FERN SYSTEMATICS

Toward a monophyletic *Notholaena* (Pteridaceae): resolving patterns of evolutionary convergence in xeric-adapted ferns

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Cheilanthoid ferns (Pteridaceae) are a diverse and ecologically important clade, unusual among ferns for their ability to colonize and diversify within xeric habitats. These extreme habitats are thought to drive the extensive evolutionary convergence, and thus morphological homoplasy, that has long thwarted a natural classification of cheilanthoid ferns. Here we present the first multigene phylogeny to focus on taxa traditionally assigned to the large genus *Notholaena*. New World taxa (*Notholaena* sensu Tryon) are only distantly related to species occurring in the Old World (*Notholaena* sensu Pichi Sermolli). The circumscription of *Notholaena* adopted in recent American floras is shown to be paraphyletic, with species usually assigned to *Cheilanthes* and *Cheiloplecton* nested within it. The position of *Cheiloplecton* is particularly surprising—given its well-developed false indusium and non-farinose blade, it is morphologically anomalous within the "notholaenoids". In addition to clarifying natural relationships, the phylogenetic hypothesis presented here helps to resolve outstanding nomenclatural issues and provides a basis for examining character evolution within this diverse, desert-adapted clade.

KEYWORDS: *atpA*, cheilanthoids, *Cheiloplecton*, ferns, molecular phylogenetics, morphological homoplasy, *Paragymnopteris marantae*, *rbcL*, *trnG-trnR*

INTRODUCTION

With over 1,000 species, Pteridaceae comprises approximately 10% of extant fern diversity and is notable for its extreme morphological and ecological disparity. In addition to species-rich and predominantly forest-dwelling genera such as *Adiantum* and *Pteris*, the family includes floating, freshwater aquatics (*Ceratopteris*), mangrove specialists (*Acrostichum*), obligate epiphytes (*Vittaria*), and epipetric xerophytes (*Cheilanthes*, *Notholaena*, *Pellaea*). The ability of some species of Pteridaceae to flourish in arid environments (Yatskievych & Hooper, 2001) is particularly striking given that moisture dependence is often considered an ecologically limiting factor for ferns (Page, 2002). Pteridaceae includes both facultative and obligate xerophytes, nearly all of which are members of the well-supported cheilanthoid clade (sensu Schuettpelz & al., 2007), which contains an estimated 400 species.

Cheilanthoids exhibit extensive disparity in both their gross morphology (leaf shape) and their reproductive structures (particularly sporangial arrangement and type of indusium). For example, *Hemionitis palmata* has undivided palmate leaves with unprotected sporangia spread along the veins (Fig. 1A); *Pellaea intermedia* has bipinnate leaves and sporangia near vein tips where they are protected by an inrolled leaf margin (false indusium, Fig. 1B); *Astrolepis sinuata* has linear leaves, with sporangia densely covered with scales (Fig. 1C); *Notholaena rosei* has linear-lanceolate leaves and submarginal sporangia nestled among dense flavonoid deposits ("farina", Fig. 1D); and *Adiantopsis radiata* has palmately compound leaf architecture, and sporangia protected by discrete, flap-like false indusia (Fig. 1E).

Counterintuitively, these highly divergent morphologies do not correspond with monophyletic genera. Cheilanthoids, rather, have been called "the most contentious group of ferns with respect to a practical and natural generic classification" (Tryon & Tryon, 1982). This historic inability to identify monophyletic genera among cheilanthoid ferns is frequently attributed to convergent evolution (morphological homoplasy) driven by their adaptation to arid habitats (Tryon & Tryon, 1973; Lellinger, 1989; Gastony & Rollo, 1998; Kirkpatrick, 2007; Prado & al., 2007).

Generic circumscriptions among cheilanthoid ferns are thus notoriously unstable, varying radically by author and geographic region. One of the best examples of this taxonomic confusion involves the genus *Notholaena* R. Br. Circumscriptions of this genus range from narrow (including only those species with farinose sporophytes and gametophytes, e.g., Windham, 1993a) to very broad (including taxa as disparate as *Argyrochosma*, *Astrolepis*, and certain species of *Cheilanthes*; Tryon, 1956). Others have not recognized *Notholaena* at all, instead reducing it to synonymy under an expanded *Cheilanthes* (Copeland, 1947; Mickel, 1979). These contradictory treatments reflect the difficulties inherent in discerning natural groups within xeric-adapted ferns.

The taxonomic challenges inherent in *Notholaena* are further compounded by nomenclatural issues. *Notholaena* has three lectotypifications—*N. trichomanoides* (Smith, 1875), *N. distans* (Underwood, 1899), and *N. marantae* (Christensen, 1905–1906)—two of which are in current use (Yatskievych & Smith, 2003). The preferred lectotype tends to fall along regional lines: European researchers usually favour the Old World *N. marantae* (Pichi Sermolli, 1989; Jermy & Paul, 1993), whereas North American workers typically adopt the Caribbean *N. trichomanoides* (Windham, 1993a; Mickel & Smith, 2004). Yatskievych & Smith (2003) discussed this problem in depth and concluded that there was no justification for overturning the first lectotypification based on *N. trichomanoides*. Thus, we will treat the taxa closely related to *N. trichomanoides* as *Notholaena* and those aligned with *N. marantae* under *Paragymnopteris*. The phylogenetic relationship between these two taxa (*N. trichomanoides*, *P. marantae*) would figure prominently in any decision to overturn the *N. trichomanoides* lectotypification, and resolving this relationship is one of the main goals of this study.

In the circumscription adopted here, *Notholaena* contains approximately 30 species, most of which exhibit an unusual morphological feature: dense flavonoid deposits on the abaxial surfaces of the leaves. This “farina” is exuded by glandular hairs, and is frequently bright white or yellow. The chemical composition of farina is variable among and often within *Notholaena* species (Seigler & Wollenweber, 1983; Wollenweber, 1984; Wollenweber & Schneider, 2000), and is usually considered to be an adaptation to reduce water loss through increased reflectance of sunlight (Hevly, 1963). When suffering water stress, *Notholaena* plants curl their leaves to expose their abaxial (farina-covered) surfaces to irradiation, despite the fact

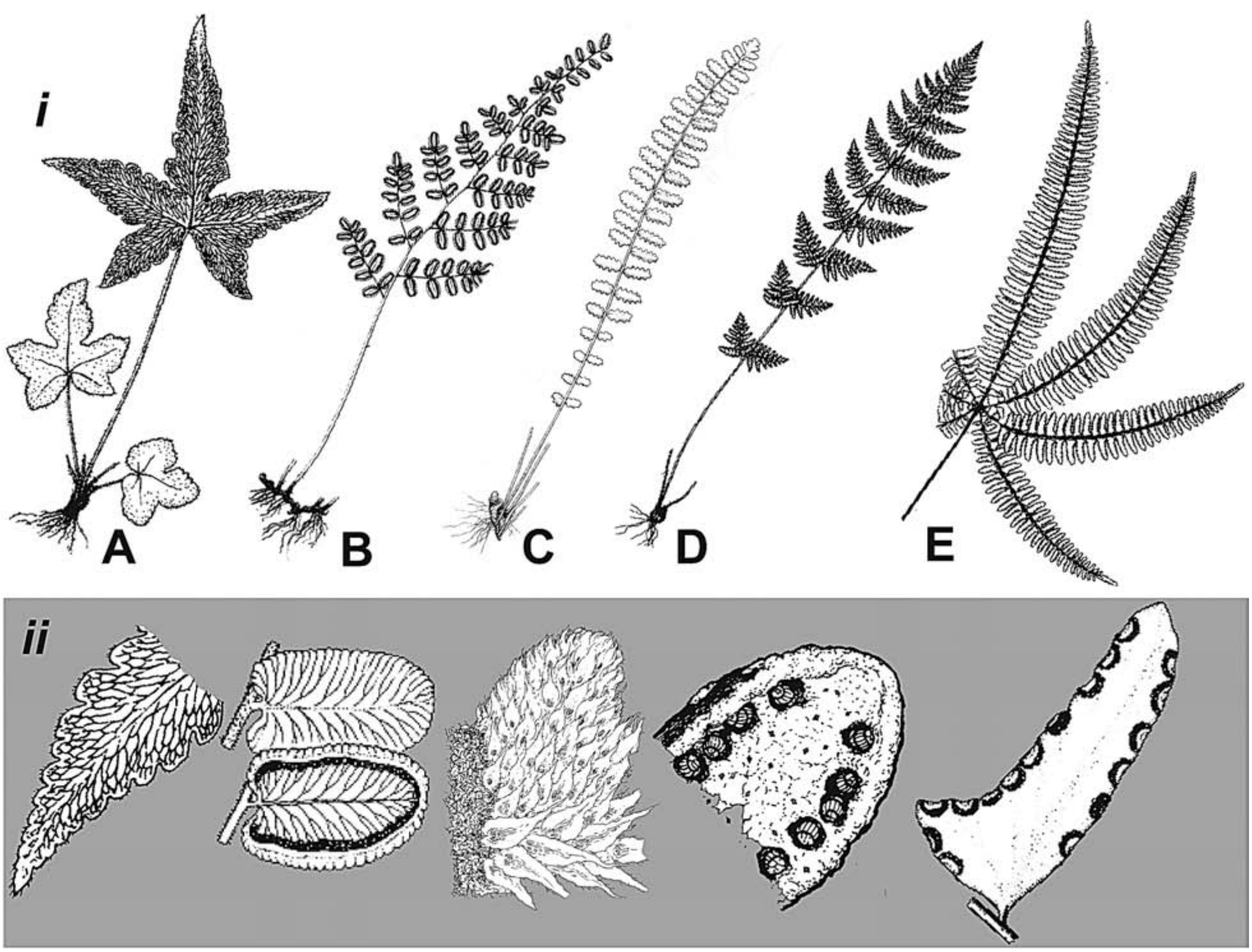

Fig. 1. General habit (i) and fertile abaxial leaf surface (ii) for: A, *Hemionitis palmata*; B, *Pellaea intermedia*; C, *Astrolepis sinuata*; D, *Notholaena rosei*; E, *Adiantopsis radiata*. Not to scale. Line drawings reprinted with permission from *The Pteridophytes of Mexico*, Mickel & Smith, Copyright 2004, The New York Botanical Garden, Bronx, New York.

that this surface is where the stomata are situated (Hevly, 1963; Lellinger, 1985).

The systematics of cheilanthoid ferns, and the genus *Notholaena* in particular, have proven largely intractable based on morphological data alone (Tryon & al., 1990). Recent molecular phylogenetic studies (Gastony & Rollo, 1995, 1998; Kirkpatrick, 2007; Prado & al., 2007; Schuettpelz & al., 2007; Zhang & al., 2007) have demonstrated the utility of DNA data for clarifying patterns of descent in cheilanthoids. Most of these studies were based on sequences from a single plastid locus (*rbcL*) and none focused specifically on *Notholaena*.

Here we construct the first multigene phylogeny for *Notholaena*, based on DNA sequences from three plastid genes—*rbcL*, *atpA*, and the *trnG-trnR* intergenic spacer—in order to explore some of the outstanding questions in this group. Specifically, we seek to determine which taxa are properly included within *Notholaena*, and the status of any natural groups within the genus. Additionally, we explore the phylogenetic relationships among the three *Notholaena* lectotypes and thus the nomenclatural consequences of overturning the *N. trichomanoides* lectotypification. Resolving these issues is necessary for a revised circumscription of the group, to determine patterns of morphological homoplasy, and to provide the necessary framework for future investigations of character evolution within these xeric-adapted ferns.

MATERIALS AND METHODS

Taxon sampling. — Fifty-eight ingroup taxa were selected to maximize the representation of *Notholaena* sensu Windham (1993a) and of groups previously considered to be allied with *Notholaena*. Multiple accessions for a small number of species were included in those cases where they were deemed most important (such as in the case of potentially non-monophyletic species). Sampling was designed to include all three *Notholaena* lectotypes and the type species of related genera whenever possible. The remaining taxa were selected to provide a broad framework of cheilanthoid ferns. As outgroups, we selected *Cryptogramma* and *Pityrogramma*, representing the "cryptogrammoid" and "pteridoid" clades of Pteridaceae respectively (Schuettpelz & al., 2007). Information on specimen vouchers and provenance are provided in the Appendix.

DNA protocols and phylogenetic analyses. — This study utilizes sequences from three plastid loci: *rbcL*, *atpA*, and *trnG-trnR* intergenic spacer (Table 1). The extraction, amplification, and sequencing of *rbcL* follow the protocol of Pryer & al. (2004). Corresponding protocols for *atpA* and *trnG-trnR* follow Pryer & al. (2004), but using the primers of Schuettpelz & al. (2006) and Nagalingum & al. (2007), respectively.

DNA sequence data were manually edited and aligned in Sequencher 4.5 (Gene Codes Corporation, 2005) and MacClade 4.07 (Maddison & Maddison, 2005), respectively. Ambiguously aligned areas (limited almost exclusively to *trnG-trnR*) were excluded prior to phylogenetic analyses; indels ("–") were treated as missing data. The complete alignment is available in TreeBase (S1925; M3546).

A total of four Bayesian inference (BI) analyses were performed using the parallel version of MrBayes (Ronquist & Huelsenbeck, 2003; Altekar & al., 2004): one for each of the individual loci, and one for the combined dataset. Each analysis included four independent runs of four chains (one cold, three hot), for 10 million generations with trees sampled every 1,000 generations. The BI analyses incorporated a GTR + I + G model; in the analysis of the combined data, each locus was assigned an individual partition, and the rate prior was set to vary among partitions (ratepr = variable). The resulting parameter files were jointly visualized in Tracer (Rambaut & Drummond, 2004) to ensure convergence, and to determine an appropriate burn-in period. In all cases, convergence was achieved before 1 million generations, and to be conservative, 2.5 million generations were discarded as burn-in. Each analysis thus yielded 30,000 trees. To assess congruence, the 95% majority-rule consensus trees for each locus were generated using PAUP* (Swofford,

Table 1. Summary of character data.

		Included Characters			
	Alignment length	Total included	Constant	Parsimony-informative[a]	Missing data[b]
rbcL	1,315	1,299	940	237	4.7%
atpA	1,860	1,680	1,170	332	14.2%
trnG-trnR	1,443	1,136	474	428	23.1%
Total	4,618	4,115	2,584	997	13.7%

[a]Parsimony-informative characters were calculated using an equally weighted parsimony model.
[b]The missing data column shows the summed percentages of "?"s and "–"s in the matrix.

2002) and manually compared for supported conflicts (Mason-Gamer & Kellogg, 1996).

The combined data were also analyzed under maximum likelihood (ML), using Garli 0.951 (Zwickl, 2006). The ML analyses were performed using a GTR + I + G model, with four discrete rate categories, and state frequencies and proportion of invariant sites were estimated. Three single "best tree" searches were performed from different random starting trees to ensure that the ML results were not unduly influenced by the starting tree; runs proceeded until they met Garli's default termination conditions. Support was assessed with 500 bootstrap searches under the same parameters as the "best tree" searches.

Morphological and cytogenetic character mapping. — Morphological character states for sporophytes were derived from the literature and, in most cases, confirmed by direct examination of herbarium specimens. The presence or absence of farina-producing glands on gametophytes was determined from published sources (Tryon, 1947; Giauque, 1949) and from cultured specimens (Gastony, unpub.; Windham, unpub.). Chromosome base numbers were obtained from Windham & Yatskievych (2003); more than half of the DNA samples sequenced herein were derived from voucher specimens cited in the latter.

RESULTS

Comparing the individual locus BI analyses revealed a single well-supported (> 0.95 posterior probability, PP) topological conflict between two loci: *rbcL* data support a clade of *Cheilanthes covillei* and *C. newberryi* (100% PP) whereas *atpA* groups *C. covillei*, *C. lendigera*, and *C. myriophylla* together in a polytomy with 98% PP (*trnG-trnR* does not provide support for either resolution). This conflict lies far outside our main clade of interest (the notholaenoids; Fig. 2), and we thus concatenated the three data sets for subsequent analyses. The concatenated dataset includes 4,115 characters (Table 1) and 145 newly contributed sequences (Appendix).

Both Bayesian and ML analyses of the combined data yield a phylogenetic hypothesis (or, in the case of BI, a probability distribution of hypotheses; Fig. 2) with strong support across the tree, especially along the backbone. The ingroup taxa are resolved into six highly supported "major clades" that are approximately equivalent to the clades identified by Kirkpatrick (2007): *(1) Doryopteris ludens*; *(2) Bommeria hispida*; *(3)* "myriopteroids" (Kirkpatrick's "American *Cheilanthes*"); *(4)* "pellaeoids" (differing from Kirkpatrick's pellaeoids by the inclusion of *Argyrochosma*); *(5)* "hemionitidoids" (part of Kirkpatrick's "distant cheilanthoids"); and *(6)* "notholaenoids" (part of Kirkpatrick's "distant cheilanthoids"). The focal clade of this study—notholaenoids—is sister to the diverse and globally distributed hemionitidoid clade (Fig. 2). Together these two clades are sister to the myriopteroids (composed entirely of New World *Cheilanthes* species) plus pellaeoids (*Pellaea* sect. *Pellaea* and its relatives; Fig. 2). This overall phylogenetic structure is consistent with the conclusions of Gastony & Rollo (1998) and Schuettpelz & al. (2007), but with a greatly expanded sampling in the notholaenoid clade.

DISCUSSION

***Notholaena*: What's out. —** The phylogenetic results of this study considerably clarify the concept of *Notholaena*, as typified by *N. trichomanoides*. The *Notholaena* of Tryon (1956) is clearly non-monophyletic, encompassing taxa included here under *Argyrochosma* (Windham, 1987), *Astrolepis* (Benham & Windham, 1992), and *Cheilanthes* (Fig. 3). *Argyrochosma* and *Astrolepis* are each resolved as monophyletic in this analysis (Fig. 2), at least to the extent that our sampling permits, with *Astrolepis* sister to a subset of *Pellaea* (including the type of *Pellaea*, i.e., *P. atropurpurea*), and *Argyrochosma* strongly supported as sister to the rest of the pellaeoids (PEL; Fig. 2). Neither *Argyrochosma* nor *Astrolepis* is phylogenetically proximate to *Notholaena*, in agreement with previous molecular studies by Gastony & Rollo (1995, 1998) and Kirkpatrick (2007).

The taxonomic transfer of *Notholaena newberryi* into *Cheilanthes* (by Domin, 1913: 133; Tryon & Tryon, 1982; cf. Lellinger, 1985) finds strong support in our dataset (Fig. 2 arrow a), as does the decision to move *N. aurea* into *Cheilanthes*, where it becomes *C. bonariensis* (Fig. 2 arrow b). Both taxa (*C. newberryi* and *C. bonariensis*) are strongly supported within the New World myriopteroid clade (MYR; Fig. 2) where the latter was also placed in the *rbcL* and ITS phylogenies of Gastony & Rollo (1998).

***Notholaena*: What's in. —** The majority of *Notholaena* s.str., the "core *Notholaena* group" (cN; Fig. 2), is strongly supported as monophyletic in our analyses, and is divided approximately equally into two clades, roughly corresponding to species with scaly versus glabrous leaf blades (Figs. 2, 4C). All core *Notholaena* taxa are farinose, have fertile leaves with margins that are at most weakly modified (as opposed to the prominent false indusium characteristic of most *Cheilanthes*, *Pellaea*, etc.), and have a chromosome base number of $x = 30$ (Windham & Yatskievych, 2003). These findings support earlier conclusions by Gastony & Rollo (1995, 1998), but incorporate greater taxon and character sampling.

The phylogenetic position of *N. standleyi*, however, is novel and noteworthy. This species was not previously sampled, and its robust placement near the base of the notholaenoids (NOTH; Fig. 2) renders *Notholaena* s.str. paraphyletic by the inclusion of *Cheiloplecton* and the *Cheilanthes aurantiaca*/*aurea*/*brachypus* clade (Fig. 2). Although single-locus analyses (Gastony & Rollo, 1998) previously demonstrated a close relationship between *Cheiloplecton*, *Cheilanthes aurea*, and *Notholaena*, *N. standleyi* was not included, so *Cheiloplecton* and *Cheilanthes aurea* appeared as early diverging lineages, rather than embedded within *Notholaena*. *Notholaena standleyi* is morphologically very similar to *N. sulphurea*, with which it was once lumped and for which it can easily be mistaken (Tryon, 1956; Mickel & Smith, 2004); *N. sulphurea* is strongly supported well within core *Notholaena* in this analysis (cN; Fig. 2 arrow c).

The taxa bracketed by *Notholaena standleyi* and the core *Notholaena* group (Fig. 2) include two farinose species (*Cheilanthes aurea*, *C. aurantiaca*) with distinct false indusia, as well as two non-farinose species (*Cheilanthes*

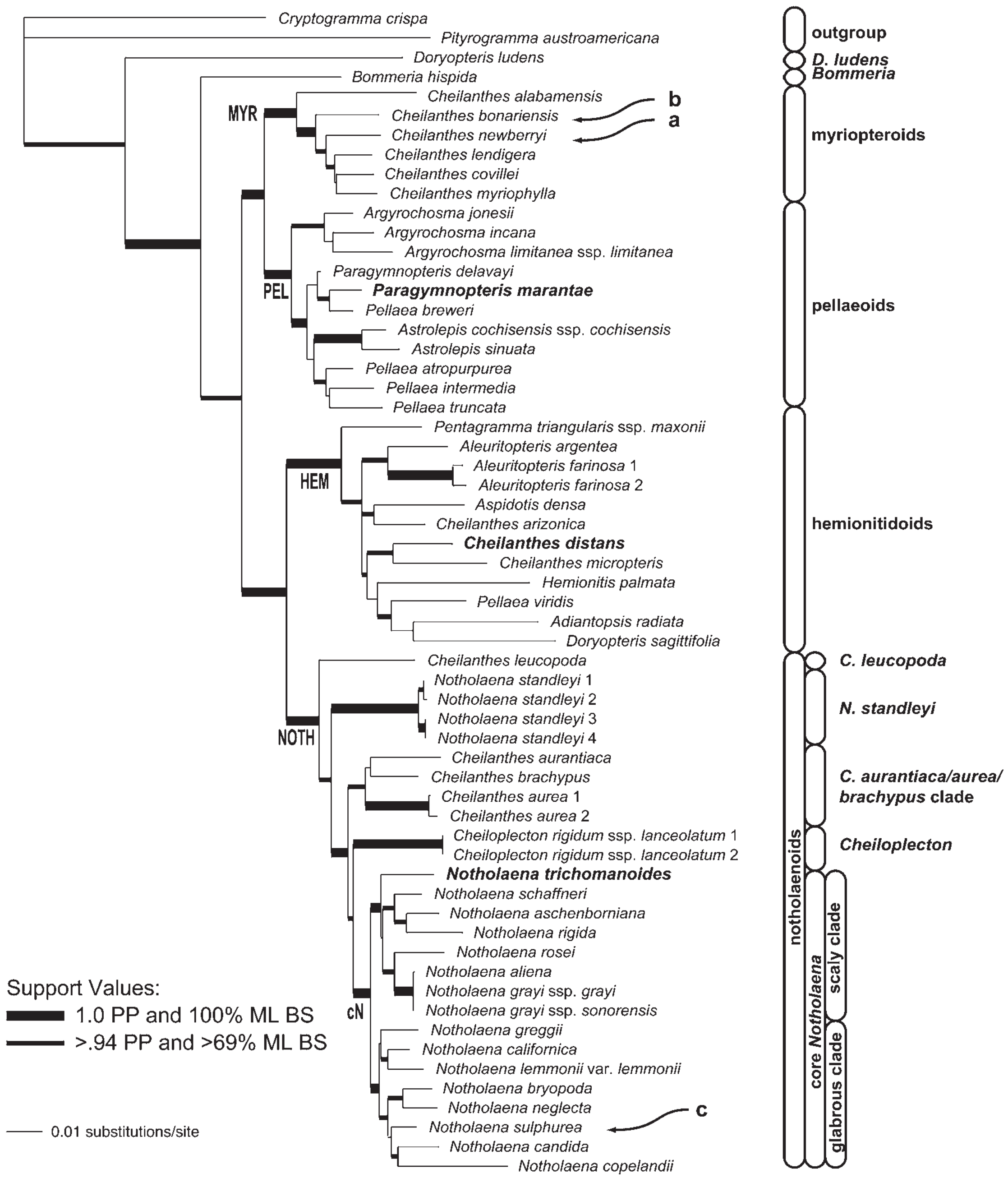

Fig. 2. Maximum likelihood phylogram from *atpA*, *rbcL* and *trnG-trnR* data; tree rooted with *Pityrogramma* and *Cryptogramma*. Taxa in bold face are the three *Notholaena* lectotypes. Major clade abbreviations: MYR (myriopteroids), PEL (pellaeoids), HEM (hemionitidoids), NOTH (notholaenoids), and cN (core *Notholaena*). For explanation of arrows a–c see text.

brachypus, *Cheiloplecton rigidum*). *Cheilanthes brachypus* has always been an anomaly in *Cheilanthes*, and early investigators tended to place it in *Notholaena* (Fig. 3A; Tryon, 1956). It is highly unusual within *Notholaena* s.str., however, in its overall aspect, dense scaly indument, membranous fertile leaf margins, and absence of farina (Tryon, 1956). *Cheiloplecton* is even more anomalous, and while it has valid names under both *Cheilanthes* and *Pellaea*, it has never been included in *Notholaena*. In addition to being non-farinose, it has a prominent inrolled false indusium completely unlike the unmodified fertile margins of the core *Notholaena* group. Tryon & Tryon (1982) placed both *Cheilanthes brachypus* and *Cheiloplecton rigidum* within *Cheilanthes*, but as "morphologically isolated species".

Cheilanthes leucopoda is resolved as sister to the rest of the notholaenoids, a position consistent with the results of Gastony & Rollo (1998), but with improved support. While *C. leucopoda* does not fit well into any subgeneric scheme for *Cheilanthes* (e.g., it is placed as a "morphologically isolated species" in Tryon & Tryon, 1982), its inclusion in that genus has never been disputed. In agreement with most New World *Cheilanthes* (the myriopteroids: MYR; Fig. 2), *C. leucopoda* has a vestiture of hairs and discontinous sori covered by a weakly differentiated false indusium. Nevertheless, it is strongly supported as an early diverging member of the notholaenoids, and is thus phylogenetically distant from the myriopteroids.

Notholaena bryopoda is well embedded within the core *Notholaena* (cN; Fig. 2), despite its association with species of *Argyrochosma* by Tryon & Tryon (1982) and its placement in that genus by Hassler & Swale (2003). Windham (1987) did not include *N. bryopoda* in *Argyrochosma*, and *N. bryopoda* does not, in fact, have a validly published name under that genus. Features uniting *N. bryopoda* with the other core *Notholaena* species include a chromosome base number of $x = 30$ ($x = 27$ in *Argyrochosma*; Fig. 5B) and the presence of farina on the gametophyte (absent in *Argyrochosma*; Fig. 5C).

Among the species-level relationships, those of *Notholaena grayi* and *N. aliena* are worth highlighting. In the phylogram (Fig. 2), the two *N. grayi* subspecies and *N. aliena* together form a polytomy, with negligible branch lengths differentiating the taxa. *Notholaena aliena*, a purported apogamous triploid (Windham, 1993a), is similar to *N. grayi* but distinguished by the presence of hairs on abaxial blade surfaces. Our data thus suggest that *N. aliena* may be an allopolyploid, the first reported for *Notholaena* s.str. Because chloroplasts are maternally inherited in cheilanthoid ferns (see Gastony & Yatskievych, 1992), it would appear that *N. grayi* was the maternal parent of *N. aliena*. Our data are also consistent with the hypothesis first proposed by Gastony & Windham (1989) that *N. grayi* subsp. *grayi* (an apogamous triploid) is an autopolyploid derived from sexual diploid *N. grayi* subsp. *sonorensis*.

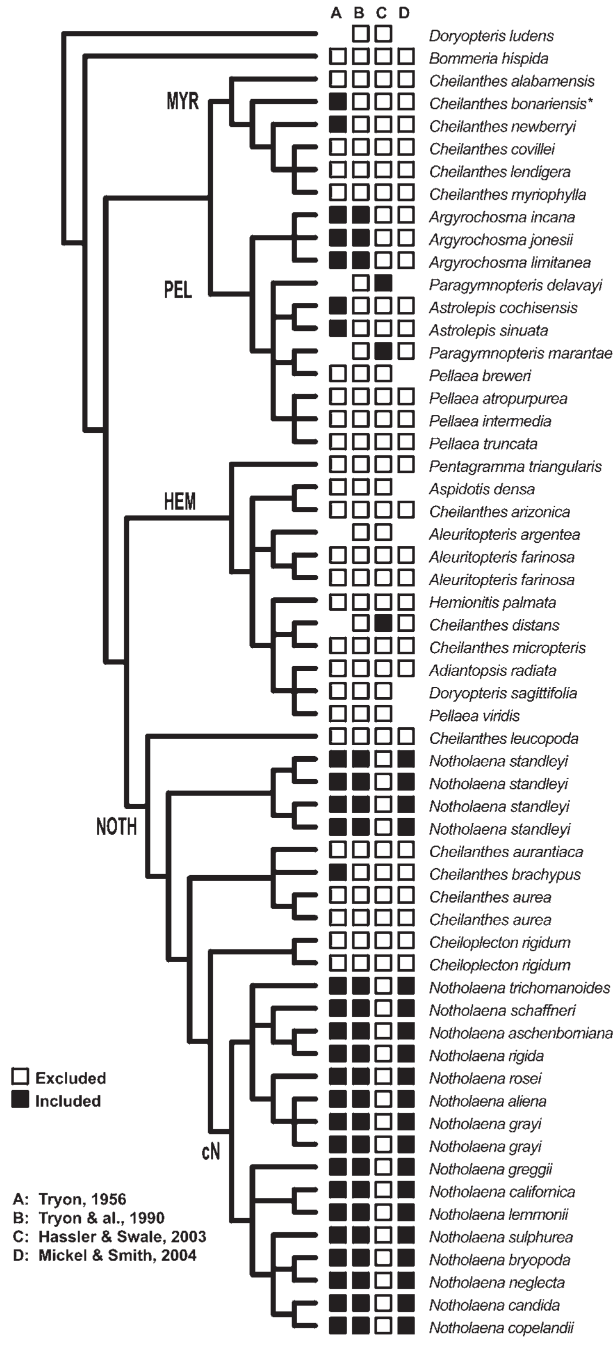

Fig. 3. Selected historical treatments of the *Notholaena* generic concept, mapped onto the 0.95 posterior probability consensus ingroup cladogram from the analyses summarized in Fig. 2. A, Tryon, 1956; B, Tryon & al., 1990; C, Hassler & Swale, 2003; D, Mickel & Smith, 2004. Clade name abbreviations follow Fig. 2.

Informative morphological characters. — There are few clear morphological synapomorphies among cheilanthoid ferns, as suggested by the non-monophyletic generic concepts employed in the group (Fig. 3). One feature that warrants further attention is the presence of farina on the gametophytes, a character that Tryon & Tryon (1982) described as being "exceptional" among ferns; very few morphological characters are expressed in both the gametophytic and sporophytic generations. Of the notholaenoids and pellaeoids examined to date, farina has been observed on the gametophytes of all the notholaenoids but on none of the pellaeoids (Fig. 5C; Tryon, 1947; Giauque, 1949; Windham, 1987; Gastony unpub.; Windham unpub.). Further investigations in light of the new phylogenetic information presented here are needed to determine whether this character remains a synapamorphy for the notholaenoids.

Even within the notholaenoids, morphological features such as blade indument (scales, hairs, and farina) and the presence of a false indusium are homoplastic (Fig. 4A–C). However, these characters, and several others, are not completely labile. A covering of farina on the underside of the leaves is the common condition in the notholaenoid clade, with only *C. leucopoda*, *C. brachypus*, and *Cheiloplecton* (all early-diverging taxa) lacking farina (Fig. 4A). Similarly, most of the taxa have a vestiture that includes some scales or hairs, with *N. standleyi*, *N. rigida*, *N. rosei*, and the *N. greggii–N. copelandii* clade being the only glabrous taxa (Fig. 4C). Leaf shape, which is extremely variable in cheilanthoids, also carries some phylogenetic information within the notholaenoids. The linear-lanceolate taxa are almost entirely in the *N. trichomanoides–N. grayi* clade (all of which are linear-lanceolate); the linear-lanceolate form otherwise occurs only in *C. brachypus*, *C. aurantiaca*, and *N. lemmonii* (Fig. 4D).

Morphological homoplasy and geography. — Of the eight genera with multiple species included in this study (*Aleuritopteris*, *Argyrochosma*, *Astrolepis*, *Cheilanthes*, *Doryopteris*, *Notholaena*, *Paragymnopteris*, *Pellaea*), only three (*Aleuritopteris*, *Argyrochosma*, *Astrolepis*) are resolved as monophyletic in this analysis. Based on more extensive sampling, Zhang & al. (2007) additionally rejected the monophyly of *Aleuritopteris*. Thus, only the small genera *Argyrochosma* (ca. 20 species) and *Astrolepis* (ca. 10 species) are potentially monophyletic under the current morphology-based classification (Windham, 1993b; Mickel & Smith, 2004; Smith & al., 2006). This result reflects the extent to which morphological homoplasy is present in cheilanthoid ferns, especially in characters that have historically been used to delimit genera (e.g., the presence or absence of false indusia; Fig. 4B).

The phylogeny does, however, show considerable geographical structure, as two of the major clades (myriopteroids and notholaenoids; MYR and NOTH in Fig. 2) are composed entirely of New World species (Fig. 5A).

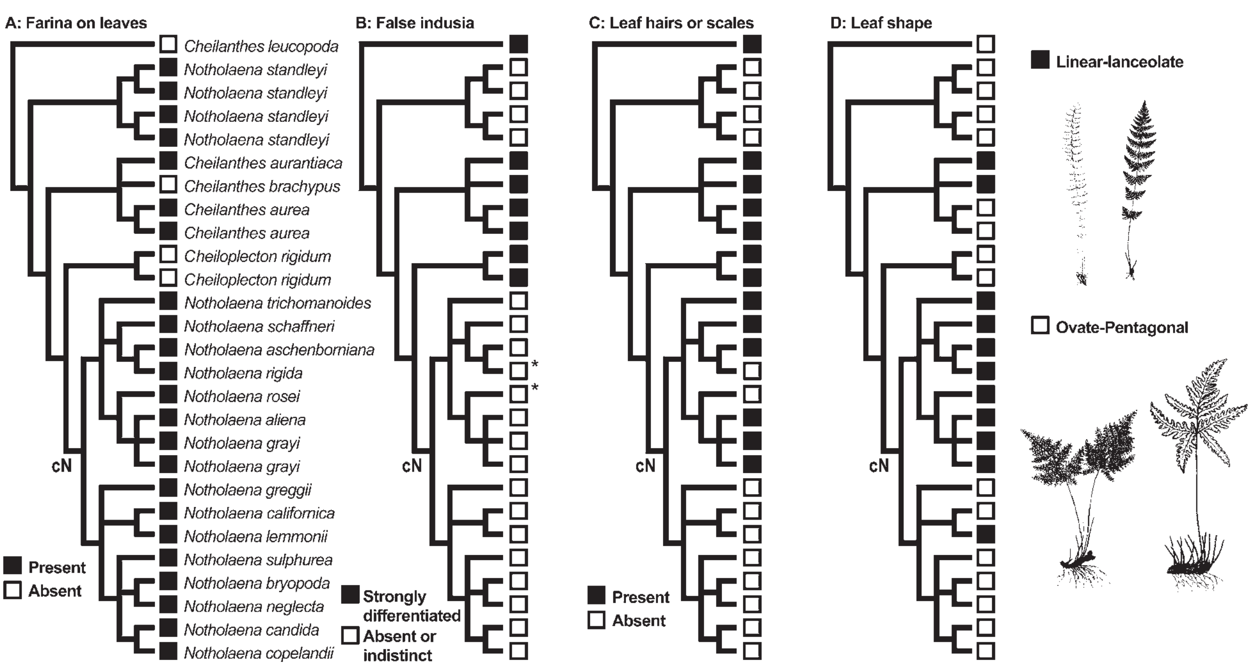

Fig. 4. Potentially informative morphological characters in the notholaenoids, mapped onto the 0.95 posterior probability consensus notholaenoid cladogram from the analyses summarized in Fig. 2. A, presence of farina on abaxial leaf surfaces. B, presence of a strongly differentiated false indusium on fertile leaves. *Both *Notholaena rigida* and *N. rosei* have fertile leaf margins that approach "strongly differentiated". C, presence of hairs and/or scales on leaf blades. D, leaf blade shape. Clade name abbreviations follow Fig. 2. Line drawings reprinted with permission from *The Pteridophytes of Mexico*, Mickel & Smith, Copyright 2004, The New York Botanical Garden, Bronx, New York.

Other data (Kirkpatrick, 2007; Schuettpelz & al., 2007) further support this pattern: cheilanthoid taxa are frequently more closely related to geographically proximate taxa from other genera than to geographically distant congeners. The clearest example of this pattern in our phylogeny (Fig. 2) concerns the genus *Doryopteris*, in which the New World species *Doryopteris sagittifolia* is sister to New World *Adiantopsis radiata* and phylogenetically distant from the Old World *Doryopteris ludens*.

Consequences for typification. — The typification of *Notholaena* has been controversial (Pichi Sermolli, 1989), a situation thoroughly reviewed by Yatskievych

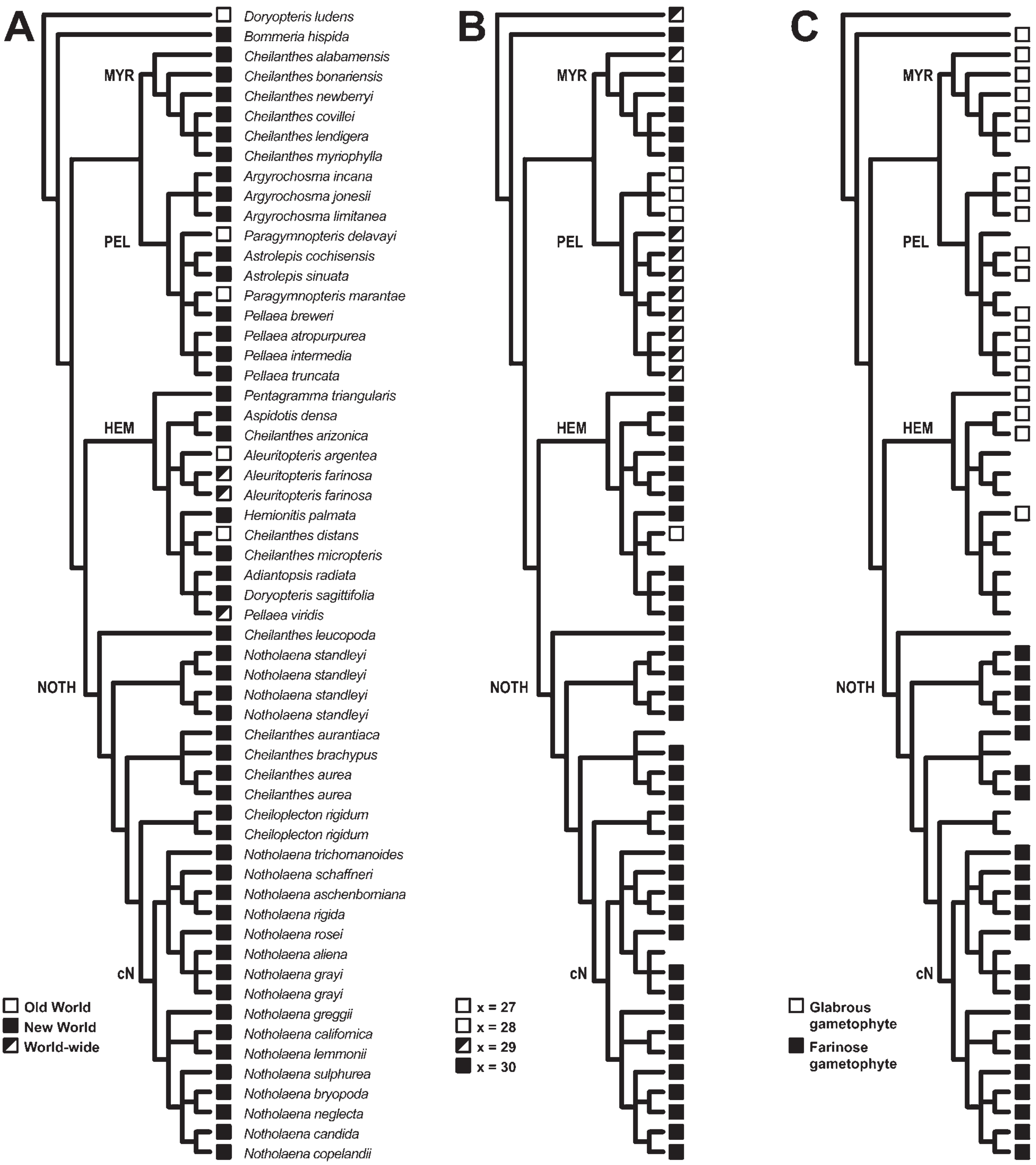

Fig. 5. Characters from geography, cytogenetics and gametophytes, mapped onto the 0.95 posterior probability consensus ingroup cladogram from the analyses summarized in Fig. 2. A, geographic range; B, chromosome base number; C, gametophyte indument. Clade name abbreviations follow Fig. 2.

& Smith (2003). Our three-gene phylogeny is the first to include all three lectotypes—*N. trichomanoides* (Smith, 1875), *N. distans* (Underwood, 1899), and *N. marantae* (Christensen, 1905–1906). As shown in Fig. 2, these taxa are quite distantly related, belonging to three different major clades (the notholaenoids, hemionitidoids, and pellaeoids, respectively). If one wished to include these three taxa in a single monophyletic genus, that taxon would encompass all cheilanthoids except *Bommeria* and *Doryopteris ludens*. A genus circumscribed in this manner would include approximately 400 morphologically, ecologically, and genetically disparate species (e.g., Figs. 1–2). The oldest generic name available for this taxon is *Hemionitis*, the use of which would require nearly 400 new combinations.

If cheilanthoid ferns are to be subdivided into more narrowly circumscribed genera, one or more of the *Notholaena* lectotypes chosen by previous authors must be excluded from the latter genus. The three-gene phylogeny presented in Fig. 2 allows us to examine the nomenclatural consequences of each lectotypification. The earliest lectotype (*N. trichomanoides*) belongs to the exclusively New World clade herein referred to as the notholaenoids (NOTH; Fig. 2). Retaining this typification, as recommended by Yatskievych & Smith (2003), involves minimal nomenclatural instability. Aside from *Cheilanthes leucopoda* and *Cheiloplecton rigidum*, all currently recognized members of this clade have valid names in *Notholaena* (Yatskievych & Arbeláez, 2008). If we follow the earliest lectotypification, *N. distans* and its relatives would be included in *Cheilanthes* and *N. marantae* and its allies in *Paragymnopteris*. Essentially all of the combinations needed for this taxonomic treatment are currently available.

The second lectotype (*N. distans*) is an Australian taxon in the hemionitidoid clade (HEM; Fig. 2), sister in this analysis to *C. micropteris*, the type of *Cheilanthes*. Because *Cheilanthes* is the earlier name, *Notholaena* would almost certainly be reduced to synonymy if *N. distans* were taken as the type species. This would have a significant impact on the nomenclature of species in the notholaenoid clade, requiring up to 30 new combinations under *Cheiloplecton* (see discussion below). Fortunately, *N. distans* has been rejected as a possible lectotype of *Notholaena*. Underwood selected *N. distans* as the type because it was the first taxon in Brown's (1810) original list, a method considered "mechanical" and thus invalid under the *Code* (Yatskievych & Smith, 2003).

The third lectotype (*N. marantae*) is more problematic. Despite strong arguments in favor of rejecting *N. marantae* as the type of *Notholaena* (summarized by Yatskievych & Smith, 2003), this lectotypification continues to find adherents (Hassler & Swale, 2003; van den Heede & al., 2004; Bäumler & al., 2005; Jelaska & al., 2005; Eggenberg & Landolt, 2006; Smarda & Bures, 2006; Selvi, 2007; Vanderpoorten & al., 2007). With the three-gene phylogeny presented in Fig. 2, we are finally in a position to assess the nomenclatural consequences of adopting *N. marantae* as the type of *Notholaena*.

First, if the concept of *Notholaena* (typified with *N. marantae*) were expanded beyond *N. marantae* itself and perhaps a few very close relatives, it would encompass the node subtending *Pellaea atropurpurea*, the type of *Pellaea* (Fig. 2). Because *Notholaena* is the older name, approximately 30 taxa of *Pellaea* sect. *Pellaea* would require new names under *Notholaena* (such names do not currently exist). Additionally, "*Notholaena*" would then be unavailable for the New World taxa related to *N. trichomanoides*. The taxa of the "*N. grayi*–*N. standleyi* alliance" (Fig. 2; Windham 1993a) largely have valid names published in the segregate genus *Chrysochosma* (typified with *N. sulphurea*; Pichi Sermolli, 1989), but the position of *Cheiloplecton* within that clade (Fig. 2) renders that name unavailable. *Cheiloplecton* was published in 1857 (Fée, 1857), 57 years before *Chrysochosma* (Kümmerle, 1914). While the taxa of the *N. grayi*–*N. standleyi* alliance have validly published names in *Notholaena*, *Chrysochosma*, and *Cheilanthes*, they have none under *Cheiloplecton*, and thus an additional ~30 names would be necessary. An alternative approach with *N. marantae* as type would be to recognize the core *Notholaena* clade as *Chrysochosma*, retain a monotypic *Cheiloplecton*, and create segregate genera for each of the *C. aurantiaca*/*C. aurea*/*C. brachypus* clade, *Notholaena standleyi*, and *C. leucopoda* (Fig. 2). We consider this latter approach (splitting the notholaenoids into five genera, three of which would have to be new) to be inadvisable given the apparent lack of morphological synapomorphies for the *C. aurantiaca*/*C. aurea*/*C. brachypus* clade and the close similarity of *N. standleyi* and *N. sulphurea* (the type of *Chrysochosma*) in nearly every feature examined.

The nomenclatural consequences of overturning *N. trichomanoides* in favor of *N. marantae* add additional weight to the arguments of Yatskievych & Smith (2003). Indeed, the nomenclatural instability that would result from adopting *N. marantae* as the type, as demonstrated by our phylogeny, is sufficient to violate the *Code's* "do the least damage" provisions (McNeill & al., 2006: Art. 14.2), rendering this option increasingly untenable. While the precise delimitation of *Notholaena* will require further research—research that will undoubtedly reveal additional interesting patterns of character evolution in these desert ferns—that circumscription should not include *N. marantae* and its allies.

Summary and future prospects. — By providing phylogenetic information independent of morphology, our three-gene phylogeny allows us to resolve long-standing debates about the position of enigmatic taxa within the cheilanthoids. *Cheilanthes newberryi* and *C. bonariensis* are in the myriopterioid clade of New World *Cheilanthes*

(MYR; Fig. 2 arrows a, b) and should be excluded from *Notholaena*. *Argyrochosma* and *Astrolepis* are in the pellaeoid clade (PEL; Fig. 2) and thus their segregation from *Notholaena* is supported. Finally, *N. bryopoda* is indeed a *Notholaena*, as suggested by its chromosome base number of $x = 30$ and farinose gametophytes (Figs. 2, 5B–C).

The majority of *Notholaena* s.str. (farinose taxa with poorly developed false indusia—the "core *Notholaena* group") forms a monophyletic clade in our analyses (cN; Fig. 2), a clade that contains the earliest *Notholaena* lectotype, *N. trichomanoides*. The position of *N. trichomanoides* within this New World clade, and far from the other two *Notholaena* lectotypes—*N.distans* (in the hemionitidoids; HEM; Fig. 2) and *N. marantae* (in the pellaeoids; PEL; Fig. 2)—adds further support in favor of its conservation, specifically against *N. marantae*, which has frequently been considered the type of *Notholaena*.

Our phylogeny highlights the extensive morphological homoplasy that (combined with extreme morphological disparity) has historically made *Notholaena*, and cheilanthoids in general, such a taxonomic challenge (Figs. 3–5). *Cheiloplecton rigidum* and the *Cheilanthes aurea*/*C. aurantiaca*/*C. brachypus* clade, for example, are embedded within *Notholaena* s.str. (Fig. 2). This novel result reflects complex patterns of evolution within the notholaenoids involving characters pertinent to both adaptation to xeric habitats (farina, leaf shape and division), and to reproduction and dispersal (sporangial arrangement, presence of false indusia; Fig. 4).

The amount of morphological homoplasy among cheilanthoids is comparable to that detected by Ranker & al. (2004) in their study of grammitid ferns, and similarly includes characters commonly used for generic-level classifications. Such homoplasy is taxonomically widespread (Lowrey & al., 2001; Manuel & al., 2003; Mueller & al., 2004) and potentially useful from a research perspective, in that it may indicate repeated independent evolutionary "experiments" resulting in similar morphological outcomes, and thus may provide greater power for elucidating evolutionary processes via the comparative method (Harvey & Pagel, 1991).

The well-supported phylogenetic relationships presented here additionally permit a reexamination of informative characters in this group. Several characters previously considered to be important for generic-level classifications (false indusia, presence of farina) are homoplastic within the notholaenoids, but do show some phylogenetic structure (Fig. 4). Additional characters—leaf shape and indumentum, chromosome base number, geography—exhibit surprising levels of phylogenetic information (Figs. 4–5), while farinose gametophytes may be a synapomorphy for the notholaenoids (Fig. 5C).

These potential synapomorphies require further investigation. For many taxa, we do not know whether the gametophytic generation has farina, and increased taxon sampling is necessary to confirm these patterns of morphological evolution, particularly if other as-yet unsampled taxa are aligned with the notholaenoids. A phylogeny containing most or all extant species would allow a more-detailed investigation of morphological evolution within these morphologically disparate ferns, and provide the necessary phylogenetic framework for a comprehensive taxonomic revision.

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Appendix. Taxon; voucher specimen (herbarium); collection locality; Fern DNA Database number; GenBank accession number (and citations, for previously published data) for *rbcL*; *atpA*; *trnG-trnR* (in that order). Fern DNA Database: pryerlab.net/DNA_database.shtml; "–" indicates missing data.

Adiantopsis radiata (L.) Fée; *Christenhusz 4033* (TUR); Guadaloupe; 3313; EF452131 (Schuettpelz & al., 2007); EU268718; EU268664. *Aleuritopteris argentea* (S.G. Gmelin) Fée; *Yatskievych 01-23* (MO); China; 3734; EF452137 (Schuettpelz & al., 2007); EU268719; EU268665. *Aleuritopteris farinosa* 1 (Forssk.) Fée; *Windham & al. 541* (DUKE); Oaxaca, Mexico; 4057; EU268770; EU268720; EU268666. *Aleuritopteris farinosa* 2; *Kayombo 2700* (DUKE); Tanzania; 4406; –; EU268721; EU268667. *Argyrochosma incana* (Presl) Windham; *Schuettpelz 491 & al.* (DUKE); Arizona, U.S.A.; 3198; EU268771; –; –. *Argyrochosma jonesii* (Maxon) Windham; *Windham 3437 & Pryer* (DUKE); California, U.S.A.; 3844; EU268772; –; –. *Argyrochosma limitanea* (Maxon) Windham subsp. *limitanea*; *Schuettpelz & al. 472* (DUKE); Arizona, U.S.A.; 3179; EF452139 (Schuettpelz & al., 2007); EU268722; EU268668. *Aspidotis densa* (Brack.) Lellinger; *Pryer & al. 06-02* (DUKE); Oregon, U.S.A.; 3870; EU268773; EU268723; EU268669. *Astrolepis cochisensis* (Goodd.) D.M. Benham & Windham subsp. *cochisensis*; *Schuettpeltz & al. 453* (DUKE); Arizona, U.S.A.; 3160; EU268774; –; –. *Astrolepis sinuata* (Lag. ex Sw.) D.M. Benham & Windham; *Schuettpelz & al. 310* (DUKE); cult., orig. from Texas, U.S.A.; 2955; EF452141 (Schuettpelz & al., 2007); EU268724; EU268670. *Bommeria hispida* (Mett. ex Kuhn) Underw.; *Schuettpelz & al. 467* (DUKE); Arizona, U.S.A.; 3174; EF452142 (Schuettpelz & al., 2007); EU268725; EU268671. *Cheilanthes alabamensis* (Buckley) Kunze; *Windham 3448 & Yatskievych* (DUKE); Oklahoma, U.S.A.; 4511; EU268775; EU268726; EU268672. *Cheilanthes arizonica* (Maxon) Mickel; *Schuettpelz & al. 461* (DUKE); Arizona, U.S.A.; 3168; EU268776; EU268727; EU268673. *Cheilanthes aurantiaca* (Cav.) Moore; *Yatskievych & Gastony 89-285* (IND); Morelos, Mexico; 4515; EU268777; EU268728;

Appendix. Continued.

EU268674. *Cheilanthes aurea* 1 Baker; *Windham & al. 544* (DUKE); Oaxaca, Mexico; 4055; EU268778; EU268729; EU268675. *Cheilanthes aurea* 2; *Yatskievych & Gastony 89-256* (IND); Oaxaca, Mexico; 4514; EU268779; EU268730; EU268676. *Cheilanthes bonariensis* (Willd.) Proctor; *Schuettpelz & al. 466* (DUKE); Arizona, U.S.A.; 3173; EU268780; EU268731; EU268677. *Cheilanthes brachypus* Kunze; *Yatskievych & Gastony 89-236* (IND); Jalisco, Mexico; 4517; EU268781; EU268732; EU268678. *Cheilanthes covillei* Maxon; *Schuettpelz & al. 443* (DUKE); Arizona, U.S.A.; 3150; EU268782; EU268733; EU268679. *Cheilanthes distans* (R. Br.) Mett.; *Nagalingum 23* (DUKE); Australia; 3894; EU268783; EU268734; EU268680. *Cheilanthes lendigera* (Cav.) Sw.; *Schuettpelz 460* (DUKE); Arizona, U.S.A.; 3167; EU268784; EU268735; EU268681. *Cheilanthes leucopoda* Link; *Villarreal 5801 & Carranza* (ARIZ); Durango, Mexico; 4506; EU268785; EU268736; EU268682. *Cheilanthes micropteris* Sw.; *Deginani & al. 1363* (MO); Argentina; 3709; EF452145 (Schuettpelz & al., 2007); –; EU268683. *Cheilanthes myriophylla* Desv.; *Brown 83-31-4* (IND); San Luis Potosi, Mexico; 4484; EU268786; EU268737; EU268684. *Cheilanthes newberryi* Domin; *Metzgar & al. 174* (DUKE); California, U.S.A.; 3827; EU268787; EU268738; EU268685. *Cheiloplecton rigidum* 1 (Sw.) Fée subsp. *lanceolatum* C.C. Hall ex Mickel & Beitel; *Windham & al. 522* (UT); Puebla, Mexico; 4056; EU268788; –; –. *Cheiloplecton rigidum* 2 subsp. *lanceolatum*; *Yatskievych & Gastony 89-284* (IND); Puebla, Mexico; 4518; EU268789; EU268739; EU268686. *Cryptogramma crispa* (L.) R. Br. ex Hook.; *Christenhusz & Katzer* 3871 (TUR; DUKE); Scotland; 2949; EF452148 (Schuettpelz & al., 2007); EU268740; EU268687. *Doryopteris ludens* (Wall. ex Hook.) J. Sm.; *Schneider s.n.* (GOET); cult., orig unknown; 3510; EF452150 (Schuettpelz & al., 2007); EU268741; EU268688. *Doryopteris sagittifolia* (Raddi) J. Sm.; *Schuettpelz 562 & Schneider* (GOET); cult., orig unknown; 3617; EF452151 (Schuettpelz & al., 2007); EU268742; EU268689. *Hemionitis palmata* L.; *Schuettpelz 297* (DUKE); cult., orig unknown; 2557; AY357708 (Ranker & Geiger, unpub.); EU268743; EU268690. *Notholaena aliena* Maxon; *Windham & Yatskievych* 761 (DUKE); Texas, U.S.A.; 4059; EU268790; EU268744; EU268691. *Notholaena aschenborniana* Klotzsch; *Schuettpelz & al. 476* (DUKE); Arizona, U.S.A.; 3183; EF452159 (Schuettpelz & al., 2007); EU268745; EU268692. *Notholaena bryopoda* Maxon; *Windham & al. 485* (DUKE); Nuevo Leon, Mexico; 4058; EU268791; EU268746; EU268693. *Notholaena californica* D.C. Eaton; *Schuettpelz & al. 436* (DUKE); Arizona, U.S.A.; 3143; EU268792; EU268747; EU268694. *Notholaena candida* (M. Martens & Galeotti) Hk.; *Windham & al. 521* (DUKE); Puebla, Mexico; 4062; EU268793; EU268748; EU268695. *Notholaena copelandii* C.C. Hall; *Windham & al. 472* (DUKE); Nuevo Leon, Mexico; 4504; –; –; EU268696. *Notholaena grayi* Dav. subsp. *grayi*; *Schuettpelz & al. 480* (DUKE); Arizona, U.S.A.; 3187; EU268794; EU268749; EU268697. *Notholaena grayi* Dav. subsp. *sonorensis* Windham; *Schuettpelz & al. 490* (DUKE); Arizona, U.S.A.; 3197; EU268795; EU268750; EU268698. *Notholaena greggii* (Mett.) Maxon; *Yatskievych & McCrary 85-10* (DUKE); Texas, U.S.A.; 4060; EU268796; EU268751; EU268699. *Notholaena lemmonii* Eat. var. *lemmonii*; *Schuettpelz & al. 457* (DUKE); Arizona, U.S.A.; 3164; EU268797; EU268752; EU268700. *Notholaena neglecta* Maxon; *Schuettpelz & al. 477* (DUKE); Arizona, U.S.A.; 3184; EU268798; EU268753; EU268701. *Notholaena rigida* Dav.; *Windham & al. 491* (DUKE); Tamaulipas, Mexico; 4408; EU268799; EU268754; EU268702. *Notholaena rosei* Maxon; *Windham & al. 542* (DUKE); Oaxaca, Mexico; 4409; EU268800; EU268755; EU268703. *Notholaena schaffneri* (E. Fourn.) Underw. ex Dav.; *Windham & al. 526* (DUKE); Oaxaca, Mexico; 4061; EU268801; EU268756; EU268704. *Notholaena standleyi* 1 Maxon; *Schuettpelz & al. 435* (DUKE); Arizona, U.S.A.; 3142; EU268802; EU268757; EU268705. *Notholaena standleyi* 2; *Metzgar & al. 129* (DUKE); New Mexico, U.S.A.; 3783; EU268803; EU268758; EU268706. *Notholaena standleyi* 3; *Windham & al. 94-164* (DUKE); Arizona, U.S.A.; 4503; EU268804; EU268759; EU268707. *Notholaena standleyi* 4; *Windham & al. 94-161* (DUKE); Texas, U.S.A.; 4502; EU268805; EU268760; EU268708. *Notholaena sulphurea* (Cav.) J. Sm.; *Windham & al. 488* (DUKE); Tamaulipas, Mexico; 4411; EU268806; EU268761; EU268709. *Notholaena trichomanoides* (L.) R. Br.; *Ranker 860 & Trapp* (UT); Jamaica; 4054; EU268807; EU268762; EU268710. *Paragymnopteris delavayi* (Baker) K.H. Shing; *Zhang 268* (PE); Sichuan, China; –; DQ432654 (Zhang & al., 2007); –; –. *Paragymnopteris marantae* (L.) K.H. Shing; *Yatskievych & al. 02-35* (MO); China; 3736; EF452161 (Schuettpelz & al., 2007); EU268763; EU268711. *Pellaea atropurpurea* (L.) Link; *Schuettpeltz 312* (DUKE); cult., orig. from Virginia, U.S.A.; 2957; EF452162 (Schuettpelz & al., 2007); –; –. *Pellaea breweri* D.C. Eaton; *Windham 3447 & Pryer* (DUKE); Utah, U.S.A.; 3930; EU268808; EU268764; EU268712. *Pellaea intermedia* Mett. ex Kuhn; *Schuettpelz & al. 481* (DUKE); Arizona, U.S.A.; 3188; EF452163 (Schuettpelz & al., 2007); EU268765; EU268713. *Pellaea truncata* Goodd.; *Schuettpelz & al. 430* (DUKE); Arizona, U.S.A.; 3137; EF452164 (Schuettpelz & al., 2007); EU268766; EU268714. *Pellaea viridis* Sw.; *Janssen 2701* (P); Ile de la Reunion, France; 3555; EF452147 (Schuettpelz & al., 2007); EU268767; EU268715. *Pentagramma triangularis* (Kaulf.) Yatsk., Windham & Wollenw. subsp. *maxonii* (Weath.) Yatsk., Windham & Wollenw.; *Schuettpelz & al. 445* (DUKE); Arizona, U.S.A.; 3152; EF452165 (Schuettpelz & al., 2007); EU268768; EU268716. *Pityrogramma austroamericana* Domin; *Schuettpelz 301* (DUKE); cult., orig. unknown; 2561; EF452166 (Schuettpelz & al., 2007); EU268769; EU268717.