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Patterns of Diversification in the Xeric-adapted Fern Genus *Myriopteris* (Pteridaceae)

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Abstract—Strong selective pressures imposed by drought-prone habitats have contributed to extensive morphological convergence among the 400 + species of cheilanthoid ferns (Pteridaceae). As a result, generic circumscriptions based exclusively on macromorphology often prove to be non-monophyletic. Ongoing molecular phylogenetic analyses are providing the foundation for a revised classification of this challenging group and have begun to clarify its complex evolutionary history. As part of this effort, we generated and analyzed DNA sequence data for three plastid loci (*rbcL*, *atpA*, and the intergenic spacer *trnG–trnR*) for the myriopterid clade, one of the largest monophyletic groups of cheilanthoid ferns. This lineage encompasses 47 primarily North and Central American taxa previously included in *Cheilanthes* but now placed in the recircumscribed genus *Myriopteris*. Here, we infer a phylogeny for the group and examine key morphological characters across this phylogeny. We also include a brief discussion of the three well-supported *Myriopteris* subclades, along with a review of reproductive mode and known ploidy levels for members of this early diverging lineage of cheilanthoid ferns.

Keywords—Apomixis, *Cheilanthes*, convergence, molecular phylogeny, myriopterid.

Cheilanthoid ferns have been called "the most contentious group of ferns with respect to practical and natural generic classification" (Tryon and Tryon 1982: 248). Members of this clade are best known for their ability to thrive in habitats too dry for most other ferns, and the taxonomic confusion plaguing the group has often been attributed to extensive morphological convergence resulting from selection imposed by arid environments (Tryon and Tryon 1973, 1982; Kramer et al. 1990; Rothfels et al. 2008). A recent series of molecular systematic studies (Gastony and Rollo 1998; Kirkpatrick 2007; Prado et al. 2007; Schuettpelz et al. 2007; Zhang et al. 2007; Rothfels et al. 2008; Windham et al. 2009; Beck et al. 2010; Eiserhardt et al. 2011; Link-Perez et al. 2011; Sigel et al. 2011; Li et al. 2012) has begun to clarify relationships among the 400 + species of cheilanthoid ferns and provides the foundation for a new, phylogenetically-based classification of the group.

These studies indicate that the most significant barrier to recognizing monophyletic genera within the cheilanthoid clade is the current circumscription of the genus *Cheilanthes* Sw. Every molecular phylogenetic analysis with broad sampling across cheilanthoids has shown that *Cheilanthes* is polyphyletic; species currently assigned to the genus reside in five of the six major cheilanthoid clades identified by Rothfels et al. (2008), Windham et al. (2009), and Eiserhardt et al. (2011). For this reason, taxonomists are working to redefine the genus by segregating out monophyletic groups that are not closely related to the generitype, *Cheilanthes micropteris* Sw. One such clade that is phylogenetically distant from *Cheilanthes* s. s. has recently been transferred to the genus *Myriopteris* (Fig. 1; see Grusz 2013; Grusz and Windham 2013). Aside from a single disjunct species endemic to southern Africa and a few widespread species that extend to South America and certain Caribbean islands, members of this group are limited to North and Central America whereas *Cheilanthes* s. s. is largely confined to the Southern Hemisphere. Previously referred to as the myriopterid ferns, this clade contains roughly 10% of all cheilanthoid species diversity (Fig. 1; Windham et al. 2009) and thus constitutes a critical group for phylogenetic analysis.

Previous studies have shown that the myriopterids constitute a well-supported clade (e.g. Windham et al. 2009; Eiserhardt et al. 2011), yet phylogenetic relationships among the species of this group are poorly known. To better understand the evolutionary history of the newly recircumscribed genus *Myriopteris*, we estimate a phylogeny for the clade and map key morphological characters across this phylogeny. Because polyploidy and apomixis are important evolutionary processes among myriopterid ferns, we also summarize the available data on reproductive mode and ploidy level for all species included in our analyses, and examine their distribution across the myriopterid tree.

Materials and Methods

Taxon Sampling—A total of 68 accessions representing 40 (of 47 total) myriopterid taxa were included in our molecular phylogenetic analyses (Table 1). Four outgroup taxa (*Argyrochosma microphylla*, *Astrolepis windhamii*, *Paragymnopteris marantae*, and *Pellaea atropurpurea*) were selected from the pellaeid clade, which was resolved as sister to *Myriopteris* in all previous molecular studies with sufficient sampling (Gastony and Rollo 1998; Kirkpatrick 2007; Rothfels et al. 2008; Windham et al. 2009; Eiserhardt et al. 2011). We included multiple accessions of wide ranging taxa within *Myriopteris*, attempting to sample across their geographic distribution.

DNA Extraction, Amplification, and Sequencing—For each individual sampled (see Appendix 1), genomic DNA was extracted from silica-dried leaf fragments or air-dried herbarium specimens using the DNeasy plant mini kit (Qiagen, Valencia, California) following the protocol described in Schuettpelz and Pryer (2007). Three plastid loci, *rbcL* (1,343 bp), *atpA* (1,872 bp), and the intergenic spacer, *trnG–trnR* (1,293 bp), were amplified for all accessions. The PCR reactions were conducted using 1 × PCR buffer IV containing $MgCl_2$ (ABgene, Epsom, U. K.), combined with 200 μM each dNTP, 100 μg/ml BSA, 50 U/ml Taq polymerase, 0.5 μM of each locus-specific primer pair (Table 2), and 1 μl template DNA for a 25 μl reaction. The PCR amplifications entailed an initial denaturation step (94°C for 5 min) followed by 35 denaturation, annealing, and elongation cycles (94°C for 1 min, 45°C for 2 min, and 72°C for 2 min) and a final elongation step (72°C for 10 min). Amplicons were visualized on a 1% agarose gel. The PCR purification and sequencing followed the protocol of Grusz et al. (2009). All 178 newly obtained sequences were subsequently deposited in GenBank (Appendix 1).

Sequence Alignment and Data Sets—Sequence fragments were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Michigan). Manual alignments of the resulting consensus sequences were

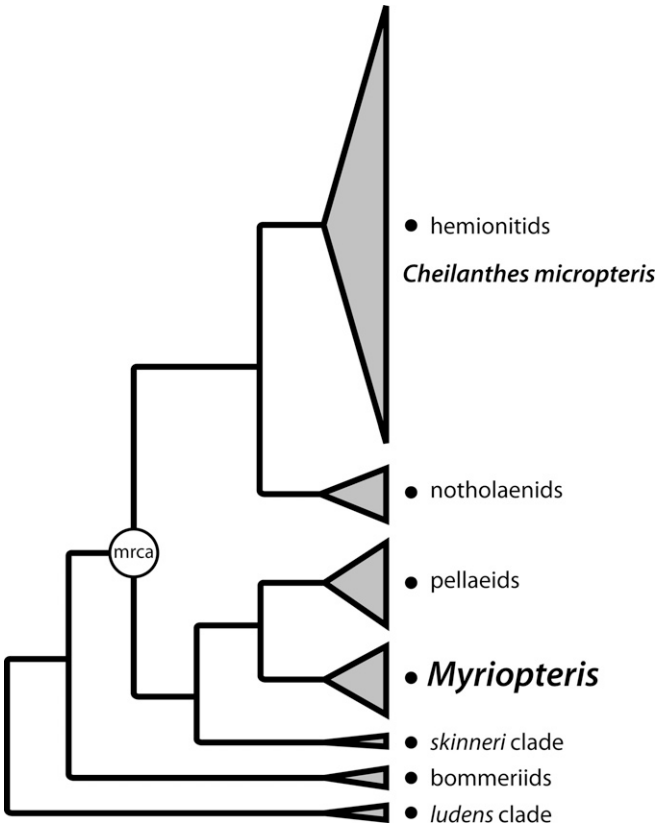

FIG. 1. Summary phylogeny for cheilanthoid ferns, indicating the placement of *Cheilanthes micropteris* (type species for *Cheilanthes*) within the hemionitid clade, only distantly related to the myriopterid clade. The six major clades of cheilanthoid ferns are shown with tip lengths roughly proportional to clade size. The most recent common ancestor (mrca) of *C. micropteris* and the myriopterid clade is indicated. Modified with permission from Windham et al. (2009).

then performed in MacClade 4.08 (Maddison and Maddison 2005). Because alignments could be completed by eye (i.e. they lacked extensive indels and/or ambiguous regions), implementation of a specific alignment criterion was unnecessary. For each alignment, portions of the 5′ and 3′ ends with large amounts of missing data were excluded; ambiguously aligned indels were also excluded.

A total of four data sets were subjected to phylogenetic analysis: the three plastid single-locus data sets (*rbcL, atpA,* and *trnG–trnR*), and a combined three-locus data set (*rbcL* + *atpA* + *trnG–trnR*).

The alignment of non-coding regions within the *trnG–trnR* spacer included a substantial number of ambiguous regions when both ingroup and outgroup taxa were included. For this reason, outgroup taxa were removed from the *trnG–trnR* single-locus alignment, as well as from the *trnG–trnR* portion of the three-locus combined alignment.

***Phylogenetic Analyses*—**Each of the four data sets was evaluated using maximum likelihood (ML; Felsenstein 1973) and Bayesian inference (BI; Yang and Rannala 1997). The ML analyses were run on CIPRES (www.phylo.org; Miller et al. 2010) and BI analyses were run on the Duke University DSCR cluster. The ML analyses were implemented in GARLI 2.0 (Zwickl 2006), where a most-likely topology was identified for each of the four data sets and branch support was assessed separately using a maximum likelihood bootstrap approach (MLBS). Initial searches using a GTR + I + Γ model of sequence evolution (the most complex yet computationally tractable model currently available, and thus interpreted to best reflect reality) failed to reach stationarity in the BI analyses; therefore, the second most complex model, GTR + Γ (not allowing for estimation of the proportion of invariant sites; invariantsites = none), was used in both ML and BI analyses. The optimal-tree search was repeated for eight replicates to ensure a most-likely topology (Garli Manual, Zwickl 2006); MLBS analyses were conducted using 1,000 bootstrap replicates, each with a single pseudoreplicate.

The BI analyses were implemented in MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). All BI analyses comprised four independent runs, each with four chains (one cold and three heated). A GTR + Γ model of sequence evolution (rates = gamma) was applied with otherwise default (i.e. flat) priors, with two exceptions: (1) rates of evolution were allowed to vary among loci (ratepr = variable) in the combined analyses, and (2) the heating parameter was decreased to 0.08 (temp = 0.08) in the three-locus combined analysis to improve the frequency of swapping between chains. Chains were run for 10 million generations and trees were sampled from the cold chain every 1,000 generations. To identify when analyses had reached stationarity, the standard deviation of the split frequencies among the independent runs (as calculated by MrBayes) was examined and the output parameter estimates were plotted using Tracer 1.2.1 (Rambaut and Drummond 2005). Based on these convergence diagnostics, the first 2.5 million generations were excluded from each analysis before obtaining a consensus phylogeny and clade posterior probabilities with the "sumt" command (contype = allcompat).

Conflict among the resulting topologies was assessed according to a 0.95 posterior probability (PP) measure for BI and a 70% MLBS criterion (Mason-Gamer and Kellogg 1996). A comparison of the phylogenies resulting from analysis of each of the three individual plastid data sets revealed no mutually well-supported incongruence between methods (ML vs. BI) or among data sets (e.g. *rbcL* vs. *trnG–trnR*). The three-locus combined data matrix and resulting trees are deposited in TreeBASE (submission ID: 15192; study number TB2:S15192).

***Character Mapping*—**To explore the distribution of individual characters, a variety of features considered to be taxonomically informative by previous authors were mapped onto a trimmed (single terminal per taxon) *Myriopteris* phylogeny. Specimens representing every species assigned to *Myriopteris* were obtained on loan from the following herbaria: ASU, B, DUKE, GH, JEPS, K, MO, NY, P, UC, UNM, US, UT, and YU. Morphological features examined included: shape of ultimate segments [bead-like (= round or oval with margins recurved such that the ultimate segments resemble small spherical beads) vs. not bead-like (= elongate, the margins recurved or not)], shape of rachis in cross-section (terete vs. flattened or grooved adaxially), vernation (circinate vs. non-circinate), and indument type (glabrous, with scales only, hairs only, or having hairs and scales). Information on chromosome base number (x = 27, 29, or 30) and ploidy level (2x, 3x, or 4x) was obtained from the relevant literature (Knobloch 1965, 1967; Reeves 1979; Tryon and Tryon 1982; Windham and Rabe 1993; Windham and Yatskievych 2003; Mickel and Smith 2004).

Alternation of generations without fertilization (i.e. apomixis) is common in myriopterid ferns [e.g. the "*Cheilanthes myriophylla* group" in Windham and Yatskievych (2003)] and may play an important role in their diversification. As part of their life cycle, apomictic ferns undergo an incomplete mitosis just prior to meiosis that results in fewer spores being produced in mature sporangia relative to sexually reproducing species. Among leptosporangiate ferns, sexual taxa usually produce 64 spores per sporangium, whereas apomicts produce either 16 or 32 spores (Manton 1950; Gastony and Windham 1989; Beck et al. 2011; Sigel et al. 2011). To determine whether apomixis is concentrated in particular evolutionary lineages, we counted spore number per sporangium for all 29 fertile accessions included in our phylogenetic analyses, as well as for 22 additional individuals not included in the phylogeny (Table 1; Appendix 1). For each fertile specimen, one to four sporangia were examined and the number of spores per sporangium was counted manually. To count spores, individual sporangia were removed from the fertile pinnae using a needle moistened with glycerol. The intact sporangium was then placed in a drop of glycerol on a microscope slide. Each sporangium was ruptured and the spores dispersed in the drop using a pair of dissecting needles. Following the removal of sporangial-wall fragments, a cover slip was placed over the drop of glycerol. Spore count images were taken using a Canon EOS Rebel XSi digital camera attached to a Leica MZ 125 dissecting microscope at either 80 × or 100 × magnification. All specimens having at least one sporangium with 64 well-formed spores were scored as sexual; individuals displaying only 32 or 16 spores per sporangium were scored as apomictic.

RESULTS

***Phylogenetic Analyses*—**Each of the four phylogenetic analyses produced well-resolved topologies, with most branches receiving strong support from both Bayesian PP and MLBS measures. Summary statistics for all phylogenetic analyses are

TABLE 1. Taxa of *Myriopteris* and related outgroups studied, along with voucher information, data on inferred reproductive mode, ploidy level, chromosome number, and DNA sequence availability. Rows in bold text summarize the information known about a given taxon. Rows not in bold text document information available for a unique voucher specimen included in this study; taxa represented by more than one voucher specimen are numbered sequentially (corresponding to numbering in Fig. 2 and Appendix 1). Reproductive mode is inferred based on spore number per sporangium (raw data are available in Appendix 1): 32 spores per sporangium is inferred as A (apomictic); 64 spores per sporangium as S (sexual); taxa (or unique voucher specimens) with sporangia containing either 32 or 64 spores as A, S (either apomictic or sexual). Where known, ploidy level for each taxon is listed; those based on chromosome counts reported in Windham and Yatskievych (2003), Windham and Rabe (1993), Mickel and Smith (2004), or Fraser-Jenkins and Dulawat (2009) are designated with one (*), two (**), three (***), or four (****) asterisks, respectively. Ploidy estimates based on spore diameter measurements from Grusz et al. (2009) are designated by a hat (^). DNA sequence data available for voucher specimens is indicated with the following abbreviations: T (*trnG–trnR*), A (*atpA*), and R (*rbcL*); a dash reflects the absence of data; GenBank accession numbers for each are reported in Appendix 1. [a]Note that Mickel and Smith (2004) doubled the original determination of $n = 87$ to erroneously report $2n = 174$ for *Cheilanthes* (= *Myriopteris*) *notholaenoides*; this species is an apomictic triploid, thus $n = 2n = 87$.

Taxon	Voucher Information	Inferred Reproductive Mode	Ploidy Level	Chromosome Count	DNA Sequence Data T A R
***M. aemula* (Maxon) Grusz & Windham**		S*	2x	$n = 29$*	
M. aemula 1	U. S. A., Texas, Beck 1037 (DUKE)	S			T A R
M. aemula 2	MEXICO, Tamaulipas, Yatskievych & Gastony 89–222 (IND)	—			T A R
***M. alabamensis* (Buckley) Grusz & Windham**		S*, A**	2x, 3x	$n = 29$*, $n = 2n = 87$**	
M. alabamensis 1	U. S. A., Arizona, Schuettpelz 468 (DUKE)	—			T A R
M. alabamensis 2	U. S. A., Missouri, Windham 3450 (DUKE)	A			T A R
M. alabamensis 3	U. S. A., North Carolina, Blomquist 9602 (DUKE)	A	—	—	—
***M. allosuroides* (Mett.) Grusz & Windham**		—	—	—	—
M. allosuroides 1	MEXICO, Jalisco, Yatskievych & Gastony 89–237 (IND)	—			T A R
***M. aurea* (Poir.) Grusz & Windham**		A*	3x	$n = 2n = 90$*	
M. aurea 1	ECUADOR, Carchi, Rothfels 3591 (DUKE)	—			T A R
M. aurea 2	MEXICO, Guerrero, Beck 1192 (DUKE)	A			T A R
M. aurea 3	U. S. A., Arizona, Schuettpelz 466 (DUKE)	—			T A R
M. aurea 4	ECUADOR, Pichincha, Schuettpelz 991 (DUKE)	A			T A R
M. aurea 5	U. S. A., Texas, Beck 1038 (DUKE)	—			T A R
***M. chipinquensis* (Knobloch & Lellinger) Grusz & Windham**		S***	2x	$n = 30$*	
M. chipinquensis 1	MEXICO, Nuevo Leon, Knobloch 1996B (IND)	—			T A R
***M. clevelandii* (D.C. Eaton) Grusz & Windham**		S**	—	—	
M. clevelandii 1	U. S. A., California, Metzgar 180 (DUKE)	S			T A R
M. clevelandii 2	U. S. A., California, Cleveland s. n. (YU, type specimen)	S			—
***M. cooperae* (D. C. Eaton) Grusz & Windham**		S**	2x	$2n = 60$**	
M. cooperae 1	U. S. A., California, Taylor 15925 (UC)	—			T A R
***M. covillei* (Maxon) Á. Löve & D. Löve**		S*	2x	$n = 30$*	
M. covillei 1	U. S. A., Arizona, Schuettpelz 443 (DUKE)	—			T A R
M. covillei 2	U. S. A., California, Windham 3436 (DUKE)	S			T A R
M. covillei 3	U. S. A., California, Beck 1090 (DUKE)	S			—
M. covillei 4	U. S. A., Arizona, Rothfels 2571 (DUKE)	S			—
M. covillei 5	U. S. A., California, Coville & Funston 593 (US, type specimen)	S			—
***M. cucullans* (Fée) Grusz & Windham**		—	—	—	
M. cucullans 1	MEXICO, Guanajuato, Beck 1137 (DUKE)	—			T A R
***M. fendleri* (Hook.) E. Fourn.**		S*	2x	$n = 30$*	
M. fendleri 1	U. S. A., Arizona, Schuettpelz 470 (DUKE)	—			T A R
***M. fimbriata* (A.R. Smith) Grusz & Windham**		S	—	—	
M. fimbriata 1	MEXICO, Oaxaca, Hallberg 1656 (DUKE)	S			T A R
***M. gracilis* Fée**		A*	3x	$n = 2n = 90$*	
M. gracilis 1	U. S. A., Arizona, Schuettpelz 416 (DUKE)	—			T A R
M. gracilis 2	U. S. A., Texas, Rothfels 2470 (DUKE)	A			—
M. gracilis 3	U. S. A., Arizona, Windham 0221A (DUKE)	A			—
***M. gracillima* (D.C. Eaton) J. Sm.**		S	—	—	
M. gracillima 1	U. S. A., Washington, Windham 3630 (DUKE)	—			T A R
M. gracillima 2	U. S. A., California, Schuettpelz 1356A (DUKE)	S			T A R
M. gracillima 3	U. S. A., Oregon, Pryer 06–03 (DUKE)	S			T A R
***M. intertexta* (Maxon) Maxon**		S	—	—	
M. intertexta 1	U. S. A., California, Greenhouse 5086 (JEPS)	—			T A R
M. intertexta 2	U. S. A., Arizona, Dudley s. n. (US, type specimen)	S			—

(*Continued*)

TABLE 1. (CONTINUED).

Taxon	Voucher Information	Inferred Reproductive Mode	Ploidy Level	Chromosome Count	DNA Sequence Data T A R
***M. jamaicensis* (Maxon) Grusz & Windham**		A***	—	—	
M. jamaicensis 1	DOM. REP., San Juan de La Maguana, Clase 3856 (US)	—			T A R
***M. lanosa* (Michx.) Grusz & Windham**		S**	2x	$2n = 60$**	
M. lanosa 1	U. S. A., Alabama, Schuettpelz 1224A (DUKE)	—			T A R
M. lanosa 2	U. S. A., North Carolina, Rothfels 2717 (DUKE)	S			T A R
M. lanosa 3	U. S. A., Indiana, Hegeman s. n. (IND)	—			T A R
***M. lendigera* (Cav.) Fée**		S*	4x	$n = 60$*	
M. lendigera 1	COSTA RICA, San Jose, Grusz 110 (DUKE)	—			T A R
M. lendigera 2	U. S. A., Arizona, Beck 1226 (DUKE)	—			T A R
M. lendigera 3	U. S. A., Arizona, Yatskievych 89–432 (IND)	S			T A R
M. lendigera 4	U. S. A., Arizona, Schuettpelz 460 (DUKE)	S			T A R
***M. lindheimeri* (Hook.) J. Sm.**		S^, A*	2x^, 3x	$n = 2n = 90$*	
M. lindheimeri 1	U. S. A., Arizona, Schuettpelz 450 (DUKE)	A			T A R
M. lindheimeri 2	U. S. A., Texas, Rothfels 2490 (DUKE)	—			T A R
M. lindheimeri 3	U. S. A., Arizona, Schuettpelz 471 (DUKE)	—			T A R
M. lindheimeri 4	U. S. A., Texas, Lindheimer 744 (K)	A			
M. lindheimeri 5	U. S. A., Texas, Lindheimer 744 (K)	S			
***M. longipila* (Baker) Grusz & Windham**		S*	2x	$n = 30$*	
M. longipila 1	MEXICO, Oaxaca, Mickel 6317 (DUKE)	—			T — R
***M. marsupianthes* Fée**		S***	2x	$2n = 60$***	
M. marsupianthes 1	MEXICO, Mexico, Jankiewicz 13 (UC)	—			T A R
***M. mexicana* (Davenp.) Grusz & Windham**		S***	2x	$n = 30$***	
M. mexicana 1	MEXICO, Guanajuato, Beck 1151 (DUKE)	—			T A R
***M. mickelii* (T. Reeves) Grusz & Windham**		—	—	—	
M. mickelii 1	MEXICO, Oaxaca, Salas et al. 1848 (NY)	S			T A R
***M. microphylla* (Sw.) Grusz & Windham**		S**, A***	4x, 3x***	$n = 2n = 87$, $2n = 116$***	
M. microphylla 1	ECUADOR, Pichincha, Schuettpelz 994 (DUKE)	—			T A R
M. microphylla 2	BOLIVIA, Cochabamba, Kessler 9568 (UC)	—			T A R
M. microphylla 3	PUERTO RICO, Guánica, Proctor (US)	—			T A R
***M. moritziana* (Kunze) Grusz & Windham**		S	—	—	
M. moritziana 1	ECUADOR, Carchi, Rothfels 3589 (DUKE)	S			T A R
M. moritziana 2	VENEZUELA, Districto Federal, Moritz 263 (GH, isolectotype)	S			—
***M. myriophylla* (Desv.) J. Sm.**		A*	3x*	$n = 2n = 90$*	
M. myriophylla 1	ECUADOR, Pichincha, Schuettpelz 989 (DUKE)	A			T A R
M. myriophylla 2	MEXICO, Guanajuato, Rothfels 3082 (DUKE)	A			T A R
M. myriophylla 3	MEXICO, Oaxaca, Rothfels 3281 (DUKE)	—			T A R
M. myriophylla 4	MEXICO, San Luis Potosí, Brown 83–31–4 (IND)	—			T A R
M. myriophylla 5	ECUADOR, Pichincha, Schuettpelz 990 (DUKE)	A			—
***M. newberryi* (D.C. Eaton) Grusz & Windham**		S*	2x	$n = 30$*	
M. newberryi 1	U. S. A., California, Metzgar 174 (DUKE)	S			T A R
***M. notholaenoides* (Desv.) Grusz & Windham**		A	3x***, [a]	$n = 2n = 87$[a]	
M. notholaenoides 1	MEXICO, Nuevo Leon, Windham et al. 481 (DUKE)	A			T A R
M. notholaenoides 2	COSTA RICA, San Jose, Grusz et al. 08–020 (DUKE)	A			T A R
***M. parryi* (D.C. Eaton) Grusz & Windham**		S**	2x	$2n = 60$**	
M. parryi 1	U. S. A., Arizona, Metzgar 149 (DUKE)	S			T A R
M. parryi 2	U. S. A., Arizona, Windham & Yatskievych 0340A (DUKE)	S			—
***M. peninsularis* (Maxon) Grusz & Windham**		—	—	—	
M. peninsularis 1	MEXICO, Baja California Sur, Leon de la Luz 9764 (MO)	—			T A R
***M. pringlei* (Davenp.) Grusz & Windham**		S*	2x	$2n = 60$*	
M. pringlei 1	U. S. A., Arizona, Schuettpelz 502 (DUKE)	—			T A R
M. pringlei 2	U. S. A., Arizona, Windham & Yatskievych 0248A (DUKE)	S			—
***M. pringlei* var. *moncloviensis* (Baker) Grusz & Windham**		S	—	—	
M. pringlei var. *moncloviensis 1*	MEXICO, Coahila, *Palmer 1378* (NY)	S			—
***M. rawsonii* (Mett. ex Kuhn) Grusz & Windham**		S	—	—	
M. rawsonii 1	NAMIBIA, Smook 11325 (MO)	S			T A R
M. rawsonii 2	NAMIBIA, Goldblatt 7014 (MO)	S			—

(*Continued*)

TABLE 1. (CONTINUED).

Taxon	Voucher Information	Inferred Reproductive Mode	Ploidy Level	Chromosome Count	DNA Sequence Data T A R
***M. rufa* Fée**		A*	3x	$n = 2n = 90$*	
M. rufa 1	U. S. A., New Mexico, Rothfels 2515 (DUKE)	A			T A R
M. rufa 2	U. S. A., Texas, Schuettpelz 323 (DUKE)	A			T A R
M. rufa 3	U. S. A., Texas, Windham 3545 (DUKE)	A			T A R
M. rufa 4	U. S. A., Texas, Rothfels 2493 (DUKE)	—			T A R
M. rufa 5	U. S. A., Arizona, Metzgar 161 (DUKE)	A			T A R
M. rufa 6	U. S. A., Virginia, Rothfels 3902 (DUKE)	A			—
M. rufa 7	U. S. A., New Mexico, Windham & Windham 0021B (DUKE)	A			—
***M. scabra* (H. Karst) Grusz & Windham**		S*	2x	$n = 29$*	
M. scabra 1	MEXICO, Nuevo Leon, Gastony 90–10–1 (DUKE)	—			T A R
M. scabra 2	U. S. A., Texas, Beck 1036 (DUKE)	S			T A R
***M. tomentosa* Fée**		A*	3x	$n = 2n = 90$*	
M. tomentosa 1	U. S. A., North Carolina, Christenhusz 3823 (DUKE)	—			T A R
***M. viscida* (Davenp.) Grusz & Windham**		A, S**	—	—	
M. viscida 1	U. S. A., California, Metzgar 169 (DUKE)	A			T A R
***M. windhamii* Grusz**		A*	3x	$n = 2n = 90$*	
M. windhamii 1	U. S. A., Arizona, Windham 458 (DUKE, paratype of *M. windhamii*)	A			T A R
M. windhamii 2	U. S. A., New Mexico, Beck 1050 (DUKE)	A			T A R
M. windhamii 3	U. S. A., Arizona, Lemmon s. n. (US, type specimen of *C. villosa*)	A			—
***M. wootonii* (Maxon) Grusz & Windham**		A*	3x	$n = 2n = 90$*	
M. wootonii 1	U. S. A., Arizona, Schuettpelz 488 (DUKE)	—	3x		T A R
***M. wrightii* (Hook.) Grusz & Windham**		S*	2x	$n = 30$*	
M. wrightii 1	U. S. A., Arizona, Schuettpelz 441 (DUKE)	—			T A R
M. wrightii 2	U. S. A., Arizona, Windham 0341A (DUKE)	S			—
***M. yatskievychiana* (Mickel) Grusz & Windham**		—	—	—	
M. yatskievychiana 1	MEXICO, Sonora, Burquez 96–302 (MO, type specimen)	—			T A R
***M. yavapensis* (T. Reeves ex Windham) Grusz & Windham**		A*	4x	$n = 2n = 120$*	
M. yavapensis 1	U. S. A., Arizona, Schuettpelz 415 (DUKE)	A			T A R
M. yavapensis 2	U. S. A., Arizona, Licher 778 (DUKE)	A			—
***Argyrochosma microphylla* (Mett. ex Kuhn) Windham**		S*	2x	$n = 27$*	
A. microphylla	U. S. A., New Mexico, Worthington 34623 (DUKE)	—			— A R
***Astrolepis windhamii* D. M. Benham**		A*	3x	$n = 2n = 87$*	
A. windhamii	U. S. A., Arizona, Schuettpelz 431 (DUKE)	—			— A R
***Paragymnopteris marantae* (L.) K. H. Shing**		S****	2x	$n = 29$****	
P. marantae	CHINA, Yunnan, Yatskievych 02–35 (MO)	—			— A R
***Pellaea atropurpurea* (L.) Link**		A**	3x	$n = 2n = 87$**	
P. atropurpurea	U. S. A., Virginia, Schuettpelz 312 (DUKE)	—			— A R

listed in Table 3. The most-likely tree (lnL = -16,790.1213) resulting from the analysis of our combined three-locus data set is presented in Fig. 2. Taxon names displayed in Fig. 2 reflect placement within *Myriopteris* (Table 1); a list of synonyms in *Cheilanthes* is provided in Appendix 2.

Our results confirm the monophyly of *Myriopteris* with maximal support (100/1.0). Myriopterid diversity is divided among three major clades (Clades A, L, C, Fig. 2), each of which is maximally supported (100/1.0). Relationships among these three groups remain uncertain, though the best likelihood topologies (for all single-locus analyses, as well as the combined three-locus data set) resolve the *alabamensis* clade (Clade A, Fig. 2) as sister to a combined *covillei* + *lanosa* clade with low support.

THE ALABAMENSIS CLADE—This lineage (Clade A, Fig. 2) includes 13 of the 40 *Myriopteris* species sampled for our study. In the ML tree based on our combined data set, *M. wrightii* (a Sonoran/Chihuahuan Desert endemic) is sister to the remainder of the clade, but with low statistical support (< 70). The remaining members of this clade fall into two well-supported monophyletic groups (Fig. 2). Clade 1, which is resolved with strong support (98/1.0), includes four species endemic to Mexico and the adjacent southwestern U. S. A.; although the relative positions of *M. mickelii* and *M. allosuroides* are uncertain, *M. peninsularis* and *M. pringlei* are unequivocally supported as sister species. The maximally supported Clade 2 encompasses eight species widely distributed across the Americas. Although the phylogenetic backbone of Clade 2 is not well resolved, there are several species groupings that receive maximal support, including a sister relationship between *M. notholaenoides* and *M. cucullans* and a similar relationship between *M. scabra* and

TABLE 2. Primers used for DNA amplification and sequencing of plastid loci for all taxa included in this study. *Asterisks indicate primers used for both the initial PCR amplification and for DNA sequencing; all other primers were used for DNA sequencing only.

DNA region	Primer	5′-3′ Primer sequence	Primer source
rbcL	ESRBCL1F*	ATGTCACCACAAACGGAGACTAAAGC	Schuettpelz and Pryer 2007
rbcL	ESRBCL654R	AGAYCGTTTCYTATTYGTAGCAGAAGC	Schuettpelz and Pryer 2007
rbcL	ESRBCL1361R*	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz and Pryer 2007
rbcL	ESRBCL628F	CCATTYATGCGTTGGAGAGATCG	Schuettpelz and Pryer 2007
trnG–trnR	TRNG1F*	GCGGGTATAGTTTAGTGGTAA	Nagalingum et al. 2007
trnG–trnR	TRNR22R*	CTATCCATTAGACGATGGACG	Nagalingum et al. 2007
trnG–trnR	TRNG63R	GCGGGAATCGAACCCGCATCA	Nagalingum et al. 2007
trnG–trnR	TRNG353R	TTGCTTMTAYGACTCGGTG	Metzgar et al. 2007
atpA	ESATPA535F	ACAGCAGTAGCTACAGATAC	Schuettpelz et al. 2006
atpA	ESATPA557R	ATTGTATCTGTAGCTACTGC	Schuettpelz et al. 2006
atpA	ESATPA856F	CGAGAAGCATATCCGGGAGATG	Schuettpelz et al. 2006
atpA	ESATPA877R	CATCTCCCGGATATGCTTCTCG	Schuettpelz et al. 2006
atpA	ESATPF412F*	GARCARGTTCGACAGCAAGT	Schuettpelz et al. 2006
atpA	ESTRNR46F*	GTATAGGTTCRARTCCTATTGGACG	Schuettpelz et al. 2006

M. fimbriata. Interestingly, *M. moritziana*, the only *Myriopteris* endemic to South America, is genetically indistinguishable from two of the three accessions of *M. microphylla* at the plastid loci analyzed.

THE LANOSA CLADE—This lineage (Clade L, Fig. 2), weakly resolved as sister to the *covillei* clade (Clade C, Fig. 2), includes seven sampled species. Relationships among taxa belonging to the *lanosa* clade are generally well resolved, though the apparent sister relationship between *M. longipila* and *M. lanosa* has low statistical support in our MLBS analysis. Our analyses indicate that two species endemic to the Californian Floristic Province (*M. viscida* and *M. cooperae*) are sequentially sister to the remaining taxa. Although members of this clade are primarily North American, the sole African representative of *Myriopteris* (*M. rawsonii*) is deeply nested within the *lanosa* clade (Clade L, Fig. 2) and maximally supported as sister to the Mohave/Sonoran Desert endemic, *M. parryi*.

THE COVILLEI CLADE—This lineage (Clade C, Fig. 2) is the most species-rich, including 20 of the 40 *Myriopteris* taxa sampled for this study. The first major split separates the *M. aurea* clade (*M. aurea* + *M. yatskievychiana*; 'au' in Fig. 2) from other members of the group with maximal support. *Myriopteris aurea* (previously *Cheilanthes bonariensis*) is the most widely distributed species in the genus and shows notable phylogenetic substructure. Among the remaining species, the Californian Floristic Province endemic *M. newberryi* is sister to the highly supported (90/1.0) core *covillei* clade ('cc', Fig. 2). The latter constitutes three well-supported monophyletic groups (Clades 3, 4, and 5, Fig. 2), the relationships among which are unresolved. Clade 3 (92/1.0) includes the eight species sampled from the western North American *M. yavapensis* complex. The phylogenetic backbone of Clade 3 is not well resolved but there are several maximally supported species pairs. Three of these pairs involve known polyploid hybrids (*M. yavapensis, M. wootonii*, and *M. intertexta*; Fig. 2; Table 1) grouping with (and nearly indistinguishable from) their known sexual diploid maternal progenitors (*M. lindheimeri, M. fendleri*, and *M. gracillima* respectively; Grusz et al. 2009). Clade 4 (100/1.0) consists of the widespread tetraploid species *M. lendigera* and its putative diploid parents, *M. mexicana* and *M. marsupianthes*. Lastly, Clade 5 (100/1.0) includes six sampled species, five of which are apomictic polyploids (Table 1) of uncertain origin. *Myriopteris myriophylla*, the most widespread among these, is maximally supported as sister to all other species now informally referred to the *M. rufa* (previously *C. eatonii*) complex. Relationships among the species in this complex are poorly resolved, but multiple accessions of single taxa occupy discrete branches with moderate to strong support. The maximally supported pairing of *M. chipinquensis* and *M. tomentosa* may indicate that the former (a known sexual diploid; Table 1) was involved in the origin of the latter (an apomictic triploid).

Mapping Characters Across Myriopteris—The distribution of various morphological, cytological, and reproductive character states across the *Myriopteris* phylogeny is shown in Figs. 3–5. The shape of ultimate segments (Fig. 3A) is the least homoplasious morphological character examined. All members of the core *covillei* clade ('cc') have bead-like ultimate segments, as does *M. gracilis* in the *lanosa* clade. All other taxa, including outgroups, lack bead-like ultimate segments.

Figure 3B illustrates the phylogenetic distribution of the three character states relating to leaf-rachis shape. The majority of myriopterid taxa have rachises that are terete (i.e. round) in cross section. This includes all members of Clade 2 within the *alabamensis* clade (Clade A, Fig. 3B), all representatives of the *covillei* clade (Clade C, Fig. 3B), and all but two

TABLE 3. Summary statistics for phylogenetic analyses in this study.

Data Set (# individuals)	Characters (base pairs)		Missing data (%)	Ingroup bipartitions with good branch support		
	Total	Variable		MLBS ≥ 70	PP ≥ 0.95	MLBS ≥ 70 and PP ≥ 0.95
rbcL (71)	1,345	172	0.2	35 (53%)	35 (53%)	33 (50%)
atpA (71)	1,873	282	1.0	38 (57%)	36 (55%)	35 (53%)
trnG–trnR (68)	1,290	228	2.3	38 (57%)	38 (57%)	34 (51%)
Combined (72)	4,508	916	9.5	50 (75%)	47 (70%)	42 (63%)

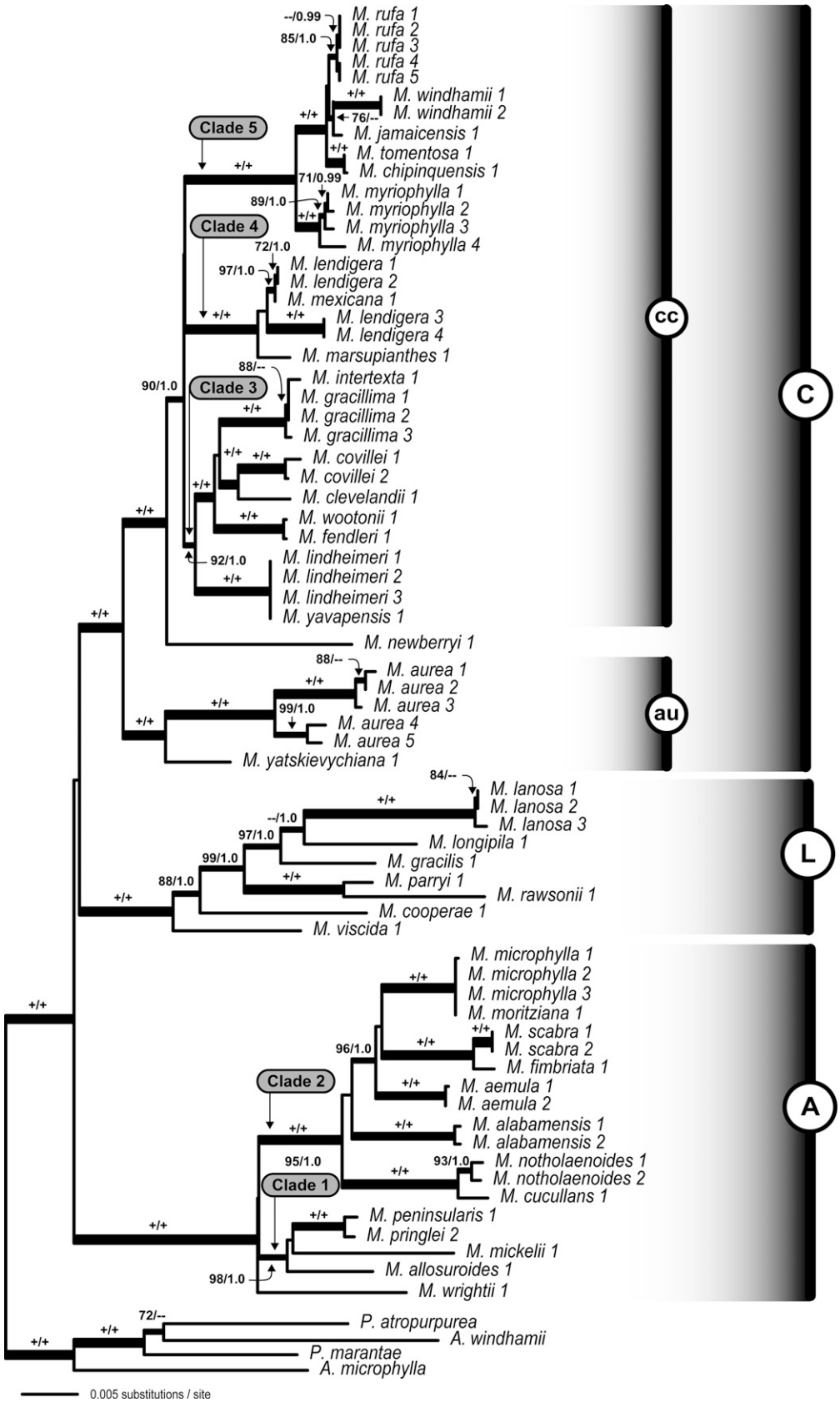

FIG. 2. Plastid phylogeny of *Myriopteris* based on combined analysis of *rbcL*, *atpA*, and *trnG–trnR*; the maximum likelihood topology is shown (ln L = -16,790.1213). Names follow the updated taxonomy for *Myriopteris* (Table 1; Appendices 1 and 2); numbers following names correspond to voucher specimens listed in Table 1. Support values are provided for branches with ≥ 70 MLBS and/or 0.95 PP (MLBS/PP, respectively). Lightly thickened branches indicate moderate support (≥ 70 MLBS and/or 0.95 PP); heavily thickened branches indicate maximal support (100 MLBS and 1.0 PP; designated as +/+). The three primary *Myriopteris* clades are designated A (= *alabamensis* clade), C (= *covillei* clade), and L (= *lanosa* clade); the *M. aurea* clade (au) is distinguished from the *core covillei* (cc) clade.

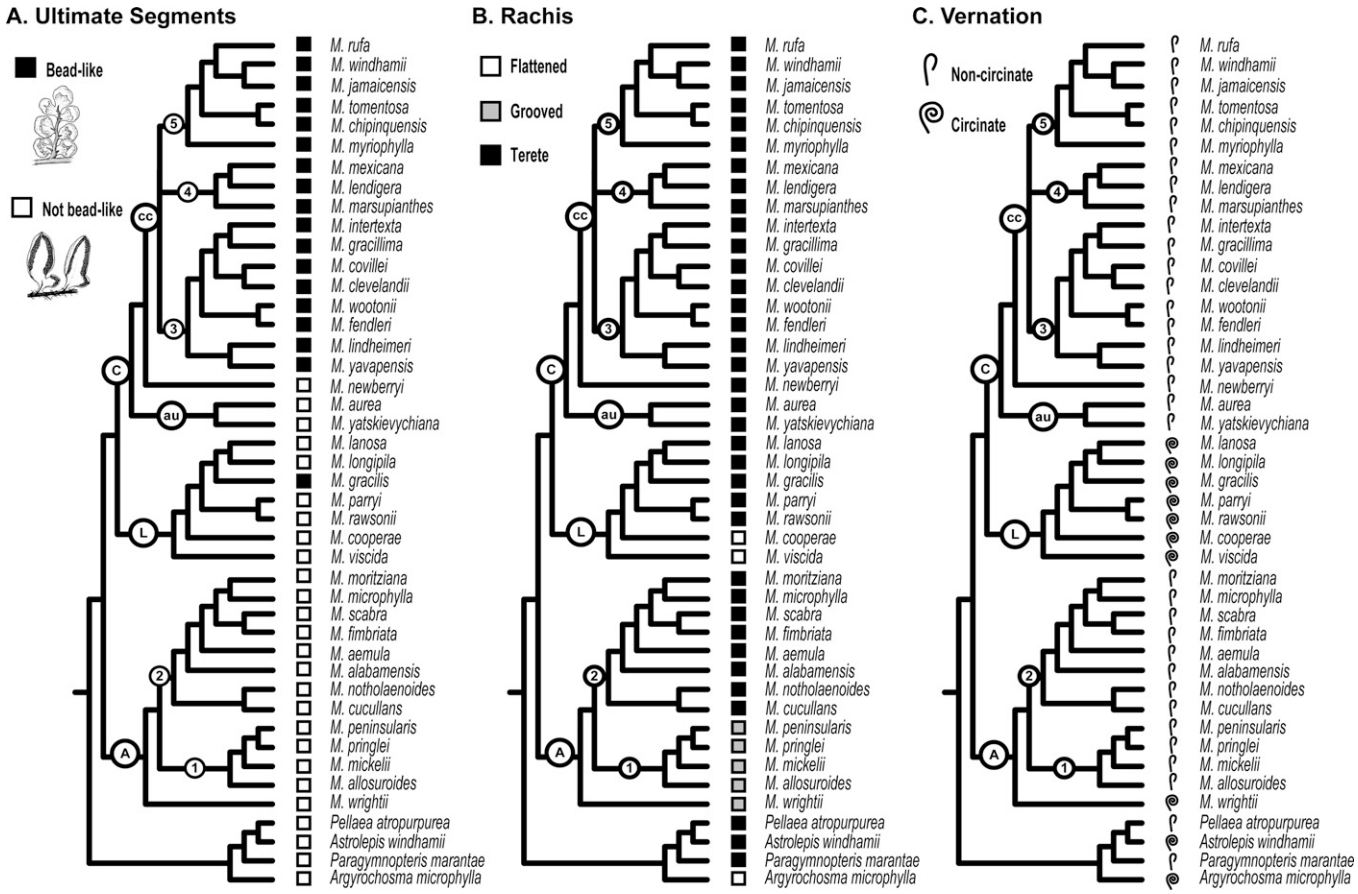

FIG. 3. Mapping leaf characters in *Myriopteris*. A. Shape of ultimate segments: black boxes = bead-like, white boxes = not bead-like. B. Cross-sectional rachis shape: white boxes = slightly flattened, grey boxes = adaxially grooved, black boxes = terete. C. Vernation: hooked = non-circinate, spiraled = circinate.

sampled species of the *lanosa* clade (Clade L, Fig. 3B). Three of the four outgroup taxa (*Pellaea atropurpurea*, *Astrolepis windhamii*, and *Paragymnopteris marantae*) also have terete rachises. Within the *alabamensis* clade (Clade A, Fig. 3B), *M. wrightii* plus all members of Clade 1 have grooved rachises. Flattened rachises are characteristic of two early-diverging members of the *lanosa* clade (*M. viscida* and *M. cooperae*) and one outgroup species (*Argyrochosma microphylla*).

The shape of young, unfurling fronds (vernation) is variable across *Myriopteris*, as well as the four outgroup species from the pellaeid clade (Fig. 3C). Of the 44 taxa included in the study, a majority exhibits non-circinate (i.e. "hooked") vernation. This includes all sampled members of the *covillei* clade, all but one representative of the *alabamensis* clade, and the outgroup species *Pellaea atropurpurea* and *Paragymnopteris marantae*. By contrast, all taxa belonging to the *lanosa* clade (Clade L, Fig. 3C) have circinate (i.e. "fiddlehead") vernation, as do *M. wrightii* (the earliest branching member of the *alabamensis* clade) and the outgroup taxa *Argyrochosma microphylla* and *Astrolepis windhamii*.

Hairs and scales, collectively referred to as indument, are commonly found on the leaves of cheilanthoid ferns. Within *Myriopteris*, variation in leaf indument (ranging from glabrous in some taxa to having both hairs and scales in others) is the most useful taxonomic character for identification of individual species (Fig. 4A). Here, we separately map the type of indument found on the adaxial (Fig. 4B) and abaxial (Fig. 4C) surfaces of the ultimate segments for each taxon represented in the phylogeny. We recognize five types of indument occurring on the surfaces of the ultimate segments proper (excluding the costae and any subtending stalks). These include simple hairs, branched hairs, skeletonized scales (differing from branched hairs in being biseriate to multiseriate for part of their length), ciliate scales, and entire scales. These indument types are often different on adaxial and abaxial surfaces and can occur alone or in combination (on the abaxial surfaces only); in a few species, indument is entirely lacking on the green tissue of the ultimate segments.

The majority of taxa in *Myriopteris* have only simple hairs on the adaxial surfaces of their ultimate segments (Fig. 4B). With the exception of *M. fendleri* (a member of Clade 3 in the *covillei* clade), ingroup species with glabrous adaxial surfaces are confined to early-diverging branches of the *alabamensis* clade (Clade A, Fig. 4B). *Myriopteris rawsonii*, the only African species of the group, differs from all other taxa in having nothing but branched hairs on adaxial leaf surfaces. Another interesting pattern involves the distribution of skeletonized scales, which appear to be a synapomorphy for Clade 3 (Fig. 4B). With the exception of *M. fendleri*, which we hypothesize has become glabrous through the loss of skeletonized scales, all members of Clade 3 exhibit this distinctive indument type on their adaxial surfaces though they may be lost when the leaves reach maturity. Outgroup taxa are

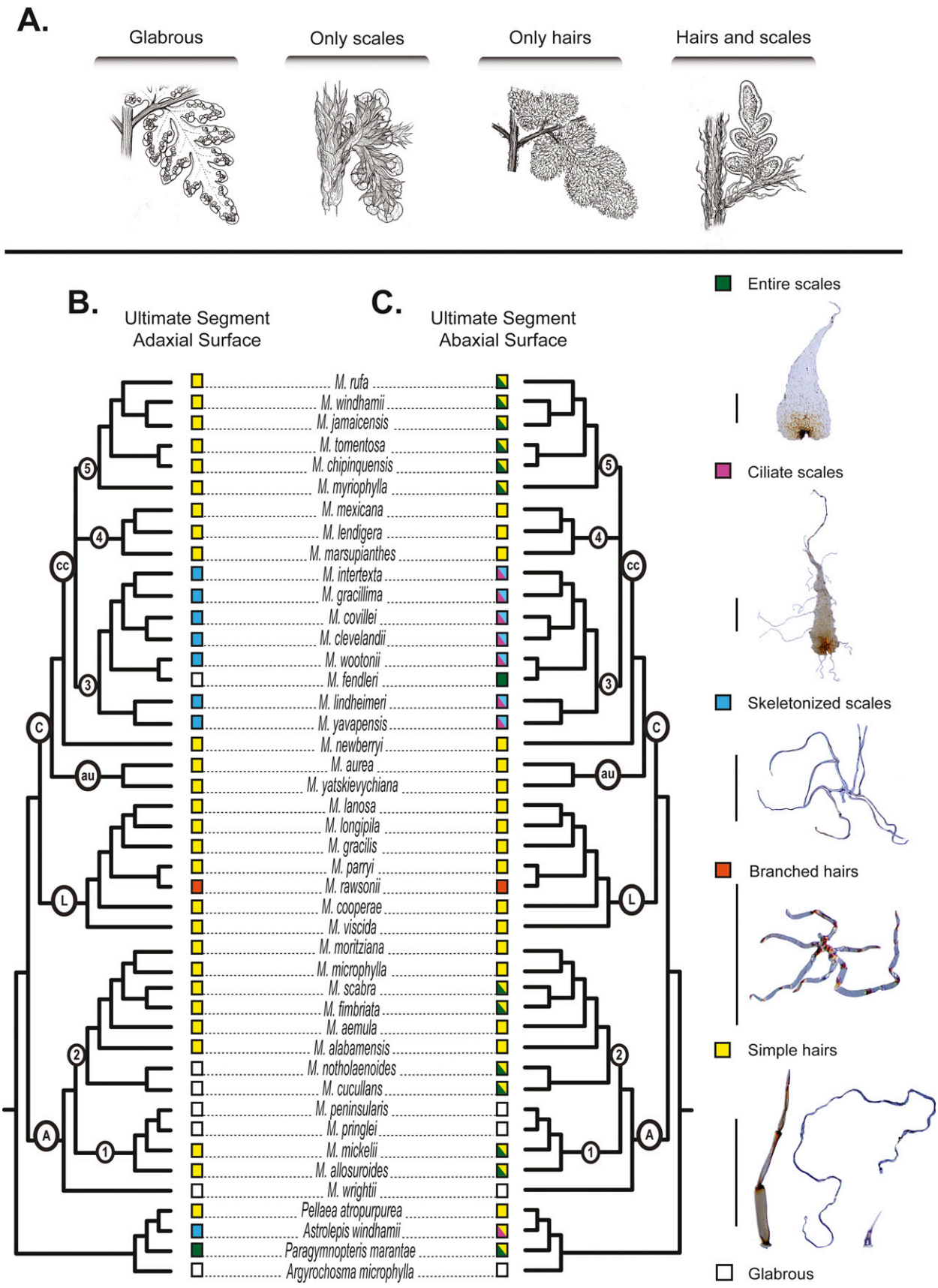

FIG. 4A–C. Mapping of indument in *Myriopteris*. A. Line illustrations of indument on the lower (abaxial) surfaces of the ultimate segments across *Myriopteris* (modified from Mickel and Smith 2004); left to right: glabrous; only scales; only hairs; both scales and hairs. B–C. Indument type on the adaxial (B) and abaxial (C) surface of the ultimate segments for members of *Myriopteris*. Indument type is coded as glabrous (= white boxes), simple hairs (= yellow boxes), branched hairs (= orange boxes), skeletonized scales (= blue boxes), ciliate scales (= purple boxes), or entire scales (= green boxes). On far right, images of each indument type are shown below its corresponding label; scale bars = 0.5 cm.

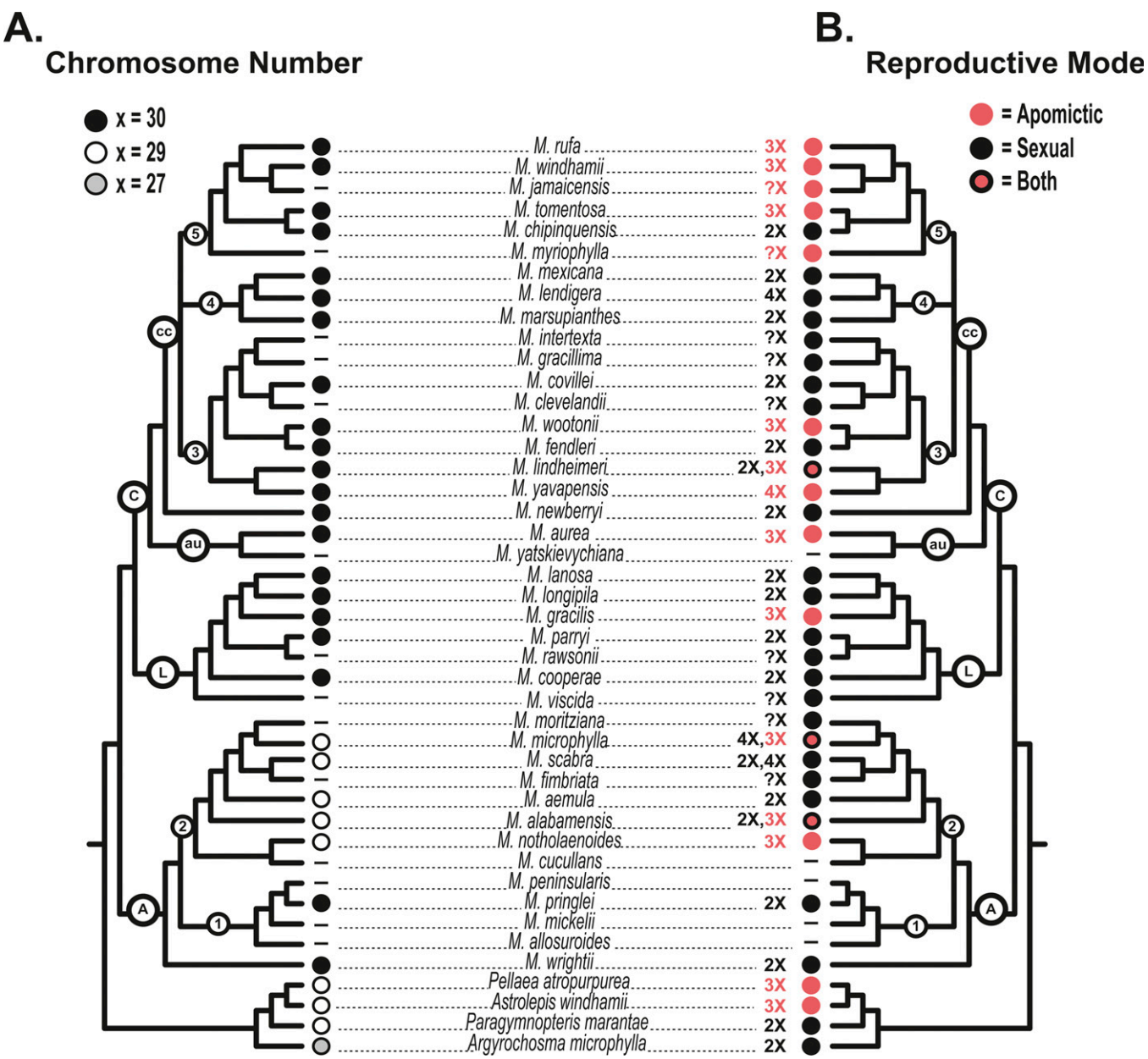

FIG. 5. Mapping of cytological and reproductive characters in *Myriopteris*. A. Chromosome base numbers, gathered from existing chromosome counts (see Table 1), are indicated as follows: $x = 27$ is indicated with grey circles, $x = 29$ with white circles, and $x = 30$ with black circles. B. Inferred reproductive mode (based on spore number per sporangium): 64 spores per sporangium = sexual (black circles), 32 spores per sporangium = apomictic (red circles); taxa exhibiting sexual and apomictic reproductive modes in different individuals/populations are indicated by red circles outlined in black. Ploidy level for each taxon is noted to the immediate left of the circle showing reproductive mode (2X = diploid, 3X = triploid, 4X = tetraploid, ?X = unknown ploidy level; black font = sexual, red font = apomictic). Missing data are indicated by a dash '—'.

variable with regard to adaxial indument; each of the four species has a different character state.

With the addition of two indument types (entire scales and ciliate scales) and the appearance of three unique combinations ('entire scales + simple hairs', 'ciliate scales + simple hairs', and 'ciliate scales + skeletonized scales'), the indument of the abaxial surfaces of the ultimate segments is even more diverse than that of the adaxial (Fig. 4C). Among the ingroup species sampled, there are some notable similarities to patterns observed for the adaxial surfaces. A plurality (but not a majority) of *Myriopteris* species produce only simple hairs on the lower surfaces of the leaves, and species with glabrous abaxial surfaces are confined to early-diverging branches of the *alabamensis* clade (Clade A, Fig. 4C). *Myriopteris rawsonii* is again distinguished from all other taxa by having only branched hairs, and Clade 3 (with the usual exception of *M. fendleri*) exhibits a singular synapomorphy of having ciliate scales (occasionally accompanied by skeletonized scales) on the abaxial surfaces of the ultimate segments. *Myriopteris fendleri* is unique in producing nothing but entire scales on the abaxial surfaces. The second most common indument type on abaxial surfaces is a combination of simple hairs and entire scales, which is scattered across the *alabamensis* clade and also appears to be a synapomorphy of Clade 5 within the *covillei* clade (Clade C, Fig. 4C). As in the case of adaxial indument, the four outgroup taxa show

four different character states. They are glabrous abaxially (*Argyrochosma microphylla*), have both simple hairs and entire scales (*Paragymnopteris marantae*), have simple hairs and ciliate scales (*Astrolepis windhamii*), or have only simple hairs (*Pellaea atropurpurea*) on the lower surfaces of the ultimate segments.

Chromosome counts, from which base numbers and ploidy levels can be inferred, are available for 26 of the 40 myriopterid taxa included in our phylogeny (Fig. 5). With the exception of Clade 2, all members of *Myriopteris* for which data are available have a chromosome base number of $x = 30$. The five members of Clade 2 that have been counted to date all show $x = 29$, a base number shared with the outgroup taxa other than *A. microphylla,* which has a unique base number of $x = 27$.

Reproductive mode was inferred for a total of 51 specimens and these data are mapped, along with published information on ploidy level, in Fig. 5. Based on our sampling of one to four sporangia per fertile specimen, 25 individuals showed approximately 64 spores/sporangium (or at least significantly more than 32) and were inferred to be sexual. Another 23 exhibited no more than 32 larger spores/sporangium and were presumed to be apomictic. Our results reaffirm that *Myriopteris* encompasses an array of sexual and apomictic taxa and, based on existing reports, a variety of ploidy levels. Sexual diploids appear in every lettered/numbered clade in the phylogeny except for the *aurea* group ('au', Fig. 5), and ongoing work by Beck et al. (unpubl.) indicates that they exist there as well. Apomictic triploids are scattered across the major clades, apparently absent only from Clade 1 (where reproductive mode and ploidy level are unknown for three of the four species included in the analysis) and Clade 4. Sexual tetraploids are relatively uncommon in *Myriopteris*; based on the current data, *M. lendigera* appears to be exclusively tetraploid whereas *M. microphylla* and *M. scabra* have sexual tetraploid populations in addition to other cytotypes. Apomictic tetraploids are even less common; the only documented example in our analysis being *M. yavapensis* in the *covillei* clade (Clade C, Fig. 5). *Myriopteris viscida*, *M. rawsonii*, *M. clevelandii*, *M. gracillima*, and *M. intertexta* are all confirmed to be sexual but do not have documented chromosome counts, and ploidy levels remain unconfirmed. *Myriopteris jamaicensis* is an apomict of unknown ploidy, though its large spores suggest that it, like all other apomicts in our analyses, is polyploid. Sexual taxa predominate in all ingroup clades except the isolated *aurea* group ('au', Fig. 5) and Clade 5. Among the outgroup taxa, *A. microphylla* and *P. marantae* are both sexual diploids, whereas *A. windhamii* and *P. atropurpurea* are apomictic triploids.

Discussion

Here, we explore evolutionary relationships among taxa belonging to the newly segregated genus, *Myriopteris* (Grusz 2013; Grusz and Windham 2013). Our sampling of the myriopterid clade represents a two-fold increase over the most comprehensive study to date (Eiserhardt et al. 2011), encompassing 40 of the 47 currently recognized taxa.

Phylogenetic Analyses—Our results agree with earlier studies (Kirkpatrick 2007; Rothfels et al. 2008; Windham et al. 2009; Eiserhardt et al. 2011) in demonstrating that members of this group form a maximally supported clade (Fig. 2) only distantly related to *Cheilanthes* s. s. (Fig. 1). Our maximum likelihood topology depicts three maximally-supported myriopterid clades (Clades A, L, C, Fig. 2), of which the *covillei* and *lanosa* clades (Clades C and L) together are weakly supported as sister to the *alabamensis* clade (Clade A). Members of Clade A show the greatest morphological resemblance to the outgroup taxa; several species therein were originally named in *Pellaea* or have, at some point, been included within it. All taxa belonging to Clade 2 (comprising the bulk of the *alabamensis* clade) that have been analyzed chromosomally show a base number of $x = 29$, a character state shared with most of the pellaeid outgroup, but otherwise absent from *Myriopteris* (Fig. 5). Finally, species belonging to the *alabamensis* clade are not known to form hybrids with members of the other two clades, whereas hybridization does occur between the *covillei* and *lanosa* clades. Morphological (Reeves 1979) and isozymic (Windham unpubl.) analyses reveal that *M. covillei* (the namesake of Clade C) and *M. parryi* (Clade L) have hybridized repeatedly to form *M.* x *parishii* (Davenp.) Grusz & Windham. The existence of such cross-clade hybrids suggests that the *covillei* and *lanosa* clades may be more closely related to one another than either is to members of the *alabamensis* clade.

All 18 myriopterid species included in the molecular analyses of Eiserhardt et al. (2011) were included in our study, along with 22 additional taxa. The phylogenetic relationships presented by Eiserhardt et al. (2011) generally match those in our maximum likelihood tree (Fig. 2); their well-supported myriopterid clade comprises three major subgroups (equivalent to our *alabamensis*, *covillei*, and *lanosa* clades), and the lineages including *M. covillei* and *M. lanosa* (as *Cheilanthes*) are also weakly supported as sister to one another. Within the *alabamensis* clade, the Eiserhardt et al. (2011) dataset provides robust support for our Clade 2, though the conflicting branching arrangement of species and the nearly identical sequences of "*Cheilanthes alabamensis*" and "*Cheilanthes notholaenoides*" in their study suggests that one of their samples was misidentified. The four species of the *lanosa* clade included in their analyses show precisely the same branching pattern in our tree (Fig. 2) and also support the unexpected sister relationship between the southern African endemic "*Cheilanthes rawsonii*" and the Sonoran/Mojave Desert endemic "*C. parryi*". Within the *covillei* clade, Eiserhardt et al. (2011) identify "*Cheilanthes bonariensis*" (= *Myriopteris aurea*) as the earliest-diverging taxon, in full accord with our analyses. Although some other relationships portrayed by Eiserhardt et al. (2011) are at odds with our reconstruction (specifically their placement of "*C. newberryi*" within the equivalent of our core 'cc' clade, Fig. 2), there is no well-supported conflict between the two studies.

Beyond the notable congruence between these two molecular studies, there also is significant agreement with some of the morphologically-based hypotheses of relationships proposed by Reeves (1979), who divided the New World species assigned to "*Cheilanthes*" into four subgenera and a fifth group of taxa he considered insertae sedis. One of the subgenera (*Othonoloma* Link ex C. Chr.) recently has been recognized as a distinct genus, *Gaga* (Li et al. 2012). The other four groups identified by Reeves (1979) are, in whole or in part, equivalent to clades within *Myriopteris* as defined herein. The "*Cheilanthes alabamensis* group" [treated as a subgenus without a formal name by Reeves (1979)] exactly corresponds to Clade 2 in our analysis, and his insertae sedis group comprises a subset of the taxa belonging to Clade 1,

plus *Myriopteris wrightii* (Fig. 2). His subgenus *Physapteris* (C. Presl) Baker exactly corresponds to our core *covillei* clade ('cc', Fig. 2), and the only patently polyphyletic subgeneric construct is his subgenus *Cheilanthes*. Reeves (1979: 47) stated "this subgenus includes most of the South American species of *Cheilanthes* together with the North American *C. parryi, C. cooperae, C. viscida, C. kaulfussii, C. leucopoda, C. feei, C. lanosa,* and *C. longipila*." The discordant elements here are: 1) the South American species of *Cheilanthes*, which include the type species of that genus, and are not closely related to *Myriopteris*; 2) *C. kaulfussii*, which belongs to the genus *Gaga* (Li et al. 2012); and 3) *C. leucopoda*, which is sister to *Notholaena* (Rothfels et al. 2008). With the removal of these taxa, Reeves' (1979) fourth subgenus (incorrectly called subg. *Cheilanthes* following elimination of the South American species) is largely congruent with our *lanosa* clade (Fig. 2).

Tryon and Tryon (1982) divided the American taxa of *Cheilanthes* s. l. into 11 informal groups, three of which contain species belonging to the myriopterid clade. With the exception of *C. regularis* Mett. [= *Adiantopsis regularis* (Mett.) Moore], the species they list as representatives of the "*C. microphylla* group" all belong to the *alabamensis* clade. And, with the exception of *C. horridula* (= *Myriopteris scabra*, another member of the *alabamensis* clade), their "*C. myriophylla* group," includes only members of the core *covillei* clade ('cc', Fig. 2). Tryon and Tryon's (1982) "*C. fraseri* group" is the largest and most diverse, containing 12 representative taxa now known to be widely dispersed across the cheilanthoid phylogeny (Eiserhardt et al. 2011; Windham et al. unpubl.). This grouping includes five species that appear in our analyses: *C. feei* (= *M. gracilis*), *C. lanosa, C. parryi*, *C. bonariensis* (= *M. aurea*), and *C. newberryi*. The first three are members of the *lanosa* clade; the other two are sequentially sister to the core *covillei* clade ('cc', Fig. 2).

Leaf Ultimate Segments—The latter finding (i.e. the robust positioning of *M. aurea* and *M. newberryi* as the earliest branches of the *covillei* clade), is one of the most surprising results of this study. Prior to the work of Tryon and Tryon (1982), these two species generally had been included in the genus *Notholaena* because of their poorly differentiated, unrecurved segment margins. With its linear, pinnate-pinnatifid fronds and large, flat ultimate segments (pinna lobes), *M. aurea* stands in stark contrast to Fée's (1852) original description of *Myriopteris*. His characterization of the genus as having laminar margins folding over the developing sporangia such that the ultimate segments often form a contracted "bead" clearly applies to a limited subset of the species in our study, including all members of the core *covillei* clade ('cc', Fig. 3A) as well as *M. gracilis*, one of the more derived members of the *lanosa* clade (Clade L, Fig. 3A). Based on the distribution of bead-like ultimate segments across our well-sampled phylogeny, it appears that this particular character state has arisen just twice during the evolution of the group.

Despite their apparent stability on a local phylogenetic scale, bead-like ultimate segments are present in fewer than half the species here assigned to *Myriopteris,* and also occur in several other, distantly related cheilanthoid genera such as *Notholaena* and *Cheilanthes* s. s. (Windham et al. unpubl.). It is no wonder that the use of this character as the primary diagnostic feature of *Myriopteris* by both Fée (1852) and Smith (1875) led to the recognition of patently non-monophyletic assemblages of species (see Grusz and Windham 2013). The taxa of *Myriopteris* that lack bead-like ultimate segments (ca. 60% of the total) all have more elongate, flatter segments but are otherwise diverse, with some taxa exhibiting recurved margins with well-differentiated, false indusia and others showing plane margins essentially lacking false indusia.

Leaf Rachises—The shape of leaf rachises in cross-section furnishes a valuable taxonomic character in several cheilanthoid genera (e.g. Anthony 1984; Link-Perez et al. 2011), including *Myriopteris*. While most species of the genus exhibit terete rachises (Fig. 3B), early-diverging members of the *alabamensis* clade (*M. wrightii* + Clade 1) have rachises that are deeply grooved adaxially, and the first two branches of the *lanosa* clade (*M. viscida* and *M. cooperae*) have flattened rachises that become shallowly grooved distally. Based on the maximum likelihood tree shown in Fig. 3B, it is tempting to view terete rachises as independently derived from grooved rachises in the *alabamensis* clade, but the low statistical support for the placement of *M. wrightii* (Fig. 2) allows for other evolutionary scenarios. Similarly, the concentration of grooved and flattened rachises on early diverging branches of the *Myriopteris* phylogeny might be an indication that terete rachises are derived (and homoplastic), but the sporadic distribution of these character states among the outgroups makes it impossible to draw any firm conclusions at this time.

Leaf Vernation—One of the most characteristic morphological features of ferns is the coiled or "fiddlehead" shape of young, unfurling fronds, also known as circinate vernation. Some ferns [e.g. *Ophioglossum* (Eames 1936); *Anemia* (Mickel 1962); *Pteris* (Knobloch 1965)] differ in having their young fronds expand in a "hook" shape, a condition variously referred to as imperfectly circinate or non-circinate vernation. Among cheilanthoids, non-circinate vernation was first reported by Wherry (1926) and Weatherby (1926) based on observations of *Cheilanthes tomentosa* (= *M. tomentosa*) and *C. eatonii* (= *M. rufa*), respectively. Knobloch (1965) observed non-circination vernation in 14 additional species here included in *Myriopteris*, and Reeves (1979) stated that all species belonging to *Cheilanthes* subgenus *Physapteris* (equivalent to the core *covillei* clade 'cc', Fig. 3C) had hooked rather than coiled vernation. To augment these observations, we documented vernation type in all remaining species of *Myriopteris.* Non-circinate vernation, while not unique to *Myriopteris* (see outgroups, Fig. 3C), characterizes the majority of ingroup taxa, with the exception of *M. wrightii* in the *alabamensis* clade (Clade A) and all members of the *lanosa* clade (Clade L). Vernation type appears to be conserved within each of the three major myriopterid clades (Clades A, L, and C), confirming Reeves' (1979) hypothesis that vernation is a useful systematic character among cheilanthoid ferns.

Leaf Indument—Leaf indument is arguably the most useful morphological feature for identifying species among myriopterid ferns (Reeves 1979; Tryon and Tryon 1982; Windham and Rabe 1993; Mickel and Smith 2004). The presence, absence, and distribution of hairs and/or scales on the laminar surfaces vary widely among species, and the character states tend to be additive in hybrids (Reeves 1979; Grusz et al. 2009). In addition to being crucial for identification purposes, mapping indument data onto our molecular phylogeny illustrates that indument type is also a phylogenetically informative character (Fig. 4B–C), with certain indument types (or combinations thereof) providing synapomorphies for well-supported clades. Evolution of indument

on the adaxial surfaces of the ultimate segments is more easily understood because there are fewer character states involved and no amalgamation of different types. Nevertheless, variability among outgroups, as well as the early-diverging branches of the ingroup, makes it difficult to ascertain the plesiomorphic adaxial character state for *Myriopteris*, which could be either simple hairs or a lack of indument. Hairs simple is slightly more parsimonious than glabrous (six vs. seven character-state changes) based on the maximum likelihood tree (Fig. 4B). In its simplest form, this scenario would involve three independent transitions from simple hairs to no indument (all within the *alabamensis* clade), one change from simple to branched hairs (on the branch leading to *M. rawsonii*), one transition from simple hairs to skeletonized scales (a synapomorphy for Clade 3), and one further change from skeletonized scales to no indument (in *M. fendleri*). We note here that Reeves (1979) scored all members of Clade 3, except *M. gracillima* and *M. intertexta*, as glabrous on the upper surfaces of the ultimate segments. Our recoding of adaxial indument shown in Fig. 4B is based on our observations that the young leaves of all Clade 3 species (aside from the truly glabrous *M. fendleri*) have scattered skeletonized scales, though these often are lost on older leaves. The evolutionary scenario that we advance here (that branched hairs and even multiseriate, scale-like structures are derived from simple hairs) is in accord with hypotheses proposed for ferns in general by Eames (1936).

The indument of abaxial surfaces in *Myriopteris* is often different (and, in those cases, more complex) than that of adaxial surfaces (compare Figs. 4B and 4C; Reeves 1979). This suggests that the observed phenotypes may involve multiple genes, as well as differential regulation/expression, with respect to the two surfaces (e.g. as with *Arabidopsis*; Hülskamp and Schnittger 1998; Szymanski et al. 2000). Setting aside pervasive (and sometimes profound) differences in density, exactly half the sampled ingroup taxa (20 of 40) have basically the same indument type on the adaxial and abaxial surfaces of the ultimate segments. This includes seven taxa belonging to the *alabamensis* clade, all members of the *lanosa* clade and Clade 4, plus the two species of the *aurea* clade ('au') and *M. newberryi* (Figs. 4B and 4C). In *M. fendleri*, glabrous adaxial surfaces contrast with abaxial surfaces producing rare, entire scales. The greatest disparity between upper and lower surfaces is observed in *M. cucullans* and *M. notholaenoides*, in which the adaxial surfaces are glabrous whereas the abaxial show a mixture of simple hairs and entire scales. The abaxial surfaces of the other 17 ingroup species exhibit combinations of two different indument types, one of which also occurs on the adaxial surfaces. These admixtures involve either simple hairs and entire scales (in six species of the *alabamensis* clade plus the entirety of Clade 5) or skeletonized scales and ciliate scales (all species of Clade 3 except *M. fendleri*). Although these indument types are quite distinctive in theory, they intergrade completely.

Reeves (1979) used the apparent transition from ciliate scales to branched trichomes (herein called "skeletonized scales") to simple trichomes among the species of "*Cheilanthes* subgenus *Physapteris*" (our core *covillei* clade; 'cc') to argue for the exclusive evolution of uniseriate trichomes from multiseriate scales in this group. The existence of a continuum does not establish character polarity but, based on our maximum likelihood phylogeny (Fig. 4B and 4C), we hypothesize that the dominant evolutionary pathway for indument is the reverse of that proposed by Reeves (1979). The early diverging branches of the *covillei* clade (i.e. the aurea clade ('au') and *M. newberryi*) have only simple hairs on the adaxial surfaces (Fig. 4B), as do many of the more derived species (Clades 4 and 5). Therefore, we interpret the skeletonized scales found on the adaxial surfaces of nearly all species in Clade 3 as derived from simple hairs. Identical skeletonized scales occur on the abaxial surfaces of these same species, where they are completely transitional to ciliate scales and, ultimately, entire scales. Based on our phylogenetic tree, it also seems likely that entire scales evolved directly from simple hairs in some lineages. Although we disagree on some particulars, we concur with Reeves (1979: 27) in that "the nature and derivation of trichomes in cheilanthoid ferns deserves (further) critical analysis."

Cytogenetic and Reproductive Variability within Myriopteris—As documented by Windham and Yatskievych (2003), *Myriopteris* species exhibit two chromosome base numbers (x = 29 and x = 30). Although variability in base number is relatively uncommon among closely related fern species (Britton 1974), such variation is known to occur in some large genera where different base numbers often prove to be phylogenetically informative [e.g. in *Thelypteris*; Smith (1971, 1990); He and Zhang (2012)]. This pattern holds true in *Myriopteris*, with all cytogenetically studied species of Clade 2 having the chromosome base number x = 29 and all other ingroup species studied to date having x = 30 (Fig. 5A).

In addition to variation in chromosome base number, both apomixis and whole genome-duplication (i.e. polyploidy) are prevelant among species of *Myriopteris*. As with most other apomictic plant lineages (Stebbins 1950; Grant 1981), these processes are closely linked, and all known apomicts in the genus are polyploid (mostly triploid). Given these circumstances, evolutionary changes in reproductive mode should be effectively unidirectional [from sexual to apomictic; Beck et al. (2011, 2012)]. This is congruent with our phylogeny (Fig. 5B) that reveals sexual diploids predominate in all but Clade 5, and that apomictic polyploids generally are nested among the sexual taxa. Based on simple parsimony, we hypothesize at least nine independent origins of apomixis within *Myriopteris*. Apomixis in ferns requires two major changes in the life cycle (Gastony and Windham 1989): 1) a non-reductive meiosis (owing to an endomitosis preceding meiosis) that results in the production of diplospores rather than haplospores ($n = 2n$); and 2) the mitotic production of sporophytes from somatic tissue (rather than from a zygote produced via the fusion of gametes). Even so, frequent switches from sexual to apomictic reproduction across the myriopterid tree indicate that this transition may involve relatively simple genetic and/or environmental controls.

Findings of Note—Our study utilizes the power of molecular sequence data to elucidate patterns of species diversification in the genus *Myriopteris*. It provides an improved view of relationships among the morphologically disparate taxa included in this newly recircumscribed genus, and allows us to assess the evolution of several morphological, cytological, and reproductive characters within this well-supported monophyletic group. Beyond these broad-scale patterns of diversification, our findings also illuminate multiple interesting sub-stories involving the geography, parentage, and

species-level distinctions of particular taxa. Here, we briefly highlight a few of these notable findings that we hope will inspire further research.

MYRIOPTERIS RAWSONII—One of the most surprising results of this study is the corroboration of evidence put forth by Eiserhardt et al. (2011) for inclusion of the southern African endemic *Cheilanthes rawsonii* (= *M. rawsonii*) within the myriopterid clade. *Myriopteris rawsonii*, the only member of the group known to occur outside the New World, is deeply nested within the *lanosa* clade (L, Fig. 2) where it is maximally supported as sister to *M. parryi*, a sexual diploid confined to the southwestern U. S. A. and adjacent Mexico. *Myriopteris rawsonii* has long been considered a disparate element in African flora, and Anthony (1984) noted that its spores are unlike those of any other *Cheilanthes* on that continent. However, the species seems no less anomalous in *Myriopteris*, where the branched hairs on the upper and lower leaf surfaces are unique. It is interesting to note that there are ecological similarities between *M. rawsonii* and its sister species *M. parryi*; these two species occupy some of the driest, most inhospitable desert habitats in their respective ranges. Based on our counts of spore number per sporangium, *M. rawsonii* appears to be sexual, but its ploidy level remains unknown (Table 1; Fig. 5B).

MYRIOPTERIS AUREA—This species, known in the literature as *Cheilanthes bonariensis* or *Notholaena aurea*, is one of the most widely distributed cheilanthoid ferns (Tryon and Tryon 1973; Tryon 1986), with a range extending from the southwestern U. S. A. and Hispañola south to Argentina and Chile. Previously known only as an apomictic triploid, recent work by Beck et al. (unpubl.) has identified a few, highly-localized populations that produce 64 spores per sporangium; these presumably represent a relictual sexual progenitor of the widespread apomict. Interestingly, the five samples of *M. aurea* included in our analysis (all apomictic) form two highly divergent sister clades ('au', Fig. 2), suggesting either multiple origins or substantial divergence following polyploidization. Both clades of *M. aurea* are widely distributed, and there is no clear geographic or morphologic distinction evident in the current dataset.

MYRIOPTERIS LENDIGERA—Reeves (1979) proposed that this tetraploid species arose through hybridization between the sexual diploids *M. marsupianthes* and *M. mexicana.* In our phylogenetic tree (Fig. 2), these three taxa constitute a maximally supported monophyletic group (Clade 4), with the four accessions of tetraploid *M. lendigera* paraphyletic to *M. mexicana*. Two accessions of *M. lendigera* (1 and 2) and the only available sample of *M. mexicana* form a well-supported (97/1.0) clade that is sister to the other two *M. lendigera* collections. From this we infer that a genotype similar to that of the sampled *M. mexicana* individual functioned as the maternal progenitor of the tetraploid lineage represented by *M. lendigera* 1 and 2. However, the two northern accessions of *M. lendigera* (3 and 4) are highly divergent at the plastid loci analyzed (Fig. 2). These results suggest that *M. lendigera*, like the majority of hybrids studied to date (see Soltis and Soltis 1999), has arisen through recurrent hybridization between genetically distinct parental lineages.

MYRIOPTERIS MORITZIANA—Our molecular results confirm previous morphologically-based hypotheses (e.g. Yatskievych and Moran 1995) that the South American endemic *M. moritziana* is closely related to the wide-ranging Caribbean taxon *M. microphylla* (Clade 2, Fig. 2). There are subtle but critical differences between the two, however. Examination of an isolectotype of *M. moritziana* from GH indicates that this taxon is sexual (i.e. produces about 64 spores per sporangium) and reveals that spore sizes approximate those documented in closely related sexual diploid taxa (Windham unpubl.). *Myriopteris microphylla*, on the other hand, has significantly larger spores and the available chromosome counts are exclusively polyploid [sexual tetraploid in Knobloch (1967) and Walker (1966); apomictic triploid in Mickel et al. (1966)]. Based on this evidence, we hypothesize that *M. mortiziana* may be a diploid progenitor of polyploid *M. microphylla*. Given the reproductive and cytogenetic disparities involved, we tentatively maintain these two entities as separate species despite their identical sequences at the three maternally inherited loci analyzed.

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Appendix 1. Taxon—Fern DNA Database number (fernlab.biology.duke.edu), Voucher collector and collector number (Herbarium Acronym); GenBank accession numbers (with citations for previously published sequences, if existing) for *trnG–trnR; atpA; rbcL* (in that order); (# sporangia studied) spore number per sporangium observed for each

sporangium studied. For selected taxa represented by more than one duplicate of the same collection, a herbarium accession number is specified next to the herbarium acronym. Taxa that were not included in the molecular analyses or those that were not included for inferring reproductive mode have a long dash '——' in place of either the GenBank accession number or spore number per sporangium observations, respectively. Those accessions not included in molecular analyses do not have a Fern DNA Database number (designated here as 'no DB #').

Argyrochosma microphylla—4583, *Worthington 34623* (DUKE); HQ846476 (Sigel et al. 2011); HQ846374 (Sigel et al. 2011); HQ846423 (Sigel et al. 2011); ——. *Astrolepis windhamii*—3138, *Schuettpelz 431* (DUKE); JF929936 (Beck et al. 2011); KF961705; KF961768; ——. *Myriopteris aemula*—1: 5653, *Beck 1037* (DUKE); KF961828; KF961701; KF961764; (2) 58, 59. 2: 4496, *Yatskievych & Gastony 89-222* (IND); KF961827; KF961700; KF961763; ——. *Myriopteris alabamensis*—1: 3175, *Schuettpelz 468* (DUKE); KF961829; KF961702; KF961765; ——. 2: 4510, *Windham 3450* (DUKE); KF961830; KF961703; KF961766; (1) 32. 3: *no DB #*, *Blomquist 9602*, (DUKE); ——; (3) 29, 29, 31. *Myriopteris allosuroides*—1: 4497, *Yatskievych & Gastony 89-237* (IND); KF961831; KF961704; KF961767; ——. *Myriopteris aurea*—1: 7355, *Rothfels 3591* (DUKE); KF961836; KF961710; KF961773; ——. 2: 6914, *Beck 1192* (DUKE); KF961835; KF961709; KF961772; (1) 23. 3: 3173, *Schuettpelz 466* (DUKE); KF961832; KF961706; KF961769; ——. 4: 4477, *Schuettpelz 991* (DUKE); KF961833; KF961707; KF961770; (1) 26. 5: 5654, *Beck 1038* (DUKE); KF961834; KF961708; KF961771; ——. *Myriopteris chipinquensis*—1: 4498, *Knobloch 1996B* (IND); KF961839; KF961714; KF961776; ——. *Myriopteris clevelandii* —1: 3833, *Metzgar 180* (DUKE); KF961840; KF961715; KF961777; (2) 62, 63. 2: *no DB #*, *Cleveland s. n.* (YU); ——; (1) 56. *Myriopteris cooperae*—1: 6445, *Taylor 15925* (UC); KF961841; KF961717; KF961778; ——. *Myriopteris covillei*—1: 3150, *Schuettpelz 443* (DUKE); EU268679 (Rothfels et al. 2008); EU268733 (Rothfels et al. 2008); EU268733 (Rothfels et al. 2008); ——. 2: 3845, *Windham 3436* (DUKE); FJ870774 (Grusz et al. 2009); KF961718; KF961779; (2) 57, 59. 3: *no DB #*, *Beck 1090* (DUKE); ——; (1) 61. 4: *no DB #*, *Rothfels 2571* (DUKE); ——; (1) 64. 5: *no DB #*, *Covillei & Funston 593* (US); ——; (4) 61, 63, 64, 64. *Myriopteris cucullans*—1: 7138, *Beck 1137* (DUKE); KF961842; KF961719; KF961780; ——. *Myriopteris fendleri*—1: 3177, *Schuettpelz 470* (DUKE); FJ870776 (Grusz et al. 2009); KJ000204; KJ000203; ——. *Myriopteris fimbriata*—1: 6321, *Hallberg 1656* (NY); KF961846; KF961723; KF961784; (2) 62, 64. *Myriopteris gracilis*—1: 3123, *Schuettpelz 416* (DUKE); KF961845; KF961722; KF961783; ——. 2: *no DB #*, *Rothfels 2470* (DUKE); ——; (4) 30, 30, 32, 32. 3: *no DB #*, *Windham 0221A* (DUKE); ——; (1) 28. *Myriopteris gracillima*—1: 6334, *Windham 3630* (DUKE); KF961849; KF961726; KF961787; (2) 61, 62. 2: 6005, *Schuettpelz 1356A* (DUKE); KF961848; KF961725; KF961786; (2) 52, 57. 3: 3871, *Pryer 06-03* (DUKE); KF961847; KF961724; KF961785; ——. *Myriopteris intertexta* —1: 7594, *Greenhouse 5086* (JEPS); KF961852; KF961729; KF961790; ——. 2: *no DB #*, *Dudley s. n.* (US); ——; (2) 60, 64. *Myriopteris jamaicensis*—1: 6444, *Clase 3856* (US); KF961853; KF961730; KF961791; ——. *Myriopteris lanosa*— 1: 5038, *Schuettpelz 1244A* (DUKE); KF961855; KF961732; KF961793; ——. 2: 6114, *Rothfels 2717* (DUKE); KF961856; KF961733; KF961794; (3) 59, 61, 64. 3: 4495, *Hegeman s. n.* (IND); KF961854; KF961731; KF961792; ——. *Myriopteris lendigera*—1: 5575, *Grusz 110* (DUKE); KF961858; KF961735; KF961796; (1) 64. 2: 7153, *Beck 1226* (DUKE); KF961859; KF961736; KF961797; (1) 61. 3: 5074, *Yatskievych 89-432* (IND); KF961857; KF961734; KF961795; ——. 4: 3167, *Schuettpelz 460* (DUKE); EU268681 (Rothfels et al. 2008); EU268735 (Rothfels et al. 2008); EU268784 (Rothfels et al. 2008); ——. *Myriopteris lindheimeri*—1: 3157, *Schuettpelz 450* (DUKE); FJ870779 (Grusz et al. 2009); KF961737; KF961798; (2) 30, 31. 2: 5364, *Rothfels 2490* (DUKE); KF961861; KF961739; KF961800; ——. 3: 3178, *Schuettpelz 471* (DUKE); KF961860; KF961738; KF961860; ——. 4: *no DB #*, *Lindheimer 744* (K: K000501493); ——; (1) 32. 5: *no DB #*, *Lindheimer 744* (K: K000501491); ——; (2) 32, 44. *Myriopteris longipila*—1: 6325, *Mickel 6317* (NY); KF961862; ——; KF961801; ——. *Myriopteris marsupianthes*—1: 6158, *Jankiewicz 13* (UC); KF961864; KF961741; KF961803; ——. *Myriopteris mexicana*—1: 7148, *Beck 1151* (DUKE); KF961865; KF961742; KF961804; ——. *Myriopteris mickelii*—1: 6327, *Salas et al. 1848* (NY); KF961866; KF961743; KF961805; ——. *Myriopteris microphylla*—1: 4480, *Schuettpelz 994* (DUKE); KF961867; KF961744; KF961806; ——. 2: 5703, *Kessler 9568* (UC); KF961868; KF961745; KF961807; ——. 3: 9246, *Proctor 39365* (US); KF961863; KF961740; KF961802; ——. *Myriopteris moritziana*—1: 7353, *Rothfels 3589* (DUKE); KF961869; KF961746; KF961808; (3) 41, 42, 47. 2: *no DB #*, *Moritz 263* (GH); ——; (1) ca. 64. *Myriopteris myriophylla*—1: 4475, *Schuettpelz 989* (DUKE); KF961870; KF961747; KF961809; (4) 28, 30, 31, 32. 2: 6520, *Rothfels 3082* (DUKE); KF961871; KF961748; KF961810; (1) 31. 3: 6674, *Rothfels 3281* (DUKE); KF961872; KF961749; KF961811; ——. 4: 4484, *Brown 83-31-4* (IND); EU268684 (Rothfels et al. 2008); EU268737 (Rothfels et al. 2008); EU268786 (Rothfels et al. 2008); ——. 5: *no DB #*, *Schuettpelz 990* (DUKE); ——; (1) 30. *Myriopteris newberryi*—1: 3827, *Metzgar 174* (DUKE); EU268685 (Rothfels et al. 2008); EU268738 (Rothfels et al. 2008); EU268787 (Rothfels et al. 2008); (1) 62. *Myriopteris notholaenoides*—1: 4494, *Windham 481* (DUKE); KF961873; KF961750; KF961812; (2) 31, 32. 2: 5134, *Grusz et al. 08-020* (DUKE); KF961874; KF961751; KF961813; (1) 31. *Myriopteris parryi*—1: 3802, *Metzgar 149* (DUKE); KF961875; KF961753; KF961815; (2) 50, 63. 2: *no DB #*, *Windham & Yatskievych 0340A* (DUKE); ——; (1) 63. *Myriopteris peninsularis*—1: 5030, *Leon de la Luz 9764* (MO); KF961876; KF961754; KF961816; ——. *Myriopteris pringlei*—1: 3209, *Schuettpelz 502* (DUKE); HM003035 (Pryer et al. 2010); HM003027 (Pryer et al. 2010); HM003031 (Pryer et al. 2010); ——. 2: *no DB #*, *Windham & Yatskievych 0248A* (DUKE); ——; (1) 42. *Myriopteris pringlei var. moncloviensis*—1: *no DB #*, *Palmer 1378* (NY); ——; (3) 40, 49, 64. *Myriopteris rawsonii*—1: 9185, *Smook 11325* (MO); KF961877; KF961756; KF961818; (1) 41. 2: *no DB #*, *Goldblatt 7014* (MO); ——; (3) 53, 58, 61. *Myriopteris rufa*—1: 5391, *Rothfels 2515* (DUKE); KF961837; KF961711; KF961774; (1) 31. 2: 5367, *Rothfels 2493* (DUKE); KF961844; KF961721; KF961782; ——. 3: 6199, *Windham 3545* (DUKE); KF961838; KF961713; KF961775; (1) 31. 4: 2968, *Schuettpelz 323* (DUKE); JQ855901 (Johnson et al. 2012); EF452084 (Schuettpelz et al. 2007); EF452144 (Schuettpelz et al. 2007); (1) 31. 5: 3814, *Metzgar 161* (DUKE); KF961843; KF961720; KF961781; (1) 32. 6: *no DB #*, *Rothfels 3902* (DUKE); ——; (1) 30. 7: *no DB #*, *Windham & Windham 0021B* (DUKE); ——; (1) 16. *Myriopteris scabra*—1: 4500, *Gastony 90-10-1* (IND); KF961850; KF961727; KF961788; ——. 2: 5652, *Beck 1036* (DUKE); KF961851; KF961728; KF961789; (1) 60. *Myriopteris tomentosa*—1: 2721, *Christenhusz 3823* (DUKE); KF961878; KF961757; KF961819; ——. *Myriopteris viscida*—1: 3822, *Metzgar 169* (DUKE); KF961880; KF961759; KF961821; (3) 32, 32, 32. *Myriopteris windhamii*—1: 4491, *Windham 458* (DUKE); KF961881; KF961760; KF961822; ——. 2: 5666, *Beck 1050* (DUKE); KF961879; KF961758; KF961820; (1) 27. 3: *no DB #*, *Lemmon s. n.* (US); ——; (1) 32. *Myriopteris wootonii*—1: 3195, *Schuettpelz 488* (DUKE); FJ870784 (Grusz et al. 2009); KF961761; KF961823; ——. *Myriopteris wrightii*—1: 3148, *Schuettpelz 488* (DUKE); HM003034 (Pryer et al. 2010); HM003026 (Pryer et al. 2010); HM003030 (Pryer et al. 2010); ——. 2: *no DB #*, *Windham 0341A* (DUKE); (2) 58, 63. *Myriopteris yatskievychiana*—1: 6333, *Burquez 96-302* (MO); KF961884; KF961712; KF961825; ——. *Myriopteris yavapensis*—1: 3122, *Schuettpelz 415* (DUKE); FJ870789 (Grusz et al. 2009); KF961716; KF961826; (1) 29. 2: *no DB #*, *Licher 778* (DUKE); ——; (3) 21, 31, 31. *Paragymnopteris marantae*—3736, *Yatskievych 02-35* (MO); EU268711 (Schuettpelz et al. 2007); EU268763 (Schuettpelz et al. 2007); EF452161 (Schuettpelz et al. 2007); ——. *Pellaea atropurpurea*—2957, *Schuettpelz 312* (DUKE); JQ855913 (Johnson et al. 2012); JQ855925 (Johnson et al. 2012); EF452162 (Schuettpelz et al. 2007); ——.

Appendix 2. List of *Myriopteris* taxa (from Grusz and Windham 2013) with names commonly applied to them in *Cheilanthes*.

Cheilanthes aemula Maxon	=	*Myriopteris aemula* (Maxon) Grusz & Windham
Cheilanthes alabamensis (Buckley) Kunze	=	*Myriopteris alabamensis* (Buckley) Grusz & Windham
Cheilanthes allosuroides Mett.	=	*Myriopteris allosuroides* (Mett.) Grusz & Windham
Cheilanthes bonariensis (Willd.) Proctor	=	*Myriopteris aurea* (Poir.) Grusz & Windham
Cheilanthes chipinquensis Knobloch & Lellinger	=	*Myriopteris chipinquensis* (Knobloch & Lellinger) Grusz & Windham
Cheilanthes clevelandii D. C. Eaton	=	*Myriopteris clevelandii* (D. C. Eaton) Grusz & Windham
Cheilanthes cooperae D. C. Eaton	=	*Myriopteris cooperae* (D. C. Eaton) Grusz & Windham
Cheilanthes covillei Maxon	=	*Myriopteris covillei* (Maxon) Á. Löve & D. Löve
Cheilanthes cucullans Fée	=	*Myriopteris cucullans* (Fée) Grusz & Windham
Cheilanthes eatonii Baker	=	*Myriopteris rufa* Fée
Cheilanthes fimbriata (A. R. Sm.) Mickel & Beitel	=	*Myriopteris fimbriata* (A. R. Sm.) Grusz & Windham
Cheilanthes feei T. Moore	=	*Myriopteris gracilis* Fée
Cheilanthes gracillima D. C. Eaton	=	*Myriopteris gracillima* (D. C. Eaton) Grusz & Windham
Cheilanthes horridula Maxon	=	*Myriopteris scabra* (C. Chr.) Grusz & Windham
Cheilanthes intertexta Maxon	=	*Myriopteris intertexta* (Maxon) Grusz & Windham
Cheilanthes jamaicensis Maxon	=	*Myriopteris jamaicensis* (Maxon) Grusz & Windham
Cheilanthes lanosa (Michx.) D. C. Eaton	=	*Myriopteris lanosa* (Michx.) Grusz & Windham
Cheilanthes lendigera (Cav.) Sw.	=	*Myriopteris lendigera* (Cav.) Fée
Cheilanthes lindheimeri Hook.	=	*Myriopteris lindheimeri* (Hook.) J. Sm.
Cheilanthes longipila Baker	=	*Myriopteris longipila* (Baker) Grusz & Windham
Cheilanthes marsupianthes (Fée) T. Reeves & Mickel	=	*Myriopteris marsupianthes* Fée
Cheilanthes maxoniana Mickel	=	*Myriopteris maxoniana* (Mickel) Grusz & Windham
Cheilanthes mexicana Davenp.	=	*Myriopteris mexicana* (Davenp.) Grusz & Windham
Cheilanthes microphylla (Sw.) Sw.	=	*Myrioperis microphylla* (Sw.) Grusz & Windham
Cheilanthes moritziana Kunze	=	*Myriopteris moritziana* (Kunze) Grusz & Windham
Cheilanthes myriophylla Desv.	=	*Myriopteris myriophylla* (Desv.) Grusz & Windham
Cheilanthes newberryi (D. C. Eaton) Domin.	=	*Myriopteris newberryi* (D. C. Eaton) Grusz & Windham
Cheilanthes notholaenoides (Desv.) Maxon ex Weath.	=	*Myriopteris notholaenoides* (Desv.) Grusz & Windham
Cheilanthes parishii Davenp.	=	*Myriopteris* × *parishii* (Davenp.) Grusz & Windham
Cheilanthes pringlei Davenp.	=	*Myriopteris pringlei* (Davenp.) Grusz & Windham
Cheilanthes rawsonii Mett. ex Kuhn	=	*Myriopteris rawsonii* (Mett. ex. Kuhn) Grusz & Windham
Cheilanthes tomentosa Link.	=	*Myriopteris tomentosa* (Link.) Fée
Cheilanthes villosa Davenp. ex Maxon	=	*Myriopteris windhamii* Grusz
Cheilanthes viscida Davenp.	=	*Myriopteris viscida* (Davenp.) Grusz & Windham
Cheilanthes wootonii Maxon	=	*Myriopteris wootonii* (Maxon) Grusz & Windham
Cheilanthes wrightii Hook.	=	*Myriopteris wrightii* (Hook.) Grusz & Windham
Cheilanthes yatskievychiana Mickel	=	*Myriopteris yatskievychiana* (Mickel) Grusz & Windham
Cheilanthes yavapensis Reeves ex Windham	=	*Myriopteris yavapensis* (Reeves ex Windham) Grusz & Windham