AMERICAN FERN JOURNAL

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Using Plastid and Nuclear DNA Sequences to Redraw Generic Boundaries and Demystify Species Complexes in Cheilanthoid Ferns

MICHAEL D. WINDHAM, LAYNE HUIET, ERIC SCHUETTPELZ, AMANDA L. GRUSZ, CARL ROTHFELS, and JAMES BECK, Department of Biology, Duke University, Durham, NC 27708-0339, GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, and KATHLEEN M. PRYER, Department of Biology, Duke University, Durham, NC 27708-0339.

Using Plastid and Nuclear DNA Sequences to Redraw Generic Boundaries and Demystify Species Complexes in Cheilanthoid Ferns.—Cheilanthoid ferns constitute a monophyletic group of 400–500 species within the Pteridaceae (Smith *et al.*, 2006; Schuettpelz and Pryer, 2007; Schuettpelz *et al.*, 2007). They are noteworthy for their ability to colonize xeric and semi-xeric habitats, niches that are rarely exploited by other ferns (Tryon and Tryon, 1979, 1982). Relationships within this lineage are highly problematic, and cheilanthoids have been called ''the most contentious group of ferns with respect to a practical and natural generic classification'' (Tryon and Tryon, 1982: 248). It is not surprising, then, that molecular phylogenetic analyses to date have revealed that most of the larger cheilanthoid genera are polyphyletic (Gastony and Rollo, 1998; Kirkpatrick, 2007; Prado *et al.*, 2007; Schuettpelz *et al.*, 2007; Zhang *et al.*, 2007; Rothfels *et al.*, 2008). Cheilanthoid ferns have long been a topic of interest for Dr. Gerald Gastony, the honoree of this collection of papers. His contributions run the gamut from studies of chromosome numbers and apomixis in *Bommeria* E. Fourn. (Gastony and Haufler, 1976), through genetic analyses of various species groups (Gastony, 1988; Gastony *et al.*, 1992), to documenting tetrasomic inheritance and gene silencing in polyploids (Gastony, 1990, 1991), and maternal inheritance of plastids in *Pellaea* Link (Gastony and Yatskievych, 1992). His phylogenetic studies of cheilanthoids (Gastony and Rollo, 1995, 1998) were the first to demonstrate that *rbcL* sequences could provide a valuable, independent tool for circumscribing genera in this taxonomically controversial group of ferns.

We are now poised to take the ''next step'' toward redefining generic boundaries among the cheilanthoids. It is clear that the number of genes and taxa analyzed must be significantly increased if we hope to obtain a robust phylogeny of the group. To this end, we have initiated a large-scale phylogenetic study using DNA sequences derived from three plastid regions (*rbcL*, *atpA*, *trnG-R*). To date, we have sequenced all three plastid regions (representing nearly 4000 base pairs) for 157 species. Maximum likelihood analyses of these data identify seven, well-supported subclades of cheilanthoid ferns (Fig. 3).

Ludens clade.—Previously published analyses (Schuettpelz *et al.*, 2007; Zhang *et al.*, 2007) revealed that *Doryopteris ludens* (Wall. ex Hook.) J. Sm. is not closely related to most taxa traditionally placed in this genus, including the type species, *D. palmata* (Willd.) J. Sm. Whereas *Doryopteris* J. Sm. in the strict sense is strongly supported as a member of the hemionitid clade (Fig. 3), *D. ludens* and its close allies appear to represent a rather isolated lineage within the Pteridaceae. Analyses by Schuettpelz *et al.* (2007) resolved *D. ludens* as sister to all other cheilanthoid ferns while those of Zhang *et al.* (2007) suggested a possible affinity to other pteroid lineages. Though the placement of this species varies depending on taxon sampling, it is clear that it

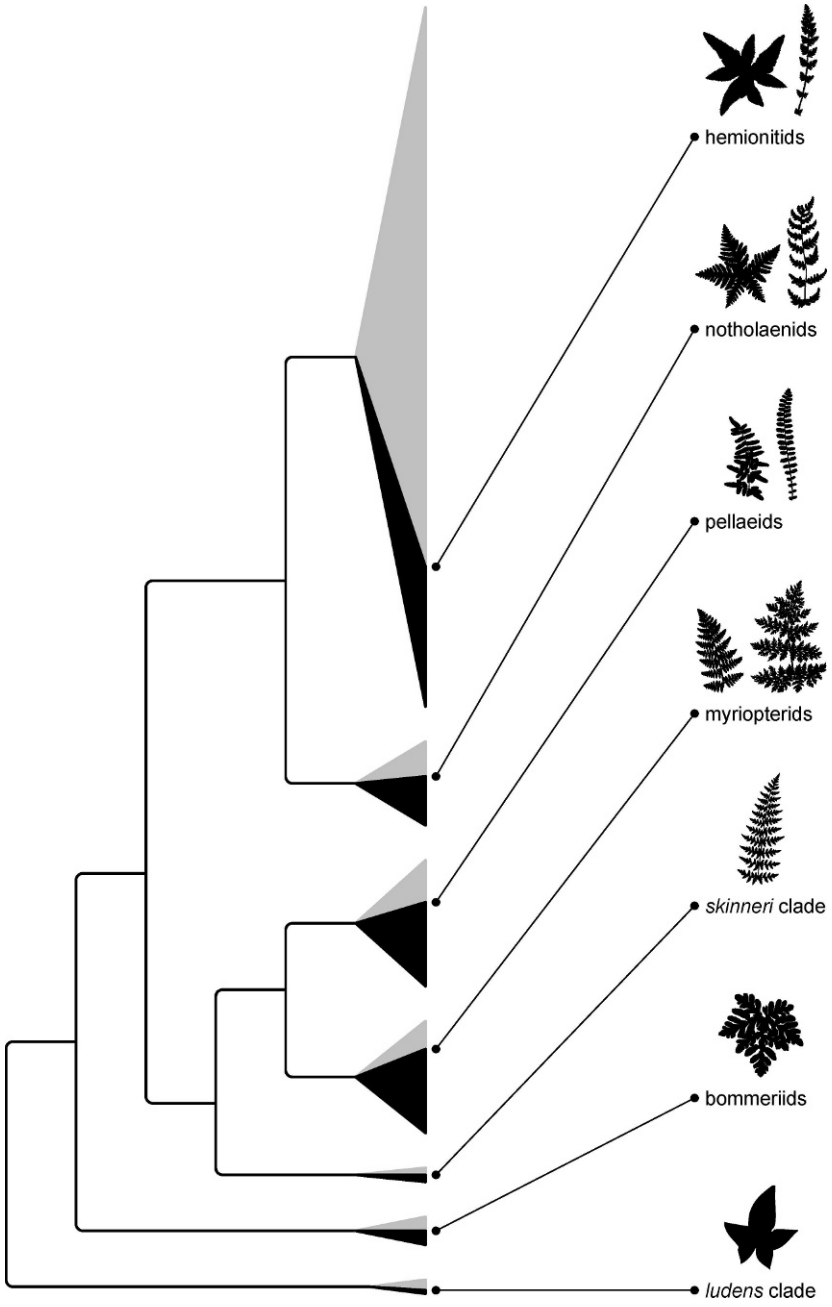

FIG. 3. Summary of phylogenetic relationships within cheilanthoid ferns. Topology results from maximum likelihood analyses of *atpA*, *rbcL*, and *trnG-R* sequence data for 157 species; tree rooted with *Doryopteris ludens*. Thumbnails identify seven, well-supported cheilanthoid clades. Triangles indicate proportion of named species belonging to each clade; darker portion of each triangle represents the proportion of species included in the current analysis.

is more closely related to cheilanthoid ferns than to any other potential outgroup sampled to date. For this reason, we have used it in our analyses to root the remaining cheilanthoid tree. The *D. ludens* clade encompasses a total of four species (only one of which is included in our sample) whose combined range extends from continental Asia to New Guinea. Because its phylogenetic divergence and geographic isolation from *Doryopteris s.s.* are substantial, this lineage is in the process of being transferred to a new genus: ''*Calciphilopteris*'' (Yesilyurt and Schneider, in press).

Bommeriids.—As shown in earlier studies (Gastony and Rollo, 1995, 1998), species of *Bommeria sensu lato* (including *B. elegans* (Davenp.) Ranker & Haufler; see Ranker and Haufler, 1990) are sister to all cheilanthoids other than the *D. ludens* clade. Our data confirm Ray Cranfill's (unpubl. data) assignment of *Cheilanthes brandegeei* D. C. Eaton to this clade, suggesting that the circumscription of *Bommeria* may need to be expanded yet again. Some species that Tryon and Tryon (1982) considered close relatives of *C. brandegeei* are strongly supported as members of the notholaenid clade in our analyses (Rothfels *et al.*, 2008), and these already have been transferred to *Notholaena* (Yatskievych and Arbelaez, 2008). The remaining members of the ''*C. brandegeei* group'' (*sensu* Tryon and Tryon, 1982) need to be sampled before the bommeriid clade can be accurately delimited. Based on available data, we estimate that this lineage ultimately will encompass about 2% of cheilanthoid species, half of which have now been included in our analyses.

Skinneri clade.—In a recent parsimony analysis of *rps4*, *rps4-trnS*, and *trnL-F* sequences by Kirkpatrick (2007), *Cheilanthes skinneri* (Hook.) R.M. Tryon & A.F. Tryon was weakly supported as sister to all cheilanthoids other than *Bommeria* (the *ludens* clade was not included in her sampling). In our studies, this taxon is strongly supported as sister to the myriopterid + pellaeid clade; together, these three clades are sister to the notholaenid + hemionitid clade (Fig. 3). Our molecular data also support a close relationship between *C. skinneri* and *C. lozanoi* (Maxon) R.M. Tryon & A.F. Tryon, an association previously proposed based on morphology (Mickel, 1987). Although these species have been transferred back and forth between *Pellaea* (in the pellaeid clade) and *Cheilanthes* (hemionitid clade) in the past, our data indicate that neither generic placement is tenable. Mickel (1987) identified several other taxa that may be related to *C. skinneri*, and these must be sampled before we can adequately circumscribe the clade and determine the correct generic name for it. Based on the available data, we estimate that this primarily North American lineage will include 4–5 species (about 1% of cheilanthoid diversity), two of which were included in the current analysis.

Myriopterids.—This clade encompasses a group of primarily North American species traditionally placed in *Cheilanthes*. A similar assemblage, also sister to the pellaeid clade, was recovered by both Gastony and Rollo (1998) and Kirkpatrick (2007). Our analyses indicate that this group is only distantly related to the type species of *Cheilanthes* (*C. micropteris* Sw., a member of the hemionitid clade) and, as such, all included taxa will need to be transferred to another genus (Grusz *et al.*, in prep.). The type species of *Myriopteris* Fée

(1852), *Cheilosoria* Trev. (1877), and *Pomatophytum* M.E. Jones (1930) all belong to this clade, so there is no shortage of potential names. The challenge will be to identify morphological features that consistently separate this group from *Cheilanthes sensu stricto*. We estimate that this lineage comprises approximately 10% of cheilanthoid diversity; 75% of recognized species have been sampled to date.

Pellaeids.—In addition to *Pellaea s.s.* (described by Link in 1841), this clade includes four genera named within the last 70 years: *Argyrochosma* (J. Sm.) Windham, *Astrolepis* D.M. Benham & Windham, *Paraceterach* Copel., and *Paragymnopteris* K.H. Shing. As revealed by earlier molecular analyses (Gastony and Rollo, 1998; Kirkpatrick, 2007), *Argyrochosma* (with ca. 30 species) is sister to all other pellaeids and can continue to be recognized as a distinct genus as proposed by Windham (1987). The other three genera, although morphologically more divergent than *Argyrochosma*, are nested within the traditional circumscription of *Pellaea* section *Pellaea*. It appears that members of this clade have switched from a typical *Pellaea* morphology (highly divided, nearly glabrous leaves) to an *Astrolepis-Paraceterach-Paragymnopteris* morphology (usually simply pinnate, densely scaly or hairy leaves) on no less than three occasions on three different continents. The taxonomic problems posed by this situation are not easily resolved; Kirkpatrick (2007) provided a good discussion of the potential synapomorphies of each pellaeid subclade and the various nomenclatural options. The pellaeid clade comprises about 12% of cheilanthoid diversity; 65% of the species are represented in our analyses and additional representatives were sampled by Kirkpatrick (2007).

Notholaenids.—This primarily North American lineage, the subject of a recent study by Rothfels *et al.* (2008), is sister to the large, cosmopolitan hemionitid clade. Most of the species included in the notholaenids are farinose, with abaxial leaf surfaces covered by ''powdery'' (predominantly flavonoid) deposits produced by underlying glandular trichomes. This feature has often been considered a synapomorphy for the genus *Notholaena* R. Br. (*sensu* Yatskievych and Smith, 2003), but our data place two nonfarinose taxa deep within the clade and a strongly glandular, but non-farinose, species as the earliest diverging branch. Additional morphological studies are underway (Rothfels *et al.*, in prep.) to identify characters that can be used to circumscribe an expanded *Notholaena*. This lineage comprises roughly 8% of cheilanthoid diversity; 60% of recognized species have been sampled to date.

Hemionitids.—This is, by far, the largest and most diverse clade of cheilanthoids; its members are found on every continent except Antarctica and the geographic ranges of two species, *Cheilanthes farinosa* (Forssk.) Kaulf. and *C. concolor* (Langsd. & Fisch.) R.M. Tryon & A.F. Tryon, cover most of the subtropics (Tryon and Tryon, 1973). The lineage includes the type species of more than a dozen genera named between 1753 (*Hemionitis* L.) and 1991 (*Pentagramma* Yatsk., Windham & E. Wollenw.). Nearly all of these generic names are associated with well-supported subclades in our analyses, but relationships among these groups are largely unresolved in the plastid tree.

The hemionitid lineage appears to have undergone a rapid radiation (possibly associated with its colonization of new habitats and continents), and much additional data will be needed to clarify generic boundaries in this group. We estimate that this lineage comprises about 67% of cheilanthoid diversity; only 20% of known species are represented in the current analysis.

Future directions.—Ultimately, we hope to include more than 60% of cheilanthoid species in our studies, with a special emphasis on under-sampled diversity hotspots in South America and Africa. The type species of all validly named genera will be sampled, as well as the majority of species of uncertain or disputed relationship. Phylogenetic analyses of these plastid DNA sequences will be used to identify well-supported monophyletic lineages. These clades can then be evaluated for morphological synapomorphies that will provide the foundation for a revised generic classification.—Michael D. Windham, Layne Huiet, Eric Schuettpelz, Amanda L. Grusz, Carl Rothfels, and James Beck, Department of Biology, Duke University, Durham, NC 27708-0339, George Yatskievych, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, and Kathleen M. Pryer, Department of Biology, Duke University, Durham, NC 27708-0339.