

BRIEF COMMUNICATION

**UNIQUE EXPRESSION OF A SPOROPHYTIC CHARACTER ON THE
GAMETOPHYTES OF NOTHOLAENID FERNS (PTERIDACEAE)¹**

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- *Premise of the study:* Not all ferns grow in moist, shaded habitats; some lineages thrive in exposed, seasonally dry environments. Notholaenids are a clade of xeric-adapted ferns commonly characterized by the presence of a waxy exudate, called farina, on the undersides of their leaves. Although some other lineages of cheilanthoid ferns also have farinose sporophytes, previous studies suggested that notholaenids are unique in also producing farina on their gametophytes. For this reason, consistent farina expression across life cycle phases has been proposed as a potential synapomorphy for the genus *Notholaena*. Recent phylogenetic studies have shown two species with nonfarinose sporophytes to be nested within *Notholaena*, with a third nonfarinose species well supported as sister to all other notholaenids. This finding raises the question: are the gametophytes of these three species farinose like those of their close relatives, or are they glabrous, consistent with their sporophytes?
- *Methods:* We sowed spores of a diversity of cheilanthoid ferns onto culture media to observe and document whether their gametophytes produced farina. To place these species within a phylogenetic context, we extracted genomic DNA, then amplified and sequenced three plastid loci. The aligned data were analyzed using maximum likelihood to generate a phylogenetic tree.
- *Key results:* Here we show that notholaenids lacking sporophytic farina also lack farina in the gametophytic phase, and notholaenids with sporophytic farina always display gametophytic farina (with a single exception). Outgroup taxa never displayed gametophytic farina, regardless of whether they displayed farina on their sporophytes.
- *Conclusions:* Notholaenids are unique among ferns in consistently expressing farina across both phases of the life cycle.

Key words: alternation of generations; cheilanthoid; *Cheilopteron*; farina; ferns; gametophyte; *Notholaena*; Pteridaceae; taxonomy; xeric habitats.

The Earth's terrestrial ecosystems are dominated by angiosperms, and ferns—a lineage that literally diversified “in the shadow of angiosperms”—are often relegated either to the understory or forest canopy (Schneider et al., 2004). There are, however, several fern lineages that have adapted to more challenging environments. Prominent among these are the cheilanthoid ferns (Pteridaceae), a group of 400+ species concentrated in open, seasonally dry habitats worldwide (Schuettpelz et al., 2007). The exceptional ability of cheilanthoid ferns to flourish in these habitats is due, in part, to sporophytic traits such as hairs, scales, thick cuticles, and waxy exudates that provide protection from intense sunlight and low humidity (Hevly, 1963; Wollenweber, 1984; Koch et al., 2009). Waxy exudates (commonly referred to as farina) occur in several cheilanthoid

lineages, most notably in the genera *Aleuritopteris*, *Argyroschisma*, *Notholaena*, and *Pentagramma* (Wollenweber and Schneider, 2000; Rothfels et al., 2008; Sigel et al., 2011). When present, farina is typically found on the abaxial leaf surface and is thought to protect the plant against overheating and desiccation. Under prolonged drought, the leaves curl up leaving only their highly reflective, farinose undersides exposed (Hevly, 1963; Wollenweber, 1984).

Consensus on generic delimitations within cheilanthoid ferns has so far proven elusive. Current evidence suggests that morphological characters traditionally used for generic circumscription in the group are homoplastic, likely due to extensive morphological convergence resulting from their colonization of extreme habitats (Gastony and Yatskievych, 1992; Rothfels et al., 2008). Although DNA data have been useful in resolving well-supported clades, these clades often do not possess obvious, nonmolecular synapomorphies. As part of an effort to identify morphological characters that differentiate these clades, we here investigate the occurrence of farina on the gametophytes of a broad sampling of cheilanthoid ferns. This trait has been considered a possible synapomorphy of the genus *Notholaena* (sensu Windham, 1993), as it occurs in every species previously sampled (Woronin, 1907; Tryon, 1947; Giauque, 1949; Pray, 1967; Knobloch et al., 1973; Wollenweber, 1984; Windham, 1993) and has not been confirmed in any other clade.

A recent phylogenetic study by Rothfels et al. (2008) sampled most species of *Notholaena*, as well as closely related taxa assigned to *Cheilanthus* and *Cheilopteron*. This study identified a monophyletic “core *Notholaena*” in which all members

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are farinose in the sporophytic phase and a broader notholaenid clade that included several species with nonfarinose sporophytes. Two nonfarinose species, *Notholaena brachypus* and *Cheiloplecton rigidum*, are nested within the genus as traditionally defined, in clades sequentially sister to core *Notholaena*. A third nonfarinose species, *Cheilanthes leucopoda*, is strongly supported as sister to all other notholaenids. With the addition of these three members to the clade, farinose sporophytes no longer serve as an obvious morphological synapomorphy for the notholaenids. Here we focus on whether farina expression in the gametophytic phase may instead be a potential unifying characteristic for the clade. By assaying gametophytes from a broad sampling of cheilanthoids, we can also examine the potential correlation between sporophytic and gametophytic farina production in notholaenid ferns.

MATERIALS AND METHODS

Taxon sampling and spore sowing—Special emphasis was placed on maximizing our sampling within the notholaenid clade and on including species with farinose sporophytes from related clades. Spores were obtained from herbarium specimens of 19 notholaenid species and from nine outgroup taxa (Table 1). All three notholaenids with nonfarinose sporophytes, *Cheilanthes leucopoda*, *Cheiloplecton rigidum*, and *Notholaena brachypus*, were included in the sampling, as were both varieties of *Cheiloplecton rigidum*. Species with farinose sporophytes were selected from both inside and outside of core *Notholaena* to represent as many notholaenid subclades as possible. Unsterilized spores were directly sown onto Hevly's medium (pH 7) in 60 × 15 mm petri plates (Hevly, 1963). The plates were sealed with Parafilm and positioned right-side up with 8 h of fluorescent light per day at approximately 23°C. Light micrographs of developing gametophytes were taken with a Canon EOS Rebel XSi digital camera mounted on a Leica MZ 12.5 dissecting scope. Linear adjustments to contrast and brightness were made using Preview (version 4.2; Softonic, San Francisco, California, USA) to make the presence or absence of farina more evident. Images were then assembled using Adobe (San Jose, California) Illustrator CS3.

Phylogenetic analysis—Our phylogenetic study included 49 taxa (30 notholaenids and 19 outgroup taxa; see Appendix 1) analyzed for three plastid loci, *atpA*, *trnG-R*, and *rbcL*. Ninety-seven sequences were obtained from GenBank and 38 were newly generated for this study; missing data constituted only 8% of the entire matrix (Appendix 1). DNA extraction, amplification, and sequencing followed the protocols of Rothfels et al. (2008). Sequences were manually edited in Sequencher (version 4.10.1) and aligned in MacClade (version 4.07). For each locus alignment, we performed maximum likelihood (ML) bootstrapping in Garli 2.0 (Zwickl, 2006) under a GTR +G +I model, with 500 bootstrap repetitions, each optimized from two random-addition-starting trees. The resulting three consensus trees were visually compared to determine if there was any significant disagreement among loci (conflicting branches with bootstrap values $\geq 70\%$). Because the consensus trees were congruent, the alignments for the three loci were concatenated using the program abioscript (Larsson, 2010), with ambiguous areas of the alignment excluded. The fully concatenated alignment was analyzed in Garli 2.0 (Zwickl, 2006) under a GTR +G +I model. Each locus was given its own partition, with substitution parameters unlinked among partitions. Ten individual searches were performed from different random starting trees. A bootstrap search of 500 replicates was performed on the concatenated data under the above model, but with only two search repetitions per bootstrap data set.

RESULTS

Spores that germinated successfully did so, on average, 1–3 wk after sowing. Gametophytes producing farina (see Fig. 1) typically initiated the process 2–3 wk after germination. Farina production was marked by the development of small, gland-tipped hairs along the margins of the gametophyte, which soon exuded a waxy substance from the terminal gland. As gametophytes

aged, they often produced identical hairs (and waxy exudate) on the upper gametophyte surface. Gametophytic farina appeared white in color, regardless of the color of the farina on its corresponding sporophyte. All notholaenids assayed in this study (Table 1) produced farina in the gametophytic phase except for the following species: *Cheilanthes leucopoda*, *Cheiloplecton rigidum*, *Notholaena aureolina*, *N. brachypus*, and *N. jaliscana*. Gametophytes of the two varieties of *Cheiloplecton rigidum* both lacked farina but differed slightly in gross morphology; the gametophytes of var. *lanceolatum* were relatively smooth along the edges (Fig. 1B), while those of var. *rigidum* were jagged (Fig. 1C). All outgroup species assayed, including those with farinose sporophytes, produced nonfarinose gametophytes (Fig. 1S–Fig. 1AA).

In our analysis of the concatenated plastid DNA data set, nearly all of the branches of the resulting tree were highly supported, with bootstrap values $\geq 70\%$ (Fig. 2). The consensus tree is mostly congruent with that of Rothfels et al. (2008), but includes an expanded sampling of species in core *Notholaena* along with a few outgroup species (e.g., *Cheilanthes bolborrhiza*) not previously analyzed.

DISCUSSION

All land plants share a life cycle that alternates between a diploid sporophytic phase and a haploid gametophytic phase. In ferns and lycophytes, however, these two phases are free-living and completely independent of one another; hence, a detailed knowledge of the morphology and ecology of both phases is critical for a comprehensive understanding of fern biology (Stokey, 1951; Atkinson, 1973). The cryptic but equally important gametophytic phase of the fern life cycle has received renewed and welcome attention in recent years (Watkins, 2008; Watkins et al., 2007), but much remains to be done.

Our study demonstrates, once again, that viable spores are readily available from herbarium specimens (Windham and Hafler, 1986; Windham et al., 1986), gametophytes are inexpensive to cultivate, and important new data can be obtained with relatively little effort. Spores from recent specimens (collected within the last 5 years) hold the most promise, but we were able to germinate spores from one specimen that was more than 20 years old (Fig. 1E, Table 1, *Notholaena aliena*). Spores that germinated almost always did so within 1 month after sowing and sometimes in as little as 1 week.

In our phylogenetic analysis of the plastid DNA data, we recovered a tree topology that is consistent with the previous molecular study of the group by Rothfels et al. (2008). Our sampling differed from theirs in having a greater number of species representing the notholaenid clade, as well as a greater variety of farinose and nonfarinose cheilanthoid outgroups. Our analysis reinforced the finding by Rothfels et al. (2008) that the notholaenids form a well-supported clade (Fig. 2). Additionally, we showed that members of the notholaenid clade (Fig. 2) are consistent in farina production across their life cycle; a species with farinose sporophytes will have farinose gametophytes, and a species with nonfarinose sporophytes will have nonfarinose gametophytes (Fig. 2). The exceptions to this pattern are the sister taxa *Notholaena aureolina* and *N. jaliscana*, which did not display farina in their gametophytes despite having farinose sporophytes (Figs. 1, 2). In this regard, it is interesting to note that the sporophyte of *N. jaliscana* is unique in being only sparsely farinose (see Mickel and Smith, 2004, as *Cheilanthes*

TABLE 1. Taxa of cheilanthoid ferns assayed for gametophytic farina.

Taxon	Cheilanthoid Fern Clade ^a	Provenance	Collector (Voucher Location)	Farinose gametophyte
<i>Aleuritopteris argentea</i> (S. G. Gmelin) Fée	hemionitid	Taiwan: Nantou	Schuettpelz 1059A (DUKE)	No
<i>A. farinosa</i> Fée	hemionitid	Costa Rica: San José	Rothfels et al., 08-021 (DUKE)	No
<i>A. subvillosa</i> (Hook.) Ching	hemionitid	China: Sichuan	Sundue 1029 (DUKE)	No
<i>Cheilanthes bolborhiza</i> Mickel & Beitel	incertae sedis	Mexico: Oaxaca	Rothfels 3294 (DUKE)	No
<i>C. bonariensis</i> (Willd.) Proctor	myriopterid	USA: Arizona	Schuettpelz 501 (DUKE)	No
<i>C. bonariensis</i>	myriopterid	Cultivation: Juniper Level Botanic Garden, NC	Rothfels et al., 2716 (DUKE)	No
<i>C. chinensis</i> (Baker) Domin	hemionitid	China: Guizhou	Zhang 684 (MO)	No
<i>C. distans</i> (R. Br.) Mett.	hemionitid	Cultivation: Juniper Level Botanic Garden, NC	Schuettpelz 320 (DUKE)	No
<i>C. distans</i>	hemionitid	Australia: New South Wales	Nagalingum 23 (DUKE)	No
<i>C. leucopoda</i> Link	notholaenid	USA: Texas	Rothfels 2477 (DUKE)	No
<i>Cheiloplecton rigidum</i> (Sw.) Fée var. <i>lanceolatum</i> C. C. Hall ex Mickel & Beitel	notholaenid	Mexico: Oaxaca	Rothfels 3278 (DUKE)	No
<i>C. rigidum</i> var. <i>lanceolatum</i>	notholaenid	Mexico: Oaxaca	Rothfels 3282 (DUKE)	No
<i>C. rigidum</i> var. <i>rigidum</i>	notholaenid	Mexico: Colima	Rothfels 3205 (DUKE)	No
<i>Notholaena affinis</i> (Mett.) T. Moore	notholaenid (core <i>Notholaena</i>)	Mexico: Querétaro	Labat 2603 (MEXU)	Yes
<i>N. aliena</i> Maxon	notholaenid (core <i>Notholaena</i>)	USA: Texas	Windham and Yatskevych 761 (DUKE)	Yes
<i>N. aschenborniana</i> Klotzsch	notholaenid (core <i>Notholaena</i>)	USA: Arizona	Schuettpelz 476 (DUKE)	Yes
<i>N. aureolina</i> Yatsk. & Arbeláez	notholaenid	Costa Rica: Guanacaste	Rothfels et al., 08-049 (DUKE)	No
<i>N. brachypus</i> (Kunze) J. Sm.	notholaenid	Costa Rica: Guanacaste	Rothfels et al., 08-054 (DUKE)	No
<i>N. brachypus</i>	notholaenid	Mexico: Jalisco	Rothfels 3115B (DUKE)	No
<i>N. brevistipes</i> Mickel	notholaenid (core <i>Notholaena</i>)	Mexico: Tamaulipas	Rothfels 3048 (DUKE)	Yes
<i>N. copelandii</i> C. C. Hall	notholaenid (core <i>Notholaena</i>)	USA: Texas	Rothfels 2478 (DUKE)	Yes
<i>N. copelandii</i>	notholaenid (core <i>Notholaena</i>)	USA: Texas	Rothfels 2481 (DUKE)	Yes
<i>N. galeottii</i> Fée	notholaenid (core <i>Notholaena</i>)	Mexico: Hidalgo	Beck 1119 (DUKE)	Yes
<i>N. jacalsana</i> Yatsk. & Arbeláez	notholaenid (core <i>Notholaena</i>)	Mexico: San Luis Potosí	Rothfels 3053 (DUKE)	Yes
<i>N. jaliscana</i> Yatsk. & Arbeláez	notholaenid (core <i>Notholaena</i>)	Mexico: Nayarit	Rothfels 3118A (DUKE)	No
<i>N. meridionalis</i>	notholaenid (core <i>Notholaena</i>)	Costa Rica: Guanacaste	Rothfels et al., 08-059 (DUKE)	Yes
<i>N. montieliae</i> Yatsk. & Arbeláez	notholaenid (core <i>Notholaena</i>)	Costa Rica: Guanacaste	Rothfels et al., 2646 (DUKE)	Yes
<i>N. nealleyi</i> Seaton ex J. M. Coult.	notholaenid (core <i>Notholaena</i>)	Nicaragua: Madrid	Stevens s.n. (DUKE)	Yes
<i>N. ochracea</i> Yatsk. & Arbeláez	notholaenid (core <i>Notholaena</i>)	USA: Texas	Rothfels 2482 (DUKE)	Yes
<i>N. rosei</i> Maxon	notholaenid (core <i>Notholaena</i>)	Mexico: Mexico	Beck 1230 (DUKE)	Yes
<i>N. standleyi</i> Maxon	notholaenid (core <i>Notholaena</i>)	Mexico: Oaxaca	Windham et al., 542 (DUKE)	Unknown ^b
<i>N. standleyi</i>	notholaenid	USA: Arizona	Rothfels 2519 (DUKE)	Unknown ^b
<i>N. standleyi</i>	notholaenid	USA: Arizona	Rothfels 2520 (DUKE)	Yes
<i>N. sulphurea</i> (Cav.) J. Sm.	notholaenid (core <i>Notholaena</i>)	USA: New Mexico	Alexander 909 (DUKE)	Yes
<i>Paragymnopteris marantae</i> (L.) K. H. Shing	notholaenid (core <i>Notholaena</i>)	Mexico: Querétaro	Rothfels 3419 (DUKE)	Yes
<i>P. marantae</i>	pellaeid	Spain: Canary Islands	Larsson 40 (DUKE)	No
<i>Pentagramma triangularis</i> Goodd.	pellaeid	Spain: Canary Islands	Larsson 54 (DUKE)	No
<i>P. triangularis</i>	hemionitid	USA: California	Rothfels 2934 (DUKE)	No
<i>P. triangularis</i>	hemionitid	USA: California	Rothfels 2938 (DUKE)	No

^a Refer to Fig. 2 for clade assignment.^b Spores did not germinate.

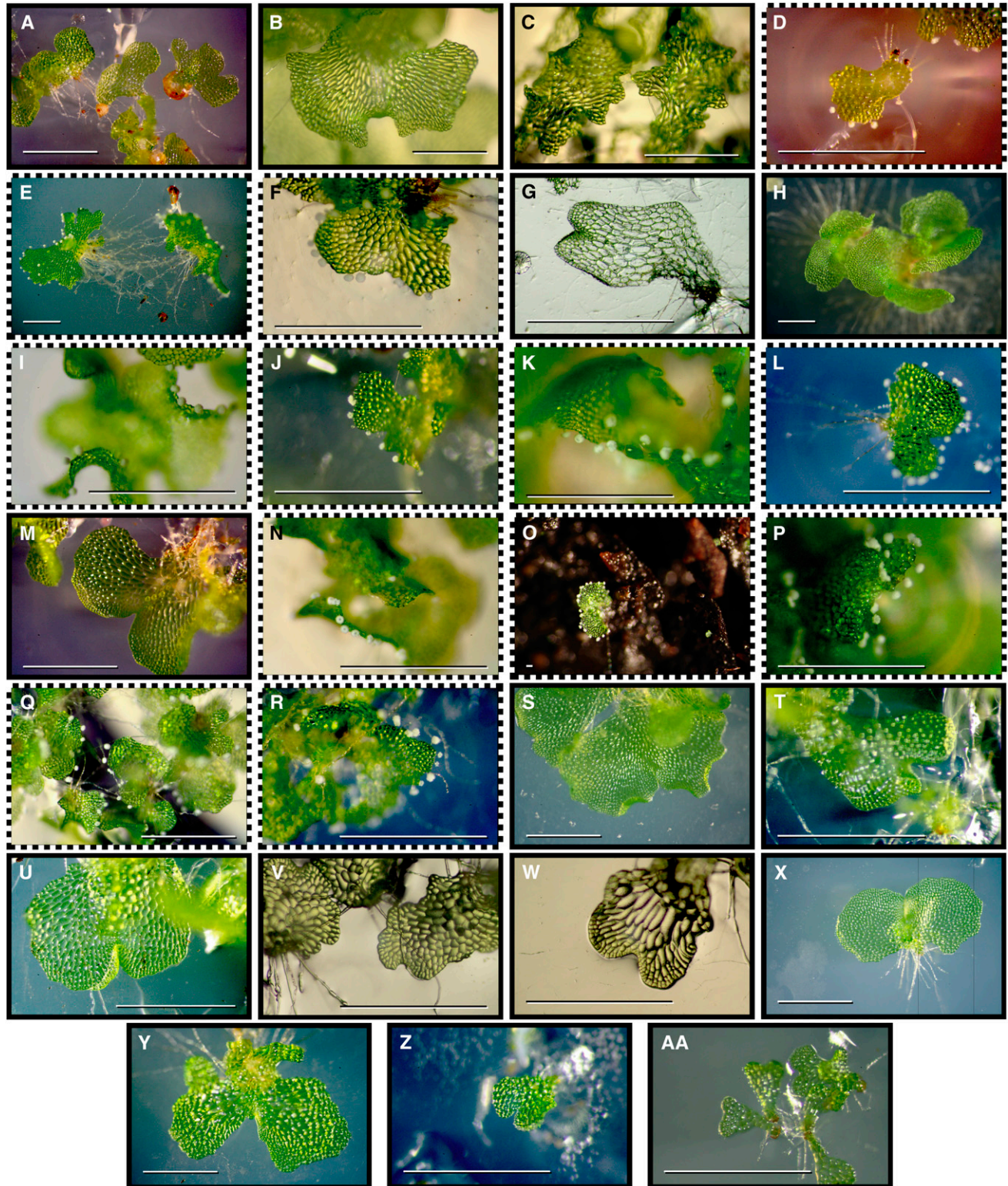


Fig. 1. Micrographs of cheilanthoid fern gametophytes grown for this study. Scale bar on each image = 1 mm. Micrographs outlined with dashed borders indicate farinose gametophytes; those with nonfarinose gametophytes have solid borders. A–R: notholaenids. S–AA: outgroup taxa. A = *Cheilanthes leucopoda*. B = *Cheiloptecton rigidum* var. *lanceolatum*. C = *C. rigidum* var. *rigidum*. D = *Notholaena affinis*. E = *N. aliena*. F = *N. aschenborniana*. G = *N. aureolina*. H = *N. brachypus*. I = *N. brevistipes*. J = *N. copelandii*. K = *N. galeottii*. L = *N. jacalensis*. M = *N. jaliscana*. N = *N. meridionalis*. O = *N. montieliae*. P = *N. nealleyi*. Q = *N. standleyi*. R = *N. sulphurea*. S = *Aleuritopteris argentea*. T = *A. farinosa*. U = *A. subvillosa*. V = *Cheilanthes bonariensis*. W = *C. bolborrhiza*. X = *C. chinensis*. Y = *C. distans*. Z = *Pentagramma triangularis*. AA = *Paragymnopteris marantae*.

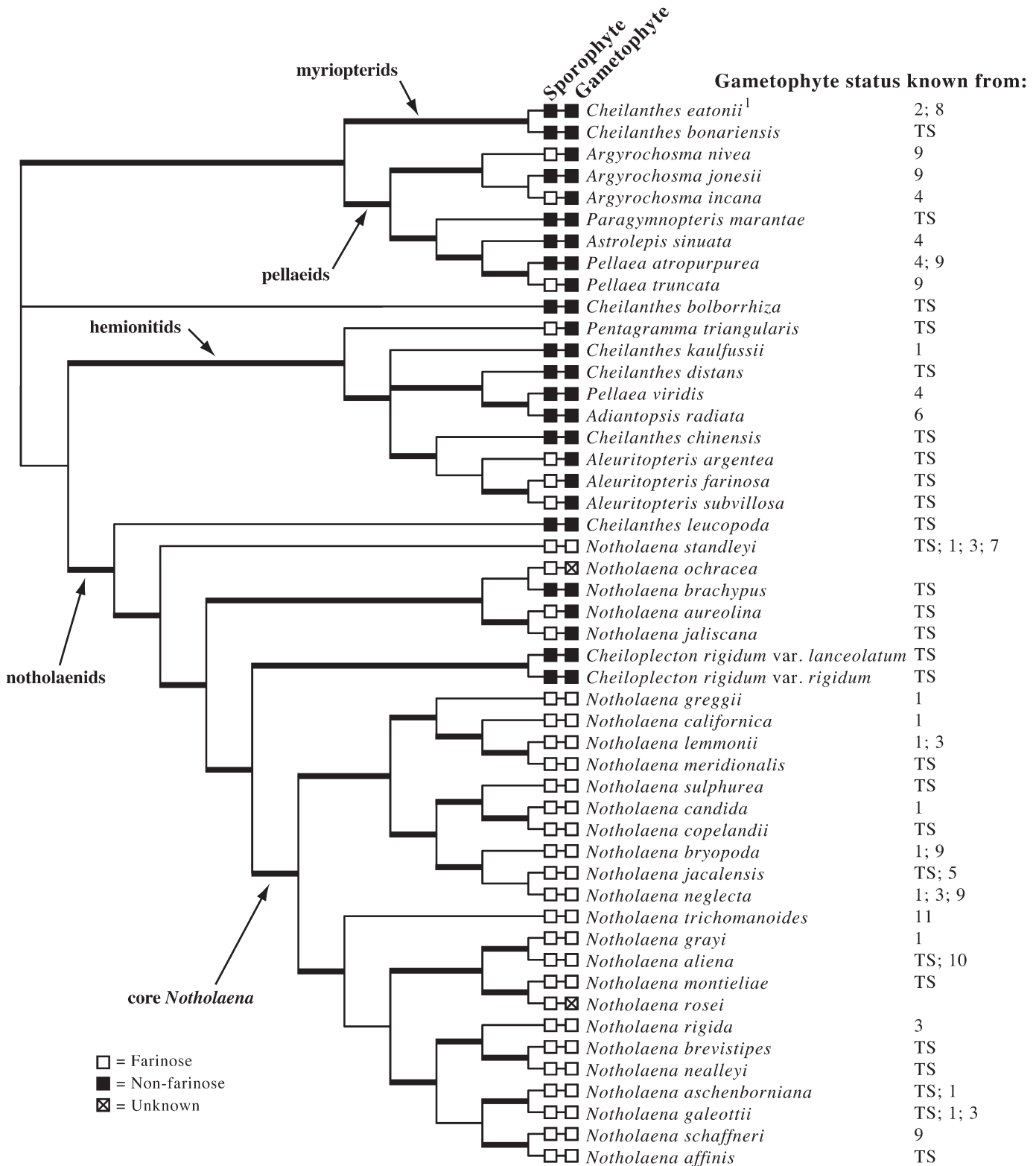


Fig. 2. Maximum likelihood topology for notholaenid ferns resulting from phylogenetic analysis of a concatenated data set of three plastid loci (*atpA*, *trnG-R*, and *rbcL*). The tree is rooted by related cheilantheid clades; clade names follow Windham et al. (2009) and Rothfels et al. (2008). Branches with bootstrap support $\geq 70\%$ are thickened. Branch tips terminate in shaded boxes that indicate the farinose condition observed for the sporophytic and gametophytic phases of that taxon. The source for determining the gametophyte status for each taxon is listed to the right of the figure: TS = cultivated for this study; 1 = Giaouque (1949); 2 = Hitt and Knobloch (1967); 3 = Knobloch et al. (1973); 4 = Nayar and Bajpai (1964); 5 = Pray (1967); 6 = Ranal (1991); 7 = Tryon (1947); 8 = Whittier (1965); 9 = Windham, personal observation; 10 = Wollenweber (1984); 11 = Woronin (1907). ¹Originally identified as *C. tomentosa* in Whittier (1965), later corrected to *C. castanea* (= *C. eatonii*) by Whittier (1970).

aurea var. *palmeri*). The gametophyte of *N. ochracea*, which is closely related to *N. aureolina* and *N. jaliscana* (Fig. 2), has yet to be observed. None of the outgroup species with farinose sporophytes had farinose gametophytes (Fig. 2). Although Giauque (1949) reported occasional wax-exuding glands on the gametophytes of *Aleuritopteris argentea* and *A. farinosa*, the samples of these two species included in our study did not produce farina on their gametophytes.

Our data suggest that the consistent expression of farina-producing trichomes across both phases of the life cycle may represent a synapomorphy for notholaenid ferns. The sharing of trichome traits between sporophytes and gametophytes has been previously reported in a few other fern lineages (e.g., *Thelypteris*; Muñiz-Díaz de León, 2008), but the underlying genetics have not yet been investigated. Our documentation of this potential synapomorphy illustrates the importance of including observations from the gametophytic phase in morphological and ecological studies of ferns, especially for ferns living in similarly extreme environments, where extensive morphological convergence in the sporophytic phase blurs and complicates classification. In a clade that includes 30+ species, only a single potential reversal of this feature was observed, along the branch uniting *Notholaena aureolina* and *N. jaliscana*. We currently lack gametophyte data for two species included in our phylogenetic analysis: *N. rosei*, a member of core *Notholaena*, and *N. ochracea*, a close relative of *N. aureolina* and *N. jaliscana* (Fig. 2). On the basis of patterns observed in the present data set, we predict that the gametophytes of *N. rosei* will prove to be farinose, whereas those of *N. ochracea* will lack farina-producing hairs. The ability to distinguish major lineages of cheilanthoid ferns based on differences in gametophyte morphology might provide an opportunity to conduct crucial in situ studies of gametophyte ecology in xeric-adapted ferns.

In addition to noting simple presence or absence of farina, the chemical composition of farina has proven to be an important phylogenetic character at various taxonomic levels among cheilanthoid ferns (Wollenweber, 1984; Windham 1987; Wollenweber and Schneider, 2000; Sigel et al., 2011). Our observation that the farina produced by gametophytes appears white, regardless of the color of the sporophytic farina, suggests that there may be chemical disparities potentially related to functionality. On sporophytic leaves, the usually dense waxy exudate is thought to “reduce transpiration by its lipophilic nature,” “[reflect] excess irradiation,” and “reduce air movement over the epidermis” (Wollenweber, 1984, p. 4). Nothing is known about the function of the sparse farina observed on gametophytes, which raises an important question: Does farina serve an adaptive function in the gametophyte, or is its presence there simply a byproduct of its occurrence on the sporophyte? A study comparing the genetic mechanisms and adaptive significance of farina production across the fern life cycle would be a major contribution to understanding the ability of cheilanthoid ferns to survive and thrive in xeric environments.

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APPENDIX 1. Vouchers and GenBank accession numbers for taxa used in molecular phylogenetic analysis.

Taxon, *Voucher specimen collector* (Herbarium acronym), Collection locality, Fern DNA database number^a, GenBank accession (with citations, for previously published data) for *rbcL*, *atpA*, *trnG-R* (in that order). A dash (–) indicates missing data.

- Adiantopsis radiata** (L.) Fée, *Christenhusz 4033* (TUR), Guadeloupe: Houelmont, 3313, EF452131 (Schuettpezel et al., 2007), EU268718 (Rothfels et al., 2008), EU268664 (Rothfels et al., 2008); **Aleuritopteris argentea** (S.G. Gmelin) Fée, *Yatskievych 01-23* (MO), China: Beijing, 3734, EF452137 (Schuettpezel et al., 2007), EF452074 (Schuettpezel et al., 2007), EU268665 (Rothfels et al., 2008); **A. farinosa** Fée, *Windham et al. 541* (DUKE), Mexico: Oaxaca, 4057, EU268770 (Rothfels et al., 2008), EU268720 (Rothfels et al., 2008), EU268666 (Rothfels et al., 2008); **A. subvillosa** (Hook.) Ching var. *tibetica* (Ching & S.K. Wu) H.S. Kung, *Anderson 22* (MO), China: Yunnan, 4569, JQ855926, JQ855914, JQ855897; **Argyrochosma incana** (C. Presl) Windham, *Schuettpezel 491* (DUKE), USA: Arizona, 3198, EU268771 (Rothfels et al., 2008), HQ846362 (Sigel et al., 2011), HQ846472 (Sigel et al., 2011); **A. jonesii** (Maxon) Windham, *Windham 3437* (DUKE), USA: California, 3844, EU268772 (Rothfels et al., 2008), HQ846365 (Sigel et al., 2011), HQ846473 (Sigel et al., 2011); **A. nivea** (Poir.) Windham var. *tenera* (Gillies ex Hook.) Ponce, *Beck St. G. 23950* (UC), Bolivia: Tarija, 5855, HQ846432 (Sigel et al., 2011), HQ846383 (Sigel et al., 2011), HQ846480 (Sigel et al., 2011); **Astrolepis sinuata** (Lag. ex Sw.) D.M. Benham & Windham subsp. *sinuata*, *Correll & Smith P795* (US), Peru: Lambayeque, 5831, JQ855927, JQ855915, JQ855898; **Cheilanthes bolborrhiza** Mickel & Beitel, *Breedlove 64102* (UC), Mexico: Jalisco, 1083, JQ855928, JQ855916, JQ855899; **C. bonariensis** (Willd.) Proctor, *Schuettpezel et al. 466* (DUKE), USA: Arizona, 3173, EU268780 (Rothfels et al., 2008), EU268731 (Rothfels et al., 2008), EU268677 (Rothfels et al., 2008); **C. chinensis** (Baker) Domin, *Zhang 684* (MO), China: Guizhou, 5321, JQ855929, JQ855917, JQ855900; **C. distans** (R. Br.) Mett., *Nagalingum 23* (DUKE), Australia: New South Wales, 3894, EU268783 (Rothfels et al., 2008), EU268734 (Rothfels et al., 2008), EU268680 (Rothfels et al., 2008); **C. eatonii** Baker, *Schuettpezel 323* (DUKE), Cultivated: Juniper Level Botanic Garden, NC, 2968, EF452144 (Schuettpezel et al., 2007), EF452084 (Schuettpezel et al., 2007), JQ855901; **C. kaulfussii** Kunze, *Windham et al. 519* (DUKE), Mexico: Morelos, 4407, JQ855930, JQ855918, JQ855902; **C. leucopoda** Link, *Villarreal 5801 & Carranza* (ARIZ), Mexico: Durango, 4506, EU268785 (Rothfels et al., 2008), JQ855919, EU268682 (Rothfels et al., 2008); **Cheilopteron rigidum** (Sw.) Fée var. *lanceolatum* C.C. Hall ex Mickel & Beitel, *Windham et al. 522* (UT), Mexico: Puebla, 4056, EU268788 (Rothfels et al., 2008), JQ855920, JQ855903; **C. rigidum** var. *rigidum*, *Rothfels et al. 3203* (DUKE), Mexico: Colima, 6617, –, –, JQ855904; **Notholaena affinis** (Mett.) T. Moore, *Labat 2603* (MEXU), Mexico: Querétaro, 7089, –, –, JQ855905; **N. aliena** Maxon, *Windham & Yatskievych 761* (DUKE), USA: Texas, 4059, EU268790 (Rothfels et al., 2008), EU268744 (Rothfels et al., 2008), EU268691 (Rothfels et al., 2008); **N. aschenborniana** Klotzsch, *Schuettpezel et al. 476* (DUKE), USA: Arizona, 3183, EF452159 (Schuettpezel et al., 2007), EU268745 (Rothfels et al., 2008), EU268692 (Rothfels et al., 2008); **N. aureolina** Yatsk. & Arbeláez, *Windham et al. 544* (DUKE), Mexico: Oaxaca, 4055, EU268778 (Rothfels et al., 2008), EU268729 (Rothfels et al., 2008), EU268675 (Rothfels et al., 2008); **N. brachypus** (Kunze) J. Sm., *Yatskievych & Gastony 89-236* (IND), Mexico: Jalisco, 4517, EU268781 (Rothfels et al., 2008), EU268732 (Rothfels et al., 2008), EU268678 (Rothfels et al., 2008); **N. brevistipes** Mickel, *Rothfels et al. 3048* (DUKE), Mexico: Tamaulipas, 6500, –, –, JQ855906; **N. bryopoda** Maxon, *Windham et al. 485* (DUKE), Mexico: Nuevo Leon, 4058, EU268791 (Rothfels et al., 2008), EU268746 (Rothfels et al., 2008), EU268693 (Rothfels et al., 2008); **N. californica** D.C. Eaton subsp. *californica*, *Schuettpezel et al. 436* (DUKE), USA: Arizona, 3143, EU268792 (Rothfels et al., 2008), EU268747 (Rothfels et al., 2008), EU268694 (Rothfels et al., 2008); **N. candida** (M. Martens & Galeotti) Hook., *Windham et al. 521* (DUKE), Mexico: Puebla, 4062, EU268793 (Rothfels et al., 2008), EU268748 (Rothfels et al., 2008), EU268695 (Rothfels et al., 2008); **N. copelandii** C.C. Hall, *Windham et al. 472* (DUKE), Mexico: Nuevo Leon, 4504, JQ855931, JQ855921, EU268696 (Rothfels et al., 2008); **N. galeottii** Fée, *Rothfels et al. 3440* (DUKE), Mexico: Querétaro, 6782, –, –, JQ855907; **N. grayi** Davenp. subsp. *grayi*, *Schuettpezel et al. 480* (DUKE), USA: Arizona, 3187, EU268794 (Rothfels et al., 2008), EU268749 (Rothfels et al., 2008), EU268697 (Rothfels et al., 2008); **N. greggii** (Mett.) Maxon, *Yatskievych & McCrary 85-10* (DUKE), USA: Texas, 4060, EU268796 (Rothfels et al., 2008), EU268751 (Rothfels et al., 2008), EU268699 (Rothfels et al., 2008); **N. jacalensis** Pray, *Rothfels et al. 3030* (DUKE), Mexico: Hidalgo, 6488, –, –, JQ855908; **N. jaliscana** Yatsk. & Arbeláez, *Rothfels, P. et al. 4* (DUKE), Mexico: Nayarit, 6808, –, –, JQ855909; **N. lemmonii** D.C. Eaton var. *lemmonii*, *Schuettpezel et al. 457* (DUKE), USA: Arizona, 3164, EU268797 (Rothfels et al., 2008), EU268752 (Rothfels et al., 2008), EU268700 (Rothfels et al., 2008); **N. meridionalis** Mickel, *Rothfels et al. 2646* (INB), Costa Rica: Guanacaste, 5592, JQ855932, JQ855922, JQ855910; **N. montieliae** Yatsk. & Arbeláez, *Stevens s.n.* (MO), Nicaragua: Madriz, 6182, JQ855933, JQ855923, JQ855911; **N. nealleyi** Seaton ex J.M. Coult., *Rothfels et al. 2482* (DUKE), USA: Texas, 5354, JQ855934, JQ855924, JQ855912; **N. neglecta** Maxon, *Schuettpezel et al. 477* (DUKE), USA: Arizona, 3184, EU268798 (Rothfels et al., 2008), EU268753 (Rothfels et al., 2008), EU268701 (Rothfels et al., 2008); **N. ochracea** Yatsk. & Arbeláez, *Yatskievych & Gastony 89-285* (IND), Mexico: Morelos, 4515, EU268777 (Rothfels et al., 2008), EU268728 (Rothfels et al., 2008), EU268674 (Rothfels et al., 2008); **N. rigida** Davenp., *Windham et al. 491* (DUKE), Mexico: Tamaulipas, 4408, EU268799 (Rothfels et al., 2008), EU268754 (Rothfels et al., 2008), EU268702 (Rothfels et al., 2008); **N. rosei** Maxon, *Windham et al. 542* (DUKE), Mexico: Oaxaca, 4409, EU268800 (Rothfels et al., 2008), EU268755 (Rothfels et al., 2008), EU268703 (Rothfels et al., 2008); **N. schaffneri** (E. Fourn.) Underw. ex Davenp., *Windham et al. 526* (DUKE), Mexico: Oaxaca, 4061, EU268801 (Rothfels et al., 2008), EU268756 (Rothfels et al., 2008), EU268704 (Rothfels et al., 2008); **N. standleyi** Maxon, *Schuettpezel et al. 435* (DUKE), USA: Arizona, 3142, EU268802 (Rothfels et al., 2008), EU268757 (Rothfels et al., 2008), EU268705 (Rothfels et al., 2008); **N. sulphurea** (Cav.) J. Sm., *Windham et al. 488* (DUKE), Mexico: Tamaulipas, 4411, EU268806 (Rothfels et al., 2008), EU268761 (Rothfels et al., 2008), EU268709 (Rothfels et al., 2008); **N. trichomanoides** (L.) Desv. var. *subnuda* Jenman, *Ranker & Trapp 860* (UT), Jamaica: Middlesex, 4054, EU268807 (Rothfels et al., 2008), EU268762 (Rothfels et al., 2008), EU268710 (Rothfels et al., 2008); **Paragymnopteris marantae** (L.) K. H. Shing, *Yatskievych et al. 02-35* (MO), China: Yunnan, 3736, EF452161 (Schuettpezel et al., 2007), EU268763 (Rothfels et al., 2008), EU268711 (Rothfels et al., 2008); **Pellaea atropurpurea** (L.) Link, *Schuettpezel 312* (DUKE), Cultivated: Juniper Level Botanic Garden, NC, 2957, EF452162 (Schuettpezel et al., 2007), JQ855925, JQ855913; **P. truncata** Goodd., *Schuettpezel 430* (DUKE), USA: Arizona, 3137, EF452164 (Schuettpezel et al., 2007), EF452110 (Schuettpezel et al., 2007), EU268714 (Rothfels et al., 2008); **P. viridis** (Forssk.) Prantl, *Janssen 2701* (P), France: Île de la Réunion, 3555, EF452147 (Schuettpezel et al., 2007), EF452086 (Schuettpezel et al., 2007), EU268715 (Rothfels et al., 2008); **Pentagramma triangularis** (Kaulf.) Yatsk., *Windham, & Wollenw.*, *Schuettpezel et al. 445* (DUKE), USA: Arizona, 3152, EF452165 (Schuettpezel et al., 2007), EU268768 (Rothfels et al., 2008), EU268716 (Rothfels et al., 2008).

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