmara & Kellogg (2010), although with a more gradual dehydration series, in 5% increments, at the same time intervals, between 50% and 100% followed by two final steps at 100% and 100% in plants with thinner cell walls. Plants were submitted to critical point drying using a Denton DCP-1 dryer (Denton Vacuum LLC, Moorestown, NJ USA) and a Bal-Tec CPD 030 dryer (Bal-Tec AG, Balzers, Liechtenstein), with liquid CO2 as the transition fluid. After drying, leaves were mounted on stubs and sputter-coated with gold and gold-palladium layers using two sputter coaters: a Hummer 6.2 (Anatech, Union City, CA, USA) and a Bal-Tec SCD 050 (Bal-Tec AG). The sputter-coated specimens were kept in a dessicator containing silica gel before SEM visualization. The samples were observed and photomicrographs were taken with JSM-840 A SM and JSM-5410 LV scanning electron microscopes (JEOL, Tokyo, Japan) at 15 kV and at a working distance of 10 mm. All cell measurements were taken from mature dorsal and lateral leaves, and at least 10 measurements of papilla dimensions were made for each specimen. Twenty-five papillose cells for each individual were also measured under SEM (Tab. 1).

**Results and discussion**

In the Pilotrichaceae genera evaluated, papillae were found only on abaxial leaf surfaces. Basipetal papilla development along the leaf was observed in all species, with younger papillae at the leaf base and mature papillae in the apical region. In addition, the papillae remained prominent even when cells collapsed due to specimen preparation, suggesting that papillae in Pilotrichaceae are solid and created by thickening of cell walls. Pluripapillose taxa had cells that were five times longer than they were wide, whereas those of the unipapillose taxa were irregularly isodiametric (Tab. 1). Seven distinct kinds of mature papillae occur and can be referred to as cylindrical, denticulate, filiform, forked, rounded, semi-conical and stellate (Fig. 1-3). These types can be distinguished as follows:

**Cylindrical papillae** are simple (not branched) protuberances that are elongate and acute, 1.5-4 μm in diameter at the base and 4-6 μm long. These papillae have "striae" that are vertical in relation to the papilla length and occur as

| Table 1. Dimensions of cells with different types of papillae. The lengths and widths are averages of 25 measurements made under scanning electron microscopy in the same region of a mature leaf. |
|-----------------|-----------------|-----------------|-----------------|
| Papilla type    | Length, μm      | Width, μm       | Length/Width    |
| Cylindrical*    | 23.9            | 10.3            | 2.3             |
| Forked          | 45.5            | 5.0             | 9.1             |
| Filiform*       | 25.5            | 5.0             | 5.1             |
| Rounded*        | 7.0             | 7.0             | 1.0             |
| Semi-conical*   | 13.3            | 13.3            | 1.0             |
| Denticulate     | 14.9            | 6.7             | 2.2             |
| Stellate*       | 64.0            | 5.0             | 12.8            |

*Unipapillose taxa.
Forked and stellate papillae have the same initial phase, with the same pattern of division when they emerge on the cell. Although both are branched when mature, they differ in that the former has two regular branches and the latter has multiple irregular branches. This development pattern was also observed by Mishler (1987) in Syntrichia papillosissima (Copp.) Loeske, as well as in Tortula papillosissima (Copp.) Broth., which presents a mature morphology similar to that of the stellate papillae. The baggy papillae observed in Taxithelium planum (Brid.) Mitt. by Câmara and Kellogg (2010) have the same kind of division found in forked and stellate papilla but do not develop into branched papillae. The filiform papillae seen in Hypnella pilifera and the baggy papillae seen in Taxithelium nepalense (Schwägr.) Broth. (Câmara & Kellogg 2010) show the same initial phase, emerging initially as a line from the cell lumen, occupying almost the entire cell width. However, in the mature form, they are distinct, the papillae being hair-like in the former and rounded in the latter.

According to Buck (1998), Callicostella colombica has a toothed margin and smooth cells. In the present study, the cells were indeed smooth under SEM, and the denticulate margin was formed by two papillae at the apex of apical and marginal cells (denticulate papillae), as shown in Fig. 3F. In order to help assess the phylogenetic significance of papilla types, we mapped them on the phylogenetic tree devised by Buck et al. (2005). The Pilotrichidaceae are divided in ten clades (Fig. 4), informally designated Pilotrichum, Hypnella, Thamniosis, Pilotrichidium, Callicostellopsis, Brymela, Lepidopilum, Trachyxiphium, Callicostella and Cyclodictyon. The Pilotrichum, Thamniosis, Brymela, Lepidopilum, Trachyxiphium and Cyclodictyon clades do not contain any papilla-bearing taxa.

The Hypnella clade (Fig. 4) is represented by one species, Hypnella pallescens, which differs from all other investigated taxa by its stellate papilla. Hypnella is traditionally recognized by its seriate papilla observed under light microscopy, as confirmed here by SEM. However, the genus is not monophyletic (Buck et al. 2005), and H. pallescens and H. diversifolia belong to two distinct clades (Fig. 4). The papillae in these species start out as simple protuberances but differ at maturity (becoming stellate and forked papillae, respectively), which suggests polyphyly of the genus. In addition, H. pilifera differed from the other two species in terms of papilla development and shape (filiform), suggesting that H. pilifera does not belong with the other two. Unfortunately, H. pilifera has yet to be sampled in phylogenetic studies.

The Pilotrichidium clade (Fig. 4) is composed of eight species: Brymela tutezona Crosby & B.H. Allen; Trachyxiphium vagum (Mitt.) W.R. Buck; Hypnella diversifolia; Thamniosis pendula (Hook.) M. Fleish.; Hemiragis aurea (Lam. ex Brid.) Kindb.; Thamniosis cruegeriana (Müll. Hal.) W.R. Buck; Pilotrichidium antillarum; and P. callicostatum. Only three species exhibit papillae: H. diversifolia, P. antillarum and P. callicostatum. The species P. antillarum and P. callicostatum, which revealed the monophyly of the genus, have the same kind of papilla, differing only...
in location (P. antillarum has papilla only in cells of the extreme apex and P. callicostatum has papillae over the entire surface of the leaf), representing a synapomorphy for the genus. However, although H. diversifolia also presents papillae, they are different in morphology and development from those of Pilotrichidium Besch., a trait suggestive of homoplasy.

The Callicostellopsis clade (Fig. 4) is composed only of Callicostellopsis meridensis. The genus is unspecific and has papillae that are distinct from those of all other taxa investigated. Callicostellopsis meridensis is the sister group to a clade comprising several genera with smooth cells (Fig. 4). The unique shape of the papillae suggests an autapomorphy.

The Callicostella clade (Fig. 4) contains only two species in the tree, as Buck et al. (2005) made a new combination, transferring C. diatomophila (Mull. Hal.) M. Fleisch. to Diploneuron E.B. Bartram. Only two of the 97 Callicostella species (C. colombica and C. pallida) were sampled from this clade, indicating the existence of at least a monophyletic core in the genus. This clade contains plants with denticulate papilla, which may be considered a synapomorphy for the genus. Callicostella pallida still exhibits one more kind of papilla (semi-conical papilla), similar to those of most species of the genus. However, Callicostella is a genus with a large number of species and it is necessary to increase the number of taxa sampled in order to clarify the evolutionary history of the group.

Within the monophyletic papillose genera sampled by Buck et al. (2005)—Callicostella and Pilotrichidium—the development and morphology of the papillae were similar, suggesting synapomorphies for the groups. In the polyphyletic genus Hypnella, the development and morphology of papillae was found to differ among species, indicating that the papillae in these species may have appeared indepen-
dently throughout the evolution of the group, suggesting autapomorphies. In Callicostellopsis, the unique shape of the papillae also distinguished it from all other clades. Therefore, our study showed that the papillae may be informative of the evolutionary history of the papillose genera within the family Pilotrichaceae, suggesting the previous use of what now appear to be non-informative features to delimit taxa, may help explain the large polyphyletic nature of the taxa shown by Buck et al. (2005).

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References


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