

## Farina Production by Gametophytes of *Argyrochosma nivea* (Poir.) Windham (Pteridaceae) and its Implications for Cheilanthoid Phylogeny

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**ABSTRACT.**—Modern molecular phylogenetic studies of the Pteridaceae have recognized a well supported cheilanthoid clade that includes four major subclades: myriopteroids, pellaoids, hemionitidoids and notholaenoids. Many of the morphological characters used in delimitation of the cheilanthoid lineages and genera appear to be the result of convergent evolution, a result of adaptation to xeric environments. Faced with the apparent lack of sporophytic synapomorphies for the cheilanthoid subclades, farina production by gametophytes has been proposed as a character of possible phylogenetic utility. All the notholaenoid species observed to date produce farina in their gametophytes, but species of the other cheilanthoid clades (pellaoids, hemionitidoids, and myriopteroids) do not. In this work we provide the first account of farina production in the gametophyte of a non-notholaenoid: two accessions of *Argyrochosma nivea* from different geographical localities were found to have farina on their gametophytes, suggesting that this gametophytic character is not a synapomorphy for the notholaenoids, and may have had several independent evolutionary origins.

**KEY WORDS.**—Pteridaceae, cheilanthoids, *Argyrochosma*, farina, gametophyte, *Notholaena*

Pteridaceae is a large family with ca. 50 genera and about 1000 species. In its modern conception it is monophyletic and includes, among others, the Adiantaceae and Vittariaceae (Smith *et al.*, 2008). However, internal relationships within the family and circumscription of many genera are still under review. Based on recent molecular analyses, five monophyletic groups can be recognized: cryptogrammoides, ceratopteridoids, pteridoids, cheilanthoids and adiantoids (Schuettpelz *et al.*, 2007; Schuettpelz and Pryer, 2008; Smith *et al.*, 2008). Cheilanthoid ferns comprise around 400 species, and form a very well supported clade (Gastony and Rollo, 1995; Schuettpelz and Pryer, 2007). Within this group, molecular studies suggest the existence of four major clades (Fig. 1): myriopteroids, pellaoids, hemionitidoids, and notholaenoids (Rothfels *et al.*, 2008; Windham *et al.*, 2009). Cheilanthoid taxonomy is particularly difficult, due to their great morphological diversity (for example, in frond architecture and presence of pseudoindusium) and to convergent evolution resulting from their adaptation to arid environments (Rothfels *et al.*, 2008). In this last sense, one of the most confusing characters is the presence of farina in the sporophytes. Farina is a white or yellow powder, produced by glandular hairs and of variable composition, but often containing flavonoids. Farina is produced by sporophytes of a number of species within each of these cheilanthoid subclades, and is thus a homoplastic character.

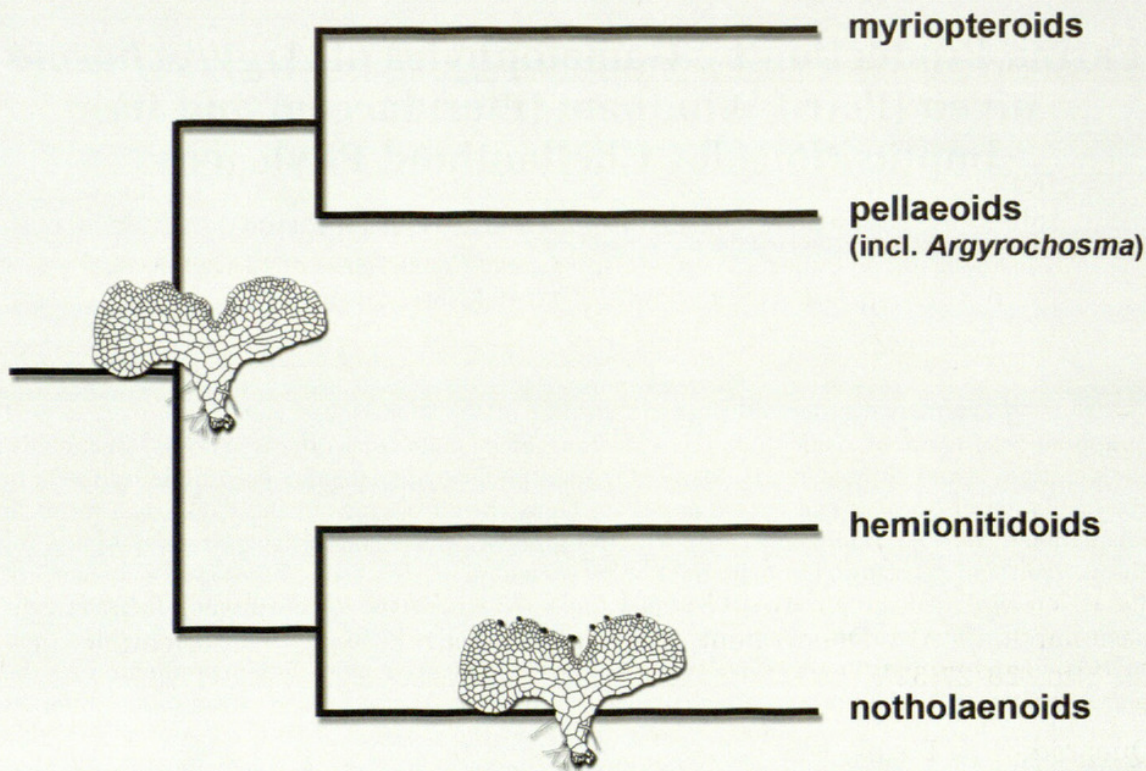


FIG. 1. Simplified to-date phylogenetic scheme of cheilanthoid clade (Pteridaceae), showing the position of *Argyrochosma* within the pellaeoids. A hypothetical history of gametophyte evolution has been represented over this tree: non-farinose gametophyte is the ancestral condition, shared by all the lineages except the notholaenoids.

Some morphological characters, however, may function as synapomorphies for some of the cheilanthoid lineages. One of these potential synapomorphies is the production of farina by the gametophyte (Rothfels *et al.*, 2008). All notholaenoid species observed to date produce farina on their gametophytes, but those of the other cheilanthoid subclades (pellaeoids, hemionitidoids, myriopteroids) do not (Fig. 1). Thus, the presence of farina in the gametophyte is potentially an important feature for defining the notholaenoid group. This is very interesting, given the traditional lack of attention paid by taxonomists to gametophytic characters, a fact that is likely attributable to their reduced levels of morphological variation.

*Argyrochosma* is a New World genus, segregated from *Notholaena* R. Brown some decades ago (Windham, 1987), and comprises around 16 species (Sigel *et al.*, 2011). *Argyrochosma nivea* (Poir.) Windham consists of a complex of three mainly apogamous taxa, considered as varieties: two of them, var. *nivea* and var. *flava* (Hook.) Ponce, produce farina on their sporophytes, and the other, var. *tenera* (Gilles ex Hook.) Ponce, has glabrous (non-farinose) sporophytes (Sigel *et al.*, 2011).

The haploid phase of *A. nivea* var. *nivea* is very well known, from studies on spore morphology (Morbelli *et al.*, 2001), spore germination and gametophyte development (Gabriel y Galán, 2011) and reproduction via apogamy (Woronin,

1907; Gabriel y Galán, 2011). These studies report the gametophyte of *A. nivea* as non-farinose, in agreement with the pellaoid gametophyte concept described above.

Nevertheless, as relatively few cheilanthoid gametophytes have been studied, more effort is required before accepting definitively the synapomorphic status of gametophytic farina for the notholaenoids. With this in mind, and within a broader framework of observing morphology, development, and reproduction of Pteridaceae gametophytes, the goal of the current study was to determine if other *A. nivea* varieties besides var. *nivea* also produce non-farinose gametophytes.

#### MATERIAL AND METHODS

Samples of *Argyroschisma* used in this study were collected from the following locations: *Argyroschisma nivea* var. *nivea*: Peru, Cuzco Department, Urubamba Province, Ollantaytambo, beyond Huilloq, 13°14'50.5"S 72°15'28.8"W, 2920 m, in rocks, JM Gabriel y Galán, 28-04-2008. Argentina, Catamarca, El Alto Department, Sierra de Ancasti, between El Portezuelo and El Alto, 28°27'52"S 65°35'10"W, 1855 m, granite rocks, Prada, 16-09-2010. *Argyroschisma nivea* var. *flava*: Argentina, Córdoba, San Alberto Department, Quebrada Los Pozos, dique La Viña, 31°52'24"S 65°01'54"W, 910 m, granite rocks, Prada, 17-09-2010. Material was identified following local floras (Tryon and Stolze, 1989; de la Sota *et al.*, 2009), and is deposited in the Herbarium of the Real Jardín Botánico de Madrid (MA).

Spore samples for cultures were taken from two different sporophytes at each location and kept dry at room temperature. Multispore cultures on mineral agar medium (Dyer, 1979) were established by manually removing spores from fertile pinnae on a weigh paper, and placing them in Petri dishes 6 cm in diameter. The sowing of each sample was replicated three times. Gametophytes were grown under fluorescent light on a 12-h light, 12-h dark cycle at  $20 \pm 2^\circ\text{C}$ , for c. 8 months. At different stages of maturity, random gametophyte samples of each culture were stained with chloral hydrate acetocarmine (Edwards and Miller, 1972). Fixed and *in vivo* materials were mounted in water and observed under a light microscope. In total, c. 150 gametophytes were assayed, c. 50 of each of the three samples (two of var. *nivea* and one of var. *flava*).

#### RESULTS

Both varieties of *Argyroschisma nivea* produced cordate gametophytes following the *Vittaria* type of development, although a notable number of prothalli acquired an irregular shape. Because description of the development and complete morphological features of the gametophytes exceeds the objectives of this work, we will focus specifically on farina production.

Gametophytes of *A. nivea* var. *flava* remained glabrous throughout the observational period. In contrast, some of the gametophytes of *A. nivea* var. *nivea*, about 20% of the total observed, produced short glandular hairs along their margin, which produced farina with time (Fig. 2). With very few

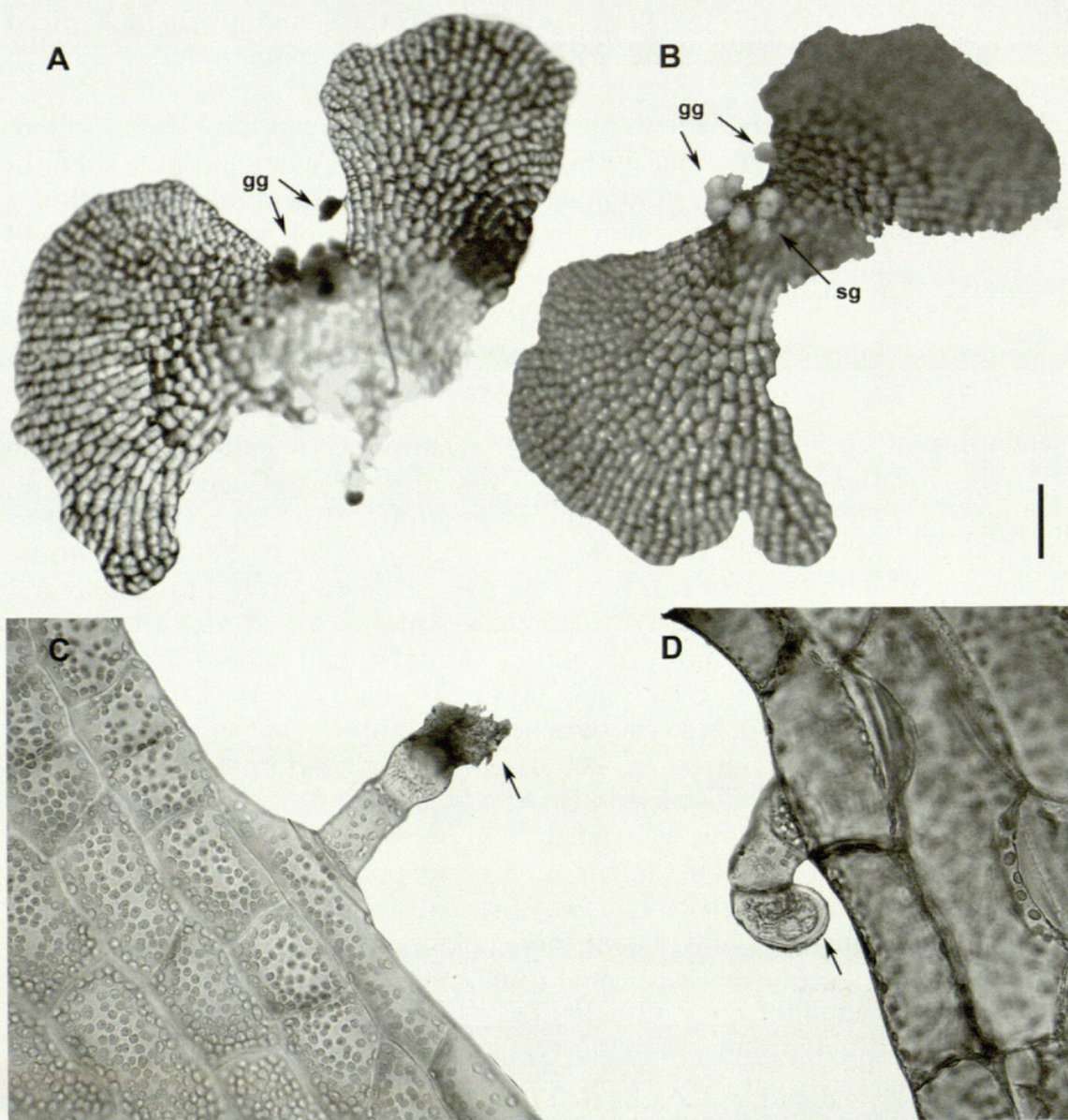


FIG. 2. Farina production in gametophytes of *Argyrochosma nivea* var *nivea*. A–B: gametophytes with glandular hairs and white farina, under transmitted light binocular microscope (A) and under incident light binocular microscope (B); gg=gametophyte glands; sg= apogamous sporophyte glands. C: detail of a glandular hair in the margin of gametophyte, with the farina secretion (arrow). D: detail of a glandular hair with the farina washed, showing the apical gland (arrow). Bar= 0.12 cm in A–B; 20  $\mu$ m in C; 14  $\mu$ m in D.

exceptions, these hairy gametophytes were those with cordate shape. The morphology of the hairs was constant in all the gametophytes: two cells, a more-or-less elongated basal one and a capitate secretory apical one. The hairs appeared more abundant near the apical notch, but they also spread towards the wings.

These marginal gametophytic trichomes could be detected towards maturity of the gametophytes, which occurred 30–40 days after sowing. Their

emergence coincided in time with the first visible indications of an apogamous sporophyte, which was the production under the notch of 2–5 very long hairs that finally reached up to 40 cells. These hairs associated with the apogamous sporophyte were very different to the ones of the gametophyte. All the gametophytes that produced sporophytes (about 75% of the total observed) also formed these long hairs associated with the sporophytes, but not all produced short marginal ones. Secretion of white farina by the marginal gametophytic hairs began within a few days following their formation. All of the observed marginal hairs produced farina. Sometimes, the long glandular hairs associated to the apogamous sporophyte produced farina also.

While the long glandular hairs elongated and the short hairs began to produce farina, proliferative areas appeared below the notch that gave rise to the new apogamous sporophyte. Some 3–5 days later, a dense cluster of cells emerged from these areas and began to produce 2–6 celled hairs that also secreted farina. Finally, as the new sporophyte elongated and acquired a leafy shape, glandular hairs appeared over its margins and lamina.

#### DISCUSSION

Observations to date of cheilanthoid gametophytes have shown that development of naked, hairless, non-glandular bodies predominates (Nayar and Kaur, 1971; Gabriel y Galán and Prada, 2009). Some gametophytes were known to produce hairs, however (Gabriel y Galán and Prada, 2010), and less frequently, even glandular secretions including farina (Tryon, 1947; Giaucque, 1949; Nayar and Kaur, 1971; Atkinson, 1973).

Following the availability of molecular phylogenies, it became clear that all the glandular farinose gametophytes observed were restricted to members of the notholaenoid lineage (Rothfels *et al.*, 2008). This was then proposed as a synapomorphic character supporting the notholaenoid clade, although with caution, due to the small number of observed gametophytes, (Rothfels *et al.*, 2008; Sigel *et al.*, 2011).

In this work we document for the first time farina production in gametophytes of the pellaeoid species *Argyroschisma nivea*. As far as is known, there are only three previous studies on gametophytes of *Argyroschisma*, one of *A. incana* (C. Presl) Windham (Nayar and Bajpai, 1964) and two of *A. nivea* (Woronin, 1907; Gabriel y Galán, 2011). All of these studies described naked, non-farinous gametophytes. The new observations made in this work report the presence of marginal glandular hairs in some of the gametophytes, which produce white farina at maturity. Timing in farina production is quite delayed in comparison with other known farinous gametophytes, as in some *Notholaena* (Nayar and Kaur, 1971): in *Argyroschisma* the phenomenon seems to occur towards gametophyte maturity. Several characters of the gametophytic farina indicate that it is independent of the sporophytic farina, including hair morphology (2-celled farinous gametophytic hairs vs. up to 40-celled first sporophytic farinous hairs), timing and amount of farina production (marginal gametophytic farina appears days before the proliferative clusters of sporophytes produce signals of farina,

and reduced farina production by the gametophyte compared to the sporophyte is similar to that in some notholaenoid species [Nayar and Kaur, 1971]), and location of farinose cells (marginal hairs develop near the notch but also in the wings). However, gametophytic farina appears identical to that produced by the sporophytes, in both color and texture, as seen under a microscope. Chemical analyses to confirm these observations should be undertaken.

Farina production in gametophytes of *A. nivea* var. *nivea* seems to be facultative, as only some individuals from each of the two different localities showed the character, while the majority remained glabrous. There is no obvious correlation between farina production and ecology by which to explain this facultative nature, as all of the sporophytes collected for this work lived under the same xeric conditions in exposed rock crevices, at more or less similar altitude. It may be that farina production in the gametophyte is triggered by the development of the apogamous sporophyte, because farina production by the gametophytes only occurs at gametophytic maturity. This deserves further investigation, but if this hypothesis is considered, it will be necessary to explain why some gametophytes produced sporophytes but not farina.

Whatever the trigger for farina production may be, its occurrence in gametophytes of *Argyrochosma* is no longer in doubt. The presence of farinose gametophytes in members of the pellaoid subclade of cheilanthoid ferns indicates that this character should no longer be considered a synapomorphy for the notholaenoids. It seems that farina production by gametophytes, like farina production by sporophytes, is a convergent character within cheilanthoids, appearing independently in at least the notholaenoid and pellaoid lineages (Rothfels *et al.*, 2008; Sigel *et al.*, 2011). In addition to observation of more gametophytes from all the Pteridaceae lineages, a more detailed ecological study is necessary in order to determine which variables may influence farina production in gametophytes of *A. nivea*.

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