Evolutionary Patterns and Processes in the Desert-Adapted Fern Genus *Myriopteris*

(Pteridaceae)

by

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Michael D. Windham

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

This dissertation investigates the processes of hybridation, polyploidy, and apomixis and their roles in the evolution of myriopterid ferns. First, I examine patterns of hybridization in members of the *Cheilanthes yavapensis* complex using a suite of techiniques, ranging from molecules to morphology—including isozymes, spore measurements, and molecular phylogenetics based on chloroplast and nuclear markers to elucidate relationships in this notorious group of ferns. Second, I utilize the rules of traditional taxonomy set by the Code of Botanical Nomenclature to recircucmscribe and resurrect the genus *Myriopteris* from within cheilanthoid ferns. This revised classification is bolstered by results from my molecular phylogenetic analysis of DNA sequence data in the subsequent chapter. Then, using morphological and cytological analyses, I examine the evolution of indument, leaf and rachis shape, vernation, chromosome number, and reproductive mode across the myriopterid tree. In my concluding chapter I develop microsatellite markers for the apomictic triploid, *M. lindheiemeri* and explore whether premeiotic chromosome duplications in this apomict result in the production of genetically variable offspring.

For my parents:

It takes a team to keep all of these eggs in one basket…

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The manuscripts prepared in fulfillment of this degree (and related publications) were thoughtfully reviewed by many colleagues, in some cases over multiple iterations; their constructive criticisms have dramatically improved the quality of my writing and research. In particular, I extend appreciation to J. B. Beck, D. O. Burge, G. J. Gastony, R. L. Huiet, F.-W. Li, J. S. Metzgar, R. C. Moran, N. S. Nagalingum, K. M. Pryer, C. J.

Rothfels, E. Schuettpelz, E. M. Sigel, M. D. Windham, and G. Yatskievych. Additionally, I thank A. R. Smith, R. Moran, K. N. Gandhi, and L. J. Dorr for assistance interpreting and/or obtaining obscure taxonomic literature. I would like to thank illustrator H. Fukuda, authors J. Mickel and A. R. Smith, and The New York Botanical Garden Press for allowing me to reproduce line drawings from their publication, *The Pteridophytes of Mexico* (e.g., Chapter 3 herein). Enzyme studies critical to Chapter 1 of this dissertation were conducted in the lab of C. H. Haufler, and to him I also extend thanks for inspiring my interest in the development of gametophytes and fern reproductive organs. For contributions to laboratory work, I acknowledge C. J. Rothfels, E. Schuettpelz, and T.-T. Kao; M. G. Johnson provided useful advice pertaining to phylogenetic analyses (and never failed to make me smile).

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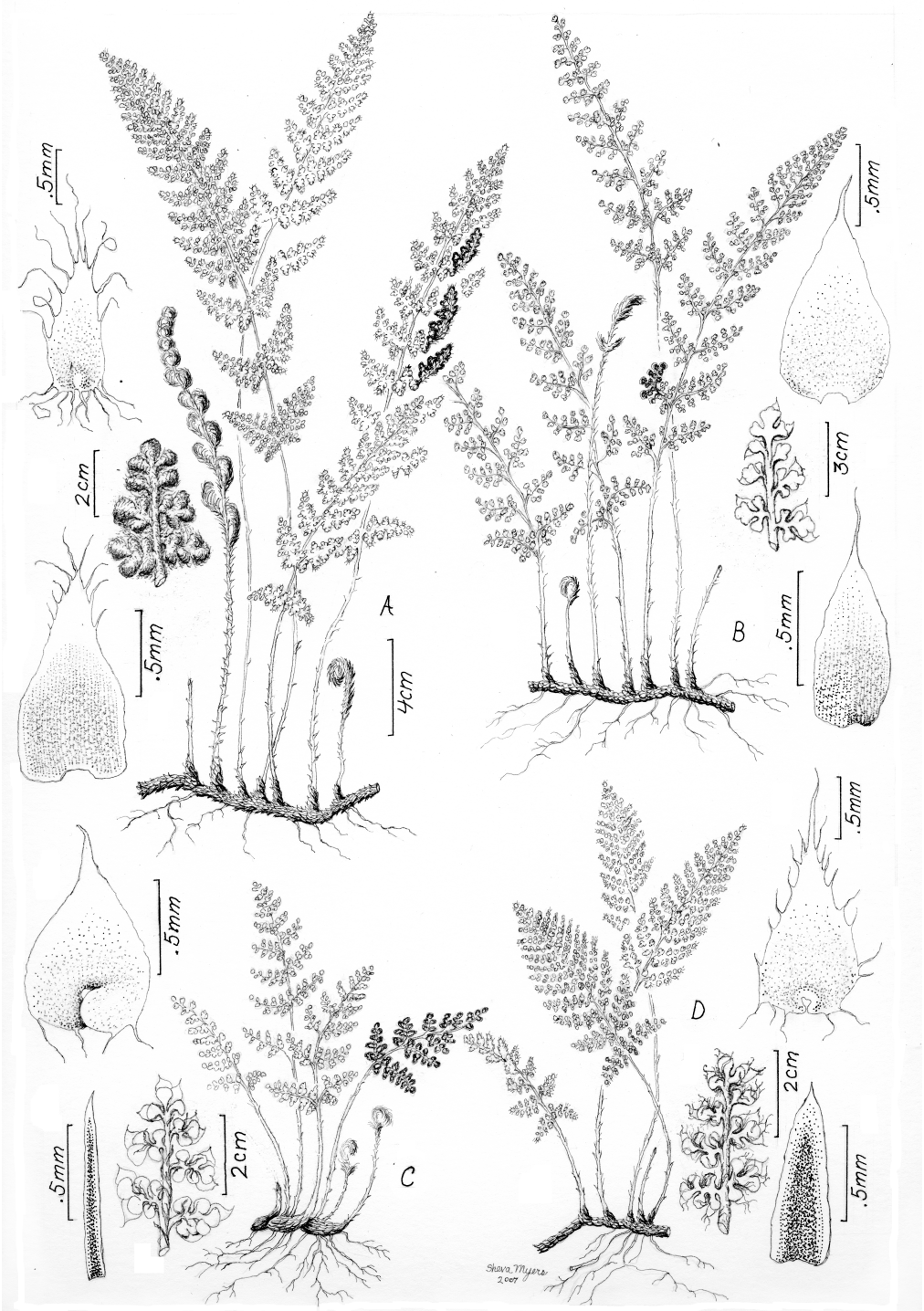
It goes without saying that I owe a huge debt to my family and friends—for their love, support, patience, flexibility, and good humor I am endlessly grateful.



The *Myriopteris yavapensis* complex. *Myriopteris lindheimeri* (A), *Myriopteris fendleri* (B), *Myriopteris covillei* (C), and *Myriopteris yavapensis* (D).

INTRODUCTION

This disseration has been a labor of love, punctuated by moments of extreme curiousity and intrigue together with long nights of sluggish persistence. It was initially borne out of a seemingly simple investigation—as are most interesting things—conducted while I was still an undergraduate at the University of North Carolina–Wilmington. The summer preceding my senior year, I was offered the opportunity to participate in an NSF-REU summer program at Duke University studying plant systematics. I was immediately drawn to ferns based on their complicated (and large) genomes, not to mention their curious morphology, ecology, and life history. During that summer, I investigated chloroplast inheritance in the apomictic tetraploid fern, *Myriopteris yavapensis*. What was expected to be a straightforward study showing *M. covillei* to be the maternal parent of that fern became a far more circuitous story (as you will see in Chapter I). I was driven to elucidate patterns of hybridization underlying the diversity of genotypes surrounding *M. yavapensis* and its relatives. Fortunately, after being accepted to the Department of Biology at Duke University, I was offered the opportunity to do just that.

Thus, Chapter I, completed in my first years of graduate school, examines the origins of apomictic polyploids in the *M. yavapensis* complex. I continue to be grateful to my coauthors (and mentors), K. M. Pryer and M. D. Windham for guiding me through the process of generating data through to publishing my first scientific article. As you read this chapter, you will notice that the species names used therein are not *Myriopteris*, but *Cheilanthes*. The reason for this discrepancy is made clear in Chapter II, wherein I formally recognize the newly recircumscribed genus *Myriopteris* Fée.

Many long hours, over the course of several years, went into the construction of Chapter II. Cheilanthoid ferns are a notoriously difficult group with respect to traditional classification. This fact has resulted in a veritable nomenclatural nightmare for many genera comprised therein. *Cheilanthes* is no exception. In order to bring the taxonomy of myriopterid *Cheilanthes* current, and corresponding with now known phylogenetic relationships, I anguished over the complicated historical nomenclature of this group to produce a refined classification of *Myriopteris*. While this exercise was completed independently, I am deeply indebted to my coathor, M. D. Windham, for his encouragement and expertise through this process. Michael is truly an expert in this group of ferns and without his enthusiasm (and oversight) it is unlikely that this chapter would have made it through to publication.

Chapter III represents a transtion in my research focus from pure systematics to a focus on evolutionary processes. Using plastid markers and molecular phylogenetics, I constructed a highly supported and well-resolved phylogeny of the newly circumscribed genus, *Myriopteris*. This phylogeny served as a starting point for further investigations of character evolution among myriopterid ferns, including morphological aspects such as leaf and rachis shape, vernation, and (perhaps most interesting of all) leaf indument. A further exploration of variation in ploidy level and reproductive mode across this phylogeny coincided with, and further motivated, the beginning of my intense focus on experiments conducted in Chapter IV.

The final chapter of this dissertation was truly a labor of love; it is a project for which I independently procured funding, designed an experimental approach, executed that experimental approach, and discovered intriguing results that are propelling me to continue studying asexuality in ferns in the next stage of my career. This investigation of the genomic underpinnings of apomixis in *M. lindheimeri* elegantly caps a journey that

began with my simple study of chloroplast inheritance in tetraploid *M. yavapensis* from its maternal progenitor, a rare sexual diploid cytotype of *M. lindheimeri*, and concludes with observations of understudied and complex patterns of genetic diversity in apomictic *M. lindheimeri*, a byproduct of which allowed me recently to discover a previously undetected apomictic diploid cytotype of that same parental plant.

These investigations track my interests from early on, with polyploidy and genome evolution, abreast to my experiments with classical taxonomony and molecular systematics, and back again to my detailed investigations of the evolutionary processes that are intimately tied to hybridization, polyploidy, and apomixis—all of which have inspired and reinforced my love of seed free vascular plants. I feel privedged to have spent these years gaining expertise in the field of fern biology and look forward to expanding my knowledge in the future.

CHAPTER I

DECIPHERING THE ORIGINS OF APOMICTIC POLYPLOIDS IN THE *CHEILANTHES YAVAPENSIS* COMPLEX (PTERIDACEAE)

Summary

Deciphering species relationships and hybrid origins in polyploid agamic species complexes is notoriously difficult. In this study of cheilanthoid ferns I demonstrate increased resolving power for clarifying the origins of polyploid lineages by integrating evidence from a diverse selection of biosystematic methods. The prevalence of polyploidy, hybridization, and apomixis in ferns suggests that these processes play a significant role in their evolution and diversification. Using a combination of systematic approaches, I investigated the origins of apomictic polyploids belonging to the *Cheilanthes yavapensis* complex. Spore studies allowed me to assess ploidy levels; plastid and nuclear DNA sequencing revealed evolutionary relationships and confirmed the putative progenitors (both maternal and paternal) of taxa of hybrid origin; enzyme electrophoretic evidence provided information on genome dosage in allopolyploids. I show here that the widespread apomictic triploid, *Cheilanthes lindheimeri*, is an autopolyploid derived from a rare, previously undetected, sexual diploid. The apomictic triploid *Cheilanthes wootonii* is shown to be an interspecific hybrid between *C. fendleri* and *C. lindheimeri*, whereas the apomictic tetraploid *C. yavapensis* is comprised of two cryptic and geographically distinct lineages. I show that earlier morphology-based hypotheses of species relationships, while not altogether incorrect, only partially explain the complicated evolutionary history of these ferns.

Introduction

Hybridization, polyploidy, and apomixis are each common processes in plants (Grant 1981). When combined in a single lineage, the resulting evolutionary complexity can frustrate even the most dedicated attempts to circumscribe species and understand relationships among them. The best-known examples of this are the polyploid agamic species complexes of such angiosperm genera as *Amelanchier* (Campbell and Wright 1996), *Antennaria* (Bayer,1987), *Boechera* (Schranz et al. 2005), *Crataegus* (Talent and Dickinson 2005), *Crepis* (Whitton et al. 2008), *Poa* (Soreng 1990), *Rubus* (Einset,1951), and *Taraxacum* (Verduijn et al. 2004). However, such taxonomic complexity is by no means limited to the angiosperms, and examples abound in the ferns as well. Polyploid agamic species complexes are especially common in the family Pteridaceae, where members of both the pteroid (Walker 1962) and cheilanthoid (Benham 1989; Windham 1993a; Windham and Rabe 1993) lineages show extensive reticulate evolution. In this study of cheilanthoid ferns I integrate evidence from a diverse selection of biosystematic approaches to provide a comprehensive example of the investigative depth required for complex speciation studies.

Here I investigate the evolutionary complexity that has resulted from the interplay of hybridization, polyploidy, and apomixis in the *Cheilanthes yavapensis* Reeves ex Windham complex. This complex, which is endemic to the southwestern United States and adjacent Mexico, consists of several sexual diploids, as well as apomictic and sexual polyploids, all of which hybridize to form various polyploid lineages (Windham and Rabe 1993). In this group, apomictic triploids and tetraploids represent a major

source of taxonomic confusion because they are able to hybridize with sexual taxa and form higher ploidy hybrids that are reproductively competent. A prime example of this complicated scenario is provided by *Cheilanthes yavapensis* itself.

Prior to 1993, *C. yavapensis* was included within the circumscription of *C. wootonii* Maxon. The first suggestion that the latter might comprise more than one taxon came from Reeves' (1979) morphological investigation of relationships within *Cheilanthes* subg. *Physapteris*. Based on disparities observed in rhizome and costa scale morphologies, Reeves (1979) concluded that *C. wootonii* harbored two distinct taxa: *C. wootonii* s.s. and an entity that he tentatively called "*C. yavapensis*." Because *C. wootonii* shares many features with *C. fendleri* Hook. and *C. lindheimeri* Hook., Reeves (1979) hypothesized that it arose through hybridization between these species (Fig. 1), which are sympatric over much of its range. *Cheilanthes* "*yavapensis*," on the other hand, appeared intermediate between *C. covillei* Maxon and *C. lindheimeri* and was hypothesized to be a fertile apomictic hybrid between them (Fig. 1).

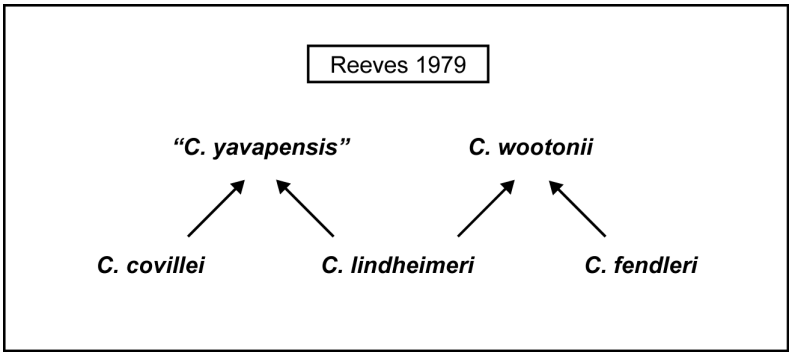


Figure 1

Proposed relationships of taxa belonging to the C. yavapensis complex as described by Reeves.(1979). Arrows point from putative parents to hypothesized offspring.

Subsequent work on this group provided additional evidence that populations referred to *C. wootonii* s.l. included at least two distinct taxa. Typical *C. wootonii* was determined to be an apomictic triploid ($n = 2n = 90$) with spores averaging < 64 μm in diameter (Windham 1983). *Cheilanthes "yavapensis"* proved to be an apomictic tetraploid ($n = 2n = 120$) with spores $\geq$ 64 μm in diameter (Windham 1993b). Results from enzyme electrophoretic analyses (Gastony and Windham 1989) supported Reeves' (1979) hypothesis that these taxa had separate origins and distinct genetic makeups. Based on these data, Windham (1993b) formally published *C. yavapensis* Reeves ex Windham.

Despite evidence that *C. wootonii* and *C. yavapensis* are distinct entities, they remain difficult to distinguish by superficial observation of morphological traits. Although Windham and Rabe (1993) provided a suite of morphological characters (pinna pubescence, costal scale ciliation, rhizome scale color and persistence) that allowed the identification of most specimens, one of the most useful characters—spore size—requires the use of a compound microscope. Identification becomes particularly difficult in the northwestern and southeastern portions of their ranges (Nevada and Texas, respectively), where intermediate morphologies occur. Using a combination of systematic approaches—spore studies, plastid and nuclear DNA sequencing, and enzyme electrophoresis—I aim to clarify the relationships between *C. wootonii* and *C. yavapensis* and decipher the events and processes that gave rise to these apomictic polyploids.

Materials and Methods

Taxon sampling. A total of twenty-one specimens representing the five members of the *C. yavapensis* complex (*C. covillei*, *C. fendleri*, *C. lindheimeri*, *C. wootonii*, and *C. yavapensis*) were selected for study (Appendix A). Based on its position as an early diverging member of the clade containing the *C. yavapensis* complex (i.e., the myriopterid cheilanthoids; Windham et al., in review; Grusz et al., unpublished data), *C. newberryi* was chosen as the most appropriate outgroup.

Spore number per sporangium and spore diameter. Only fertile (sporulating) specimens were included in my analyses. Intact, mature sporangia were removed from each specimen, placed in a drop of glycerol on a slide, and gently ruptured with a needle tip. These preparations were used to determine the number of spores per sporangium (64 or 32), which, in cheilanthoid ferns, is strongly correlated with reproductive mode (sexual or apomictic, respectively; Tryon 1968; Gastony and Windham 1989). Because spore size is a good indicator of ploidy (Barrington and Paris 1986), the diameter of twenty-five spores from each individual was measured and then compared to spores from chromosome vouchers cited by Windham and Yatskievych (2003) to establish the ploidy level of each accession.

cpDNA sequencing. For each sampled individual (Appendix A), genomic DNA was extracted from silica-dried material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the protocol described in Schuettpelz and Pryer (2007). Four plastid loci—*rbcL-atpB* (~1200 bp), *trnG-trnR* (~1138 bp), *trnP*^{UGG}*-petG* (~540 bp), and *rps4-trnS*^{GGA} (~980 bp)—were amplified by PCR using 1X PCR buffer IV

containing $MgCl_2$ (ABgene, Epsom, United Kingdom), 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 1), and 1 μl template DNA for a 25 μl reaction. 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, locus-specific annealing temperature for 1 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. PCR purification and sequencing followed the protocol of Schuettpelz et al. (2008). All plastid sequences (86 newly obtained) were subsequently deposited in GenBank (Appendix A).

Table 1 **Primers used to amplify and sequence DNA for members of the *Cheilanthes yavapensis* complex.**

DNA Region	Primer	5′-3′ Primer Sequence	Primer Source
rbcL-atpB	ESRBCL26R[a]	GCTTTAGTCTCCGTTTGTGGTGACAT	Korall et al., 2007
rbcL-atpB	ATPB609R	TCRTTDCCTTCRCGTGTACGTTC	Pryer et al., 2004
rbcL-atpB	ATPBSPACER703R[a]	CCAATGATCTGAGTAATSTATCC	Korall et al., 2007
trnG-trnR	TRNG1F[a]	GCGGGTATAGTTTAGTGGTAA	Nagalingum et al., 2007
trnG-trnR	TRNGR353F	TTGCTTMTAYGACTCGGTG	Korall et al., 2007
trnG-trnR	TRNG63R	GCGGGAATCGAACCCGCATCA	Nagalingum et al., 2007
trnG-trnR	TRNR22R[a]	CTATCCATTAGACGATGGACG	Nagalingum et al., 2007
trnP^{UGG}-petG	trnP^{UGGa}	TGTAGCGCAGCYYGGTAGCG	Small et al., 2005
trnP^{UGG}-petG	petG2[a]	CAATAYCGACGKGGYGATCAATT	Small et al., 2005
trnS^{GGA}-rps4	trnS^{GGAa}	TTACCGAGGGTTCGAATCCCTC	Shaw et al., 2005
trnS^{GGA}-rps4	rps4.5′[a]	ATGTCSCGTTAYCGAGGACCT	Souza-Chies et al., 1997
gapCp	ESGAPCP8F1[a]	ATYCCAAGYTCAACTGGTGCTGC	Schuettpelz et al., 2008
gapCp	ESGAPCP11R1[a]	GTATCCCCAYTCRTTGTCRTACC	Schuettpelz et al., 2008

Nuclear DNA sequencing. Amplification, cloning, and sequencing of the low-copy nuclear locus, *gapCp*, for each individual sampled (Appendix A) followed the protocol of Schuettpelz et al. (2008). More than one copy of *gapCp* is recovered in this group of cheilanthoid ferns, one ~900 bp copy and one ~600 bp copy. The ~900 bp copy coincides in length with a copy of nuclear *gapC*, which also occasionally amplifies using the given primers. Therefore, I chose to sequence only the ~600 bp copy (i.e., the "short copy" of *gapCp*; Schuettpelz et al. 2008).

In order to account for false sequence variation attributable to PCR error, consensus allele sequences were compiled for the "short copy" *gapCp* locus for each individual sampled. This was done by first combining all sequence clones obtained from a given *individual* into a single alignment in MacClade 4.08 (Maddison and Maddison,2005). The alignments (each corresponding to a given individual) were then analyzed separately using a maximum parsimony optimality criterion with the default parsimony settings in PAUP* (Swofford 2001). The resulting trees were used to determine unique alleles present in each individual. Alleles were recognized when one or more clones from a given individual were united by one or more shared characters. After identifying all sequence clones for a given allele, those sequences were combined in a single project in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and edited by hand using a "majority-rule" criterion to form a final consenus allele sequence; instances of PCR error and chimeras were easily identified in this way and never occurred in more than one sequence read. Consensus allele sequences (48 newly obtained) were used in all subsequent analyses of the nuclear data set and deposited in GenBank (Appendix A).

Sequence alignment and phylogenetic analyses. Manual alignments of the plastid *rbcL-atpB*, *trnG-trnR*, *trnP*^{UGG}*-petG*, *rps4-trnS*^{GGA}, and nuclear *gapCp* sequences were carried out using MacClade and are deposited in TreeBase (http://www.treebase.org; Submission P.I.N. 23241). Unambiguous indels were not recoded nor were they excluded from the alignments; ambiguous indels were excluded. A total of six data sets were analyzed: the four plastid single-gene data sets, a combined plastid four-gene data set, and the nuclear single-gene data set. The six data sets were analyzed using both a Bayesian Markov chain Monte Carlo (B/MCMC) approach employing the GTR + I + Γ model of sequence evolution, as implemented in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), as well as a likelihood approach as implemented in GARLI version 0.951 (Zwickl 2006). Conflict among the resulting phylogenies was assessed according to a 0.95 posterior probability (PP) measure for B/MCMC and a 70% maximum likelihood bootstrap (BS) criterion (Mason-Gamer and Kellogg 1996). Because a comparison of the phylogenies that resulted from each of the four individual plastid data set analyses revealed no incongruence supported across methods (e.g., ML vs. B/MCMC) or across data sets (e.g., *rbcL-atpB* vs. *trnG-trnR*), the data from the four plastid partitions were combined into a single data set.

All B/MCMC analyses comprised four independent runs, each with four chains (one cold and three heated). Default (i.e., flat) priors were used, with the exception of the rate prior that was set to allow rates of evolution to vary among loci (ratepr = variable) in the combined analysis. Chains were run for 10 million generations and trees were sampled from the cold chain every 1000 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). In GARLI, a most likely topology was identified for each of the six data sets and branch support was assessed using a maximum likelihood BS approach. Maximum likelihood BS analyses (500 replicates) employed the default model of sequence evolution and parameter values estimated by GARLI (Zwickl 2006).

Enzyme electrophoresis. Fresh leaves of each ingroup taxon were obtained from populations included in the DNA and spore studies and analyzed for electrophoretically detectable enzyme markers. Samples were extracted by crushing a small section (ca. 50 mm^2) of fresh leaf tissue in ten drops of the phosphate grinding buffer-PVP solution of Soltis et al. (1983). This extract was absorbed into paper wicks that were then inserted into 12.5% starch gels for electrophoresis. Twelve enzyme loci (list available from authors) were surveyed using standard buffers and stains (Soltis et al. 1983). Following this initial survey, my work focused exclusively on the cytosolic locus of triosephosphate isomerase (TPI-2), which was resolved on gel/electrode buffer system 6 of Soltis et al. (1983). Stained gels were photographed using a red filter and Kodak Technical Pan 2415 high contrast film.

Results

Spore number per sporangium and spore diameter. The samples of *C. fendleri* and *C. covillei* included in my analyses all had 64 spores per sporangium and spore diameters < 50 μm, which is characteristic of sexual diploids. All samples of *C. wootonii* and *C. yavapensis* had 32 spores per sporangium, in agreement with previous work (Windham and Yatskievych 2003), demonstrating that all were apomictic. Average spore diameters of *C. wootonii* ranged from 59-63 μm (characteristic of apomictic triploids), whereas those of *C. yavapensis* ranged from 64-68 μm (apomictic tetraploid). Unlike the other ingroup taxa, *C. lindheimeri* exhibited variation in both spore number per sporangium and average spore diameter. The majority of specimens had 32 spores per sporangium and spore diameters > 59 μm, consistent with earlier studies (Reeves,1979; Windham and Yatskievych 2003) that identified *C. lindheimeri* as an apomictic triploid. However, three samples yielded 64 spores per sporangium and average spore measurements in the 47-50 μm range. These represent a rare, previously undetected, sexual diploid cytotype of *C. lindheimeri* (see Schuettpelz et al. 2008).

cpDNA phylogeny. Trees resulting from maximum likelihood and Bayesian analyses of the combined plastid four-gene data set had identical topologies. Maximum likelihood analysis resulted in a single most likely tree (ln L = -6280.9384; Fig. 2). Three well-supported clades were resolved, corresponding to the *C. covillei*, *C. fendleri*, and *C lindheimeri* genomes. The *C. covillei* clade included only accessions of diploid *C. covillei* (PP = 1.0, BS = 100%), whereas the *C. fendleri* clade (PP = 1.0, BS = 100%) included all samples of diploid *C. fendleri* plus all accessions of the apomictic triploid *C. wootonii*.

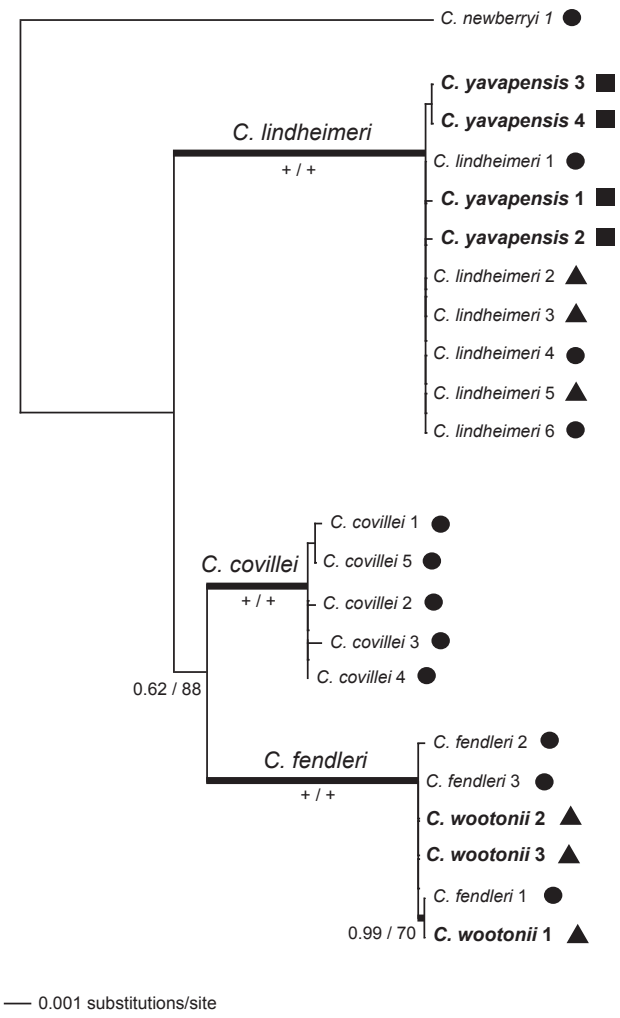


Figure 2

Plastid phylogeny for members of the *Cheilanthes yavapensis* complex based on combined analysis of *rbcL-atpB*, *trnG-trnR*, *trnP*^{UGG}*-petG*, and *rps4-trnS*^{GGA}. The best maximum likelihood topology is shown (ln L = -6280.9384); accessions of the apomictic allopolyploids *C. wootonii* and *C. yavapensis* are shown in bold. Following each taxon name is a unique identification number corresponding to a particular individual (Appendix). Circles, triangles, and squares depict ploidy level (diploid, triploid, and tetraploid, respectively). Numbers below branches correspond to posterior probability and maximum likelihood bootstrap support (PP / BS), respectively. Thickened branches indicate posterior probability ≥ 0.95 *and* maximum likelihood bootstrap support ≥ 70%. Plus signs highlight branches that have PP = 1.0 and/or BS = 100%, respectively.

The *C. lindheimeri* clade (PP = 1.0, BS = 100%) included all samples of that species (regardless of ploidy level), plus all accessions of *C. yavapensis*. Sexual diploid and apomictic triploid samples of *C. lindheimeri* have identical plastid sequences, suggesting that genetic divergence between the cytotypes is minimal.

Nuclear phylogeny. Trees resulting from maximum likelihood and Bayesian analyses of the ~600 bp "short copy" *gapCp* data set had identical topologies. Maximum likelihood analysis resulted in a single most likely tree (ln L = -12712.6944; Fig. 3) with three strongly supported clades corresponding to *C. covillei* (PP = 1.0, BS=100%), *C. fendleri* (PP = 1.0, BS = 89%), and *C. lindheimeri* (PP = 1.0, BS = 99%). All consensus allele sequences obtained from the apomictic triploid samples of *C. lindheimeri* (41 clones) formed a well-supported clade with the diploid accessions (Fig. 3), supporting an autotriploid origin for the apomictic cytotype (see Schuettpelz et al. 2008). By contrast, both *C. wootonii* and *C. yavapensis* showed strong evidence of allopolyploid origins. The three accessions of triploid *C. wootonii* each yielded sequences that clustered with *C. fendleri* and *C. lindheimeri*. The situation encountered in tetraploid *C. yavapensis* was even more complex; all four accessions contained sequences derived from *C. covillei*, *C. fendleri*, and *C. lindheimeri* (Fig. 3).

Enzyme electrophoresis. Triosephosphate isomerase (TPI) was the only enzyme surveyed that provided genome specific markers. Figure 4 summarizes my findings with respect to this enzyme. In all plants sampled, TPI was expressed as two loci (TPI-1 and TPI-2) active in the plastid and cytosol, respectively. As in most ferns (Gastony 1988), the plastid locus (TPI-1) was represented by a highly conserved (i.e., invariant across this sample), three-banded pattern in which the fastest migrating band was

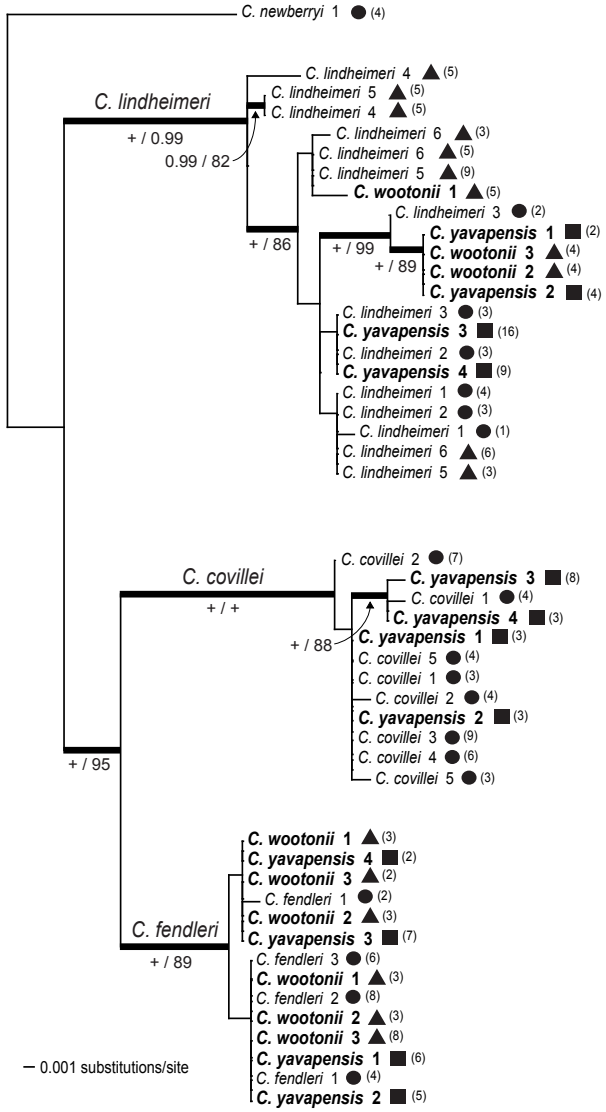


Figure 3

Nuclear phylogeny for the *Cheilanthes yavapensis* complex based on *gapCp*. The best maximum likelihood topology is shown (ln L = -12712.6944). Consensus allele sequences from accessions of *C. wootonii* and *C. yavapensis* are shown in bold. Symbols and thickened branches are as described in Fig. 3. Numbers in parentheses (following taxon names) indicate the number of clones that were compiled to determine the corresponding consensus allele sequence.

distinctly fainter than the other two. This triplet generally migrated farther into the gel than products of the cytosolic locus and is most apparent in lanes 1 and 2 of Figure 4. In contrast to the plastid locus, the cytosolic form of the enzyme (TPI-2) was variable across my study group, with each species exhibiting a distinctive band pattern.

Samples of *C. covillei* appear in lanes 1 and 2, which show a single band (labeled C-C homodimer) at the cytosolic locus. Though not represented by diploids on this gel, the marker bands for *C. fendleri* (F-F homodimer) and *C. lindheimeri* (L-L homodimer) are apparent in the samples of *C. wootonii* (lanes 5 and 6). The F-F homodimer is the fastest migrating band at the cytosolic locus and overlaps the slowest band of the plastid locus; the L-L homodimer is the slowest migrating band. Because TPI is a dimeric enzyme, the presence of both F and L in the cytosol results in a band of intermediate mobility (F-L heterodimer; see lanes 5 and 6). This F-L heterodimer migrates the same distance as the C-C homodimer (see lanes 1 and 2). In individuals where both the F-L heterodimer and the C-C homodimer are present (see lanes 3, 4, and 7), these bands are indistinguishable, yet detectable due to the additional presence of F-C and C-L heterodimers in the cytosol. The relative intensity of the three cytosolic bands in lanes 5 and 6 is useful for determining the genomic constitution of *C. wootonii*. The F-F homodimer and F-L heterodimer are approximately equal in intensity and significantly darker than the L-L homodimer. This pattern is congruent with the 4:4:1 ratio expected in a triploid that contains two genomes of *C. fendleri* and one of *C. lindheimeri*.

Cheilanthes yavapensis shows all bands that would be expected in a hybrid containing the C, F, and L genomes (Fig. 4). The F-F and L-L homodimers are apparent,

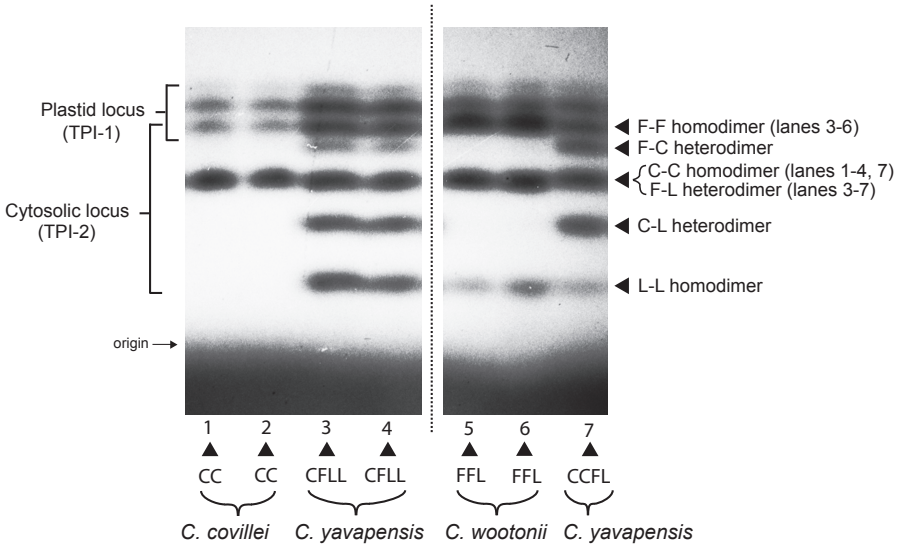


Figure 4

TPI enzyme gel for *Cheilanthes yavapensis* and relatives. Lanes 1 and 2 represent diploid *C. covillei*, lanes 3 and 4 are tetraploid *C. yavapensis* (CFLL form), lanes 5 and 6 correspond to triploid *C. wootonii* (used here also as a proxy for *C. fendleri* and *C. lindheimeri*), and lane 7 is tetraploid *C. yavapensis* (CCFL form). Note the difference in genomic dosage between CFLL and CCFL (especially notable at the L-L homodimer) as well as the partial overlap of plastid and cytosolic loci. Vertical dotted line between lanes 4 and 5 indicates an excised portion of the gel not relevant to the conclusions of this study2.

as are the F-C and C-L heterodimers. The F-L heterodimer and the C-C homodimer comigrate, but the unmistakable F-C and C-L heterodimers confirm that both are present. Despite the superficial similarity of the three separate *C. yavapensis* accessions, there are subtle differences between the samples on the left (lanes 3 and 4) and the sample on the right (lane 7). The samples in lanes 3 and 4 show enhanced expression of the L-L homodimer, indicating that these individuals may represent CFLL hybrids. In

lane 7, all bands that include C are more intense, suggesting that this plant (from the type locality of *C. yavapensis*) is a CCFL hybrid.

Discussion

Deciphering species relationships and hybrid origins in polyploid agamic species complexes is difficult due to the cryptic nature of the morphological characters that distinguish species. In this study of cheilanthoid ferns I combine spore studies, plastid and nuclear DNA sequencing, and enzyme electrophoresis to resolve the contentious origin of the apomictic tetraploid hybrid *C. yavapensis* and to determine its relationship to *C. wootonii*.

Identifying maternal parents. In all ferns analyzed to date, the chloroplast genome has been shown to be maternally inherited (e.g., Gastony and Yatskievych 1992; Vogel et al. 1998; Guillon and Raquin 2000). Thus, it is possible to use plastid phylogenies to identify the maternal parents of hybrid ferns. In my plastid phylogeny (Fig. 2), all sampled accessions of the apomictic triploid *C. wootonii* have plastid sequences that are essentially identical to those of sexual diploid *C. fendleri*, suggesting that this species is the maternal progenitor of *C. wootonii*. Conversely, plastid sequences from all sampled accessions of the apomictic tetraploid *C. yavapensis* indicate that its maternal parent is *C. lindheimeri* (Fig. 2). These results provide unequivocal evidence that *C. wootonii* and *C. yavapensis* are distinct entities.

Other genomes present in the hybrids. The phylogeny resulting from my analysis of nuclear *gapCp* allows for the identification of additional genomes (other than

the maternally-inherited chloroplast) present in these hybrids. All cloned sequences from accessions of the apomictic triploid *C. wootonii* group with either *C. lindheimeri* or *C. fendleri*, supporting Reeves' (1979) hypothesis regarding the hybrid origin of this taxon (Fig. 1). In contrast, the four accessions *C. yavapensis* contained sequences derived from *C. covillei*, *C. fendleri*, and *C. lindheimeri* (Fig. 3). These data indicate that *C. yavapensis* is a trigenomic allotetraploid, contradicting earlier hypotheses (Reeves 1979; Gastony and Windham 1989; Windham 1993b) that considered tetraploid *C. yavapensis* a hybrid between sexual diploid *C. covillei* and apomictic triploid *C. lindheimeri* (Fig. 1).

Within each of the three ingroup lineages identified by analysis of nuclear DNA sequences, subclades that appear to reflect allelic variation at the *gapCp* locus are evident (Fig. 3). The phylogenetic distribution of these alleles may provide additional insights into the origins of *C. wootonii* and *C. yavapensis*. In the case of apomictic triploid *C. wootonii*, each of the three accessions sampled includes a single allele derived from *C. lindheimeri*. Samples of the other parent, *C. fendleri*, exhibit two *gapCp* alleles, both of which are found in every sampled accession of *C. wootonii* (Fig. 3). This suggests that *C. wootonii* contains two genomes from *C. fendleri* and one from *C. lindheimeri*, which is consistent with the dosage (FFL) seen at the TPI-2 enzyme locus (Fig. 4). In addition, the presence of a distinctive *C. lindheimeri* allele in one accession of *C. wootonii* suggests that the latter species may have arisen through multiple, independent hybridization events.

The evidence for multiple origins is even more compelling in apomictic tetraploid *C. yavapensis* (Fig. 4). This species is represented in my analyses by four separate accessions that comprise two genetically distinct forms with non-overlapping geographic ranges. One form includes '*C. yavapensis* 4' (from the type locality of *C.*

yavapensis) and '*C. yavapensis* 3,' which were collected near the western and northern edges of the species' distribution, respectively. The other form comprises '*C. yavapensis* 2' and '*C. yavapensis* 1,' collected near the center of the range in central and southeastern Arizona. Within these two forms, there is little or no variation in the *gapCp* sequences representing the three constituent genomes. However, a close comparison of the two forms reveals that they have incorporated different alleles from each parental species (Fig. 4), supporting multiple origins for *C. yavapensis*. These putative independent origins correspond exactly to the genetic variants encountered in the enzyme analyses discussed below.

Determining genome dosage. Nuclear DNA evidence indicates that these accessions of *C. wootonii* contain two different genomes, whereas those of *C. yavapensis* contain three (Fig. 3). Because these accessions are triploid and tetraploid respectively, it can be deduced that, in each case, one of the constituent genomes is present as two copies. To determine genome dosage, the relative intensity of bands was examined at the TPI-2 enzyme locus (Fig. 4; Danzmann and Bogart 1982). Turning first to triploid *C. wootonii* (Fig. 4; lanes 5 and 6), the enzyme data clearly show that it is the *C. fendleri* genome that is duplicated (note the relative intensities of the F-F homodimer and the F-L heterodimer compared to the L-L homodimer). Therefore, I conclude that the genome dosage of *C. wootonii* is two *C. fendleri* to one *C. lindheimeri* (symbolized as FFL). The two distinct origins of *C. yavapensis* apparent in the nuclear DNA phylogeny (Fig. 3) find additional support in the enzyme analyses, which indicate that this "species" encompasses two geographically correlated entities characterized by different genome dosages. These two "forms" of *C. yavapensis* are designated CFLL and CCFL in the TPI

gel photo (Fig. 4). The CFLL combination is represented by lanes 3 and 4, while CCFL is seen in lane 7. These two forms have the same number of bands, but the marker alleles are present in different dosages. This is most apparent in the L-L homodimer produced by the *C. lindheimeri* genome (the band closest to the lower edge of Fig. 4). In CFLL (lanes 3 and 4), the L-L homodimer is the darkest/thickest band; in CCFL this band is less intense (about the same intensity as in the FFL samples in lanes 5 and 6), reflecting a lower dosage of the *C. lindheimeri* genome.

Origins of the apomictic polyploids. Based on the nuclear *gapCp* data, Schuettpelz et al. (2008) demonstrated that the apomictic triploids *C. lindheimeri* and *C. wootonii* had autopolyploid and allopolyploid origins, respectively. Although these authors were able to identify the genomes (*C. fendleri* and *C. lindheimeri*) present in *C. wootonii*, their data did not permit an examination of its possible origins. My analyses, summarized as a phyloreticulogram (Fig. 5), reveal two additional and important details about *C. wootonii*; namely, genome dosage (FFL) and the maternal parent (*C. fendleri*). Based on these data, I can offer two possible scenarios for the origin of *C. wootonii*. The simplest explanation involves direct hybridization between a normal (*n*) male gamete derived from diploid *C. lindheimeri* and an unreduced (2*n*) female gamete from diploid *C. fendleri*. A second, more complex, scenario involves the formation of a sterile homoploid hybrid between *C. fendleri* and *C. lindheimeri*, followed by the production of an unreduced gamete that backcrossed with a normal gamete of *C. fendleri*. My ongoing studies of other likely hybrids involving these species, coupled with analyses of geographically correlated allelic variation in *C. fendleri*, may eventually reveal both how and where *C. wootonii* was formed.

Contrary to earlier morphology-based hypotheses (Reeves, 1979; Windham, 1993b), my data indicate that apomictic tetraploid *C. yavapensis* did not arise through hybridization between sexual diploid *C. covillei* and apomictic triploid *C. lindheimeri* (Fig.1). The nuclear *gapCp* locus reveals that every sampled accession of *C. yavapensis* contains a genome derived from *C. fendleri*, in addition to the expected contributions from *C. covillei* and *C. lindheimeri* (Fig. 3). Additionally, the enzyme and *gapCp* data indicate that populations referred to *C. yavapensis* are polyphyletic; they are the result of independent hybridization events that have given rise to two entities (CCFL and CFLL) with different genomic constitutions and distinct geographic ranges.

Despite the unexpected complexity of *C. yavapensis*, it is possible to offer some robust hypotheses regarding the origins of this taxon. I have demonstrated that two distinct apomictic tetraploids are referred to *C. yavapensis*, each representing a unique genomic combination (UGC). Because each apomictic tetraploid cheilanthoid fern analyzed to date has been shown to be a hybrid between a sexual diploid and an apomictic triploid (e.g., see Gastony and Yatskievych 1992), this is a good working hypothesis for the origin of the two UGCs referred to *C. yavapensis*. As in most organisms, hybridization between sexual and apomictic ferns is highly constrained with regard to directionality. Apomictic ferns reproduce by generating a sporophyte directly from gametophytic tissue without fertilization. Though they occasionally give rise to functional antheridia, they do not produce functional archegonia (Gastony and Yatskievych 1992, and references therein). Therefore, when sexual fern species hybridize with apomicts, the sexual taxon must be the maternal parent.

The plastid phylogeny (Fig. 2) provides critical insight into the origins of the two UGCs included within *C. yavapensis*. Most importantly, it identifies *C. lindheimeri* as the maternal (i.e., sexual diploid) parent of all sampled accessions. By "subtracting" this maternal contribution (one L genome) from the two *C. yavapensis* UGCs identified above (CCFL and CFLL), it is possible to predict the genomic makeup of the paternal triploids. Based on this simple exercise, I hypothesize that the apomictic triploid (paternal) parent of the CCFL form of *C. yavapensis* had the genomic constitution CCF, whereas the sperm donor for the CFLL form was a CFL triploid (Fig. 5). Although neither of these triploids has been reported by previous authors, ongoing studies (Grusz et al., unpubl.) indicate that both exist in nature.

A full understanding of the origins of *C. yavapensis* will require tracking the various genomic components back to the sexual diploids that initiated the hybridization events. A major thrust of this effort will be to explain the origins of the two predicted triploids CCF and CFL (Fig. 5). There are two possible scenarios for the origin of CCF (analogous to the proposed origin of *C. wootonii* discussed earlier) and three distinct pathways that could yield the CFL triploid. The most likely explanation (requiring the fewest steps and no new taxa) is indicated by dashed lines in Figure 5. In this scenario, the sexual diploids *C. covillei* and *C. fendleri* hybridize to form a sterile homoploid intermediate (CF), the existence of which has been confirmed (Windham and Rabe 1993). Through the production of unreduced CF gametes, this homoploid hybrid could backcross to *C. covillei* (yielding CCF) and hybridize with sexual diploid *C. lindheimeri* (yielding CFL). Studies are ongoing to determine whether this is an accurate portrayal of the earliest stages of evolution in the *C. yavapensis* complex.

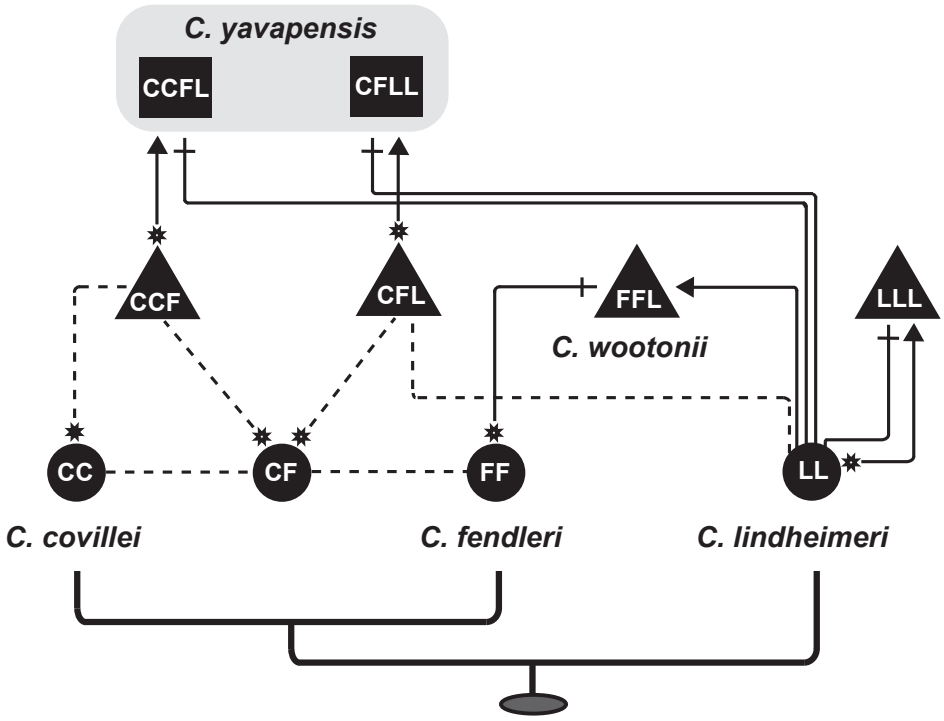


Figure 5

Phyloreticulogram summarizing hypothesized origins of apomictic polyploids in the *C. yavapensis* complex. Diploids, triploids, and tetraploids are represented by circles, triangles, and squares, respectively. All triploid taxa exhibit apomictic reproduction, all diploids are sexually reproducing, and the two tetraploid UGCs of *C. yavapensis* (CCFL and CFLL) are apomictic. Solid lines terminating in arrows point from the paternal parent to its offspring. Solid lines terminating in crosses point from the maternal parent to its offspring. Dashed lines represent hypothesized (or as yet unresolved) relationships, for which evidence of maternal and paternal parentage is yet to be determined. Lines originating in stars represent unreduced gametes.

Despite the molecular and morphological features that seem to unite *C. yavapensis*, this study provides unmistakable evidence of independent hybridization events that gave rise to two cryptic taxa (CCFL and CFLL). *Cheilanthes wootonii* was not involved in the origin of either form of *C. yavapensis*; thus, it should continue to be treated as a separate species, despite the subtlety of morphological characters that distinguish it from *C. yavapensis*. As currently defined, *C. yavapensis* encompasses two genetically distinct allotetraploids that share one parent (*C. lindheimeri*) but not the other. This situation is not unusual among plants and would typically be resolved by recognizing two species. In the case of *C. yavapensis*, however, preliminary observations of the two UGCs suggest that they differ only slightly in morphology. For practical purposes of identification, they may prove impossible to differentiate. They are, however, critical pieces of the puzzle when it comes to understanding the evolution of cheilanthoid ferns in the North American Southwest.

CHAPTER II

TOWARD A MONOPHYLETIC *CHEILANTHES*: THE RESURRECTION AND RECIRCUMSCRIPTION OF *MYRIOPTERIS* (PTERIDACEAE)

Summary

The fern genus *Cheilanthes* (Pteridaceae) has perplexed taxonomists for more than two centuries. Complex patterns of evolution involving rampant morphological convergence, polyploidy, hybridization, and apomixis have made the taxonomy of this group especially difficult. Fortunately, recent phylogenetic analyses have helped to clarify relationships among cheilanthoid taxa. Based on these findings, I formalized an updated taxonomy for one monophyletic clade comprising 47 primarily North and Central American taxa usually included in *Cheilanthes*. Because the type species of *Cheilanthes* (*C. micropteris*) is only distantly related to this clade, Michael Windham and I resurrected the genus *Myriopteris* to accommodate these taxa, and here present the revised circumscription for the group, including 36 new combinations.

Introduction

A "practical and natural" generic classification of cheilanthoid ferns (Pteridaceae) has eluded taxonomists for more than 200 years and was viewed by Tryon and Tryon (1982) as one of the most contentious issues in fern systematics. Central to the problem is the circumscription of the large genus *Cheilanthes*, which all molecular studies with sufficient sampling indicate is polyphyletic (see Gastony and Rollo 1998; Kirkpatrick 2007; Prado et al. 2007; Schuettpelz et al. 2007; Zhang et al. 2007; Rothfels et

al. 2008; Eiserhardt et al. 2011). Since the initial description of *Cheilanthes* (Swartz 1806) encompassing 16 species, various authors have moved hundreds of taxa into (e.g., Domin 1913, Mickel 1979) and out of (e.g., Fée 1852, Smith 1875, Ching 1941) the genus. Of the ca. 500 validly published species names in *Cheilanthes*, some 60% have, at some point, resided in other genera. The lack of definitive taxonomic characters in this group often is attributed to widespread convergent evolution in the drought-prone habitats occupied by these ferns (Tryon and Tryon 1973, 1982), and the problem is likely insoluble based on morphology alone. However, the same genetic evidence that highlights shortfalls in the current classification provides a key to solving this puzzle. As DNA sequence data proliferate and morphological features are reexamined in light of molecular phylogenies, it eventually becomes possible to recognize monophyletic assemblages of species that can be circumscribed as genera. We now have reached this point with certain groups of cheilanthoid ferns, at least in terms of removing taxa and clades that cannot reasonably be included within *Cheilanthes* (Link-Perez et al. 2011; Li et al. 2012).

Here, we focus on the primarily New World lineage previously referred to as the "American *Cheilanthes*" (Kirkpatrick 2007), myriopteroid (Rothfels et al. 2008), or myriopterid (Windham et al. 2009, Eiserhardt et al. 2011) ferns. Limited sampling in each of those analyses indicated that these ferns might represent a well-supported, monophyletic group, an assumption fully supported by the more complete (85%) taxon sampling of Grusz et al. (in review). In addition to suggesting the monophyly of the myriopterid lineage, the analyses of Rothfels et al. (2008) and Eiserhardt et al. (2011) conclusively demonstrated that this clade was quite distantly related to the type

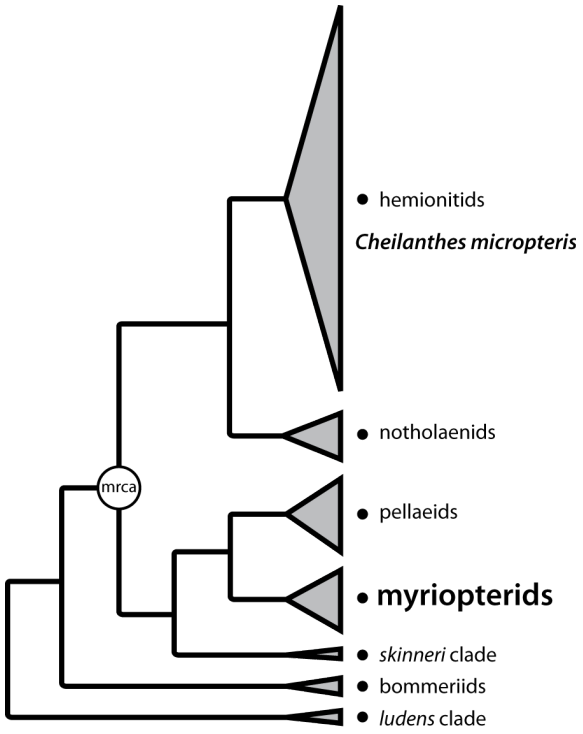


Figure 6

Summary phylogeny for cheilanthoid ferns. Indicating the placement of *Cheilanthes micropteris* (the type species for *Cheilanthes*) within the hemionitid clade which is only distantly related to the myriopterid clade. The six major clades of cheilanthoid ferns are shown with tips 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).

species of *Cheilanthes*, *C. micropteris* (results summarized in Fig. 6). This improved understanding of phylogenetic relationships among cheilanthoid ferns necessitates a taxonomic revision that can be achieved by one of two options: 1) all taxa derived from the most recent common ancestor of *C. micropteris* and the myriopterid ferns could be assigned to a single genus (which would not be called *Cheilanthes* because of the priority

of *Hemionitis*), or 2) myriopterid ferns could be transferred to a different genus, reflecting their phylogenetic distinction from *Cheilanthes s.s*. The first option would require 400+ new combinations in *Hemionitis* (or the conservation of *Cheilanthes* against it followed by more than 100 new combinations in that genus). It would also subsume a number of cohesive, well-characterized genera that are clearly distinct based on morphological, molecular, and cytological grounds, including *Adiantopsis* (Link-Pérez et al. 2011), *Argyrochosma* (Windham 1987, Sigel et al. 2011), *Astrolepis* (Beck et al. 2010), *Doryopteris* (Yesilyurt 2004), *Gaga* (Li et al. 2012), and *Notholaena* (Rothfels et al. 2008). This approach would maximize the number of nomenclatural changes while simultaneously obscuring well-documented phylogenetic relationships, resulting in the inclusion of all but six cheilanthoid species in one genus. Because we consider this option untenable, we have, instead, chosen to remove the myriopterid ferns from *Cheilanthes*.

When any species or clade is removed from *Cheilanthes*, the first issue that must be addressed involves their relationship to *Allosorus pusillus* (Willd. ex Bernh) Bernh. [= *Cheilanthes pteridioides* (Reich.) C. Chr.]. This species was designated the lectotype of *Allosorus* Bernh. by Pichi-Sermolli (1953), a choice subsequently validated by the ICBN when *Cheilanthes* was conserved over *Allosorus* (Appendix II of the Montreal Code, Stafleu et al. 1961). The only phylogenetic study published to date that includes the type species of both *Allosorus* and *Cheilanthes* is that of Eiserhardt et al. (2011). In that analysis, it is unclear whether the divergence between *C. maderensis* (= *C. pteridioides*; see Nardi and Reichstein 1985, Rothfels et al. 2012) and *C. micropteris* is sufficient to justify the recognition of two genera. The two taxa appear in distinct, well-supported clades (clade

A vs. clade C in Fig. 2B of Eiserhardt et al. 2011), but deeper relationships are poorly resolved and both clearly belong to the rapidly diversifying hemionitid lineage (clade H). The unequivocal assignment of *Allosorus* to the hemionitids by Eiserhardt et al. (2011) does, however, prevent the application of this generic name to the myriopterid clade. Any attempt to expand *Allosorus* to include myriopterids would encompass both *Cheilanthes* (conserved over *Allosorus*) and *Hemionitis* (which has priority over both).

One potentially viable option for generic placement of the myriopterid clade would be to include it within a revised circumscription of *Pellaea* Link. All recent phylogenetic studies with adequate sampling of the two groups (e.g., Kirkpatrick 2007, Rothfels et al. 2008, Eiserhardt et al. 2011) strongly support the position of myriopterids as the sister group of the pellaeid clade, which includes *Pellaea atropurpurea*, the lectotype of the oldest generic name applicable to that clade. We are not in favor of expanding the definition of *Pellaea* to encompass the myriopterids for a variety of reasons. First, the two are quite distinct, both in terms of phylogenetic divergence and morphology. The myriopterids have significantly smaller ultimate segments, pubescent and/or scaly (vs. mostly glabrous) leaf blades, and sporangia that are confined to vein tips (vs. distributed along the veins near the segment margins). Because of these differences, the two groups generally have not been considered closely related, and most myriopterids would require new combinations in *Pellaea*. Adding to this nomenclatural upheaval is the fact that other well-defined genera, including *Argyrochosma* (Sigel et al. 2011) and *Astrolepis* (Beck et al. 2010), would be subsumed within such a circumscription of *Pellaea*, which would require additional new combinations and serve only to further undermine the distinctions among the major genera of cheilanthoid ferns.

If the expansion of *Pellaea* is ruled out, there remain three other generic names typified by species belonging to the myriopterid clade: 1) *Myriopteris*, described by Fée (1852) and typified by *M. marsupianthes* Fée; 2) *Cheilosoria*, named by Trevisan (1877) and lectotypified by Copeland (1947) based on *C. allosuroides* (Mett.) Trev.; and 3) *Pomataphytum*, published by Jones (1930) and typified by *P. pocillatum* M. E. Jones (= *M. lendigera*). Phylogenetic reconstructions (Grusz et al. in review), confirm that the type species of *Myriopteris* and *Pomataphytum* fall within a single, well-supported clade. In fact, the diploid species *M. marsupianthes* is thought to be one of the parents of sexual tetraploid *M. lendigera* (see Mickel and Smith 2004). Thus, the generic name *Pomataphytum* is appropriately considered a synonym of the earlier described *Myriopteris* and can be eliminated as a potential name for the myriopterid clade. Copeland's (1947) lectotype of *Cheilosoria* belongs to the well-supported and morphologically distinctive alabamensis clade that diverges earlier in the myriopterid phylogeny (Grusz et al. in review), and the name *Cheilosoria* could be used for this particular group if the myriopterids were subdivided into two or more genera. However, *Myriopteris* predates *Cheilosoria* by 25 years and, when these species are assigned to a single genus (our preferred approach), *Myriopteris* is the correct generic name for the inclusive myriopterid clade.

Historical use of the name **Myriopteris**

The original concept of *Myriopteris* (Fée 1852) included 11 species, these split between two sections (*Eumyriopteris* and *Cheilanthastrum*) distinguished by the presence or absence of a well-developed, inframarginal false indusium. The Latin and French descriptions of the genus are only partly overlapping; shared elements include the highly divided fronds, the small, orbicular ultimate segments with recurved margins ("formant un bourrelet très-contracté"), and a tendency to be covered by hairs and/or scales. *Myriopteris* was accepted and significantly expanded by J. Smith (1875: 280) who stated "the genus consists of about 20 species, distinguished from *Notholaena* and *Cheilanthes* by their small, concave, lenticular segments." The segregation of *Myriopteris* from *Cheilanthes* was, however, rejected by most subsequent authors (e.g., Christensen 1906, Copeland 1947, Lellinger 1965, Tryon and Tryon 1982, Kramer et al. 1990), with two notable exceptions. Pichi-Sermolli (1977) advocated a narrowed circumscription of the genus, including only the two species with prominent false indusia, viz., *M. marsupianthes* and *M. lendigera*. As shown by Grusz et al. (in review), this definition of *Myriopteris* is phylogenetically indefensible because it excludes *M. mexicana*, the apparent maternal progenitor of allotetraploid *M. lendigera*. About the same time Pichi-Sermolli was narrowing the definition of *Myriopteris*, Löve and Löve (1977) expanded it slightly by proposing a new combination for the species known as *Cheilanthes covillei* Maxon. This was done without explanation, though almost certainly reflects the fact that this species has the small, bead-like ultimate segments emphasized in earlier circumscriptions of the genus.

Although this "microphyllous" leaf morphology is common within *Myriopteris*, it does not characterize the entire clade (Grusz et al. in review) and has evolved independently in other cheilanthoid lineages. Thus, the possession of small, bead-like ultimate segments does not constitute a synapomorphy for the genus as defined herein. In fact, our list of excluded names (see Taxonomic Treatment) includes seven taxa with bead-like segments previously ascribed to *Myriopteris* but more closely related to *Cheilanthes s.s.* (Windham et al. unpublished). Because all morphological characters used by previous authors to define *Myriopteris* are subject to strong, positive selection in xeric-adapted cheilanthoid lineages (Hevly 1963), it is not surprising that none of them uniquely define the genus. The totality of evidence, however, indicates that the myriopterids represent a deeply divergent clade that cannot reasonably be combined with any other in a single genus. Therefore, we propose to resurrect *Myriopteris* and recircumscribe it to encompass the entirety of this well supported cheilanthoid lineage.

Distinguishing **Myriopteris** *Fée emend. Grusz & Windham from* **Cheilanthes** *s.s.*

Ideally, morphological and/or cytological synapomorphies would substantiate phylogenetic relationships inferred from DNA sequence data. However, easily observed synapomorphies distinguishing the various clades of cheilanthoid ferns are few, and homoplastic characters abound. To paraphrase Sir William Hooker (1852: 75), "Vain is the attempt to form a definite character which shall decide the limits of [*Cheilanthes*]," a statement that applies equally well to *Myriopteris*. Highly divided (decompound) leaf blades with small ultimate segments are scattered across the cheilanthoid tree and,

indeed, are characteristic of ferns in general, and an indument of hairs and/or scales is one common strategy among plants used to reduce water loss in xeric habitats (Hevly 1963). Other characters useful for species-level identification within myriopterids, such as vernation, are, without exception, shared with other distantly related cheilanthoid ferns.

Molecular analyses spanning the diversity of cheilanthoid species (Windham et al. unpublished) illuminate one particularly useful character distinguishing *Myriopteris*, as defined herein, from *Cheilanthes* s.s. The taxa most closely related to the type species of the latter [*C. micropteris* plus all Australian *Cheilanthes* and a group of South American species including the *C. scariosa* (Sw.) C. Presl complex of Tryon and Tryon (1982), *C. obducta* Mett. ex Kuhn, and *C. fractifera* R.M. Tryon] have 32 small spores per sporangium when sexual, and 16 large spores per sporangium when apomictic. This intriguing cytological synapomorphy results from the elimination of a premeiotic mitosis in the cell lineages generating the sporocytes (Windham et al. unpublished). Aside from a few species of the distantly related genus *Notholaena*, all other cheilanthoid ferns so far examined (including every *Myriopteris* species; Grusz et al. in review) produce 64 small spores per sporangium in sexual individuals and 32 large spores per sporangium in apomicts. This character appears to provide an absolute separation between *Myriopteris* and *Cheilanthes s.s.*, and is easily observed using a dissecting microscope. In combination with differences in spore ornamentation (see Tryon and Lugardon 1991), leaf venation (Pryer et al. 2010), and geographic distribution, this feature provides a clear distinction between the two genera. For diagnostic purposes, then, *Myriopteris* Fée emend. Grusz & Windham differs from *Cheilanthes s.s.* (i.e., *C.*

micropteris and its close relatives) in its production of 64 small or 32 large (vs. 32 small or 16 large) spores per sporangium; mostly cristate or rugulose (vs. echinate, granulose, or verrucate) spore ornamentation; a lack of obvious vein endings near the margins of the ultimate segments (vs. often prominent hydathodes), and a largely North and Central American (vs. exclusively South American/Old World) distribution.

Taxonomic Treatment

Myriopteris Fée emend. Grusz & Windham

Type. *Myriopteris marsupianthes* Fée, Mém. Fam. Foug. 5: 149, t. 12A. f. 1. 1852

Description. Plants rupestral or terrestrial. Rhizomes compact to long-creeping, ascending or horizontal, scaly. Rhizome scales lanceolate to acicular, concolorous (tan to dark brown) or bicolorous (with dark central stripe and brown margins). Leaf vernation non-circinate to circinate. Petioles castaneous to black, scaly and/or pubescent, rarely almost glabrous. Rachises terete or flattened or grooved adaxially, with indument similar to that of the petioles. Blades 2- to 4-pinnate (rarely pinnate-pinnatifid), lanceolate to ovate-deltate, occasionally linear or pentagonal; adaxial surfaces glabrous or pubescent; abaxial surfaces scaly and/or pubescent or rarely glabrous. Ultimate segments round to oblong-ovate, minute to >1 cm long, the veins obscure and not ending in prominent hydathodes. Segment margins usually recurved, with a poorly differentiated false indusium (strongly differentiated in *M. lendigera* and *M.*

marsupianthes). Sori usually partly to completely covered by the recurved segment margins, the sporangia clustered at vein tips. Sporangia 64-spored (in sexual species) or 32-spored (in apomicts). Spores globose-tetrahedral, tan to brown, cristate to rugulate. Chromosome numbers $n = 29, 30, 58, 60$ (sexual species); $n = 2n = 87, 90$ (apomictic triploids); $n = 2n = 120$ (apomictic tetraploids).

Distribution. Species of *Myriopteris* range from southern Canada through the Caribbean and Central America to southern Chile, with one species (*M. rawsonii*) endemic to Namibia and South Africa. Mexico is the center of species diversity for the genus; 34 of the 44 species can be found in Mexico, and seven of these are endemic.

New and Resurrected Combinations in **Myriopteri**s

1**) Myriopteris aemula** (Maxon) Grusz & Windham, comb. nov. *Cheilanthes aemula* Maxon, Contr. U.S. Natl. Herb. 10: 495. 1908. Type: Mexico. Tamaulipas: Victoria, in river canyon, under overhanging rocks, altitude about 320 meters, February 1 to April 9, 1907, Palmer 187 (holotype: US; isotype: US).

2) **Myriopteris alabamensis** (Buckley) Grusz & Windham, comb. nov. *Pteris alabamensis* Buckley, Amer. J. Sci. Arts 45: 177. 1843. *Cheilanthes alabamensis* (Buckley) Kunze, Linnaea 20: 4. 1847. Type: USA. Alabama: Growing in tufts on limestone rocks that form the banks of the Tennessee River, at the foot of Muscle Shoals, Buckley s.n. (holotype: PH; isotypes: MO, NY).

3) **Myriopteris allosuroides** (Mett.) Grusz & Windham, comb. nov. *Cheilanthes allosuroides* Mett., Abh. Senckenberg. Naturf. Ges. 3: 78. 1859. *Pellaea allosuroides* (Mett.) Hieron., Hedwigia 62: 18. 1920. Type: Mexico, Schmitz s.n. (holotype: location unknown).

4**) Myriopteris aurea** (Poir.) Grusz & Windham, comb. nov. *Pteris aurea* Poir. Encyclopédie Méthodique, Botanique 5: 710. 1804.
Type: Peru. Elle a été recueillie au Pérou par Joseph de Jussieu s.n. (sheet 1333 in hb. Jussieu; holotype: P).

Acrostichum bonariense Willd., Sp. Pl., ed. 4, 5(1): 114. 1810. *Notholaena bonariensis* (Willd.) C. Chr., Index Filic. 459. 1906. *Cheilanthes bonariensis* (Willd.) Proctor, Bull. Inst. Jamaica, Sci. Ser. 5: 15. 1953.

In *Cheilanthes*, this has been called *C. bonariensis* (Willd.) Proctor because use of the oldest applicable epithet (based on *Pteris aurea* Poir.) was blocked by the earlier publication of *Cheilanthes aurea* Baker (Proctor 1953). With the transfer of this species to *Myriopteris* we revert to the older epithet and thus avoid the typification difficulties associated with the basionym *Acrostichum bonariense* Willd. (Ponce and Zimmer 2011).

5) **Myriopteris chipinquensis** (Knobloch & Lellinger) Grusz & Windham, comb. nov. *Cheilanthes chipinquensis* Knobloch & Lellinger, Amer. Fern J. 59: 8. 1969. Type:

Mexico. Nuevo Leon: Chipinque Mesa, outside Monterey, Knobloch 1996B (holotype: MSC; isotypes: F, GH, MEXU, MICH, UC, US).

6) **Myriopteris cinnamomea** (Baker) Grusz & Windham, comb. nov. *Notholaena cinnamomea* Baker in Hook. & Baker, Syn. Fil. ed. 2. 515. 1874. *Cheilanthes cinnamomea* (Baker) Domin., Biblioth. Bot. 20: 133. 1913. *hom. illeg. non Cheilanthes cinnamomea* D. C. Eaton, Proc. Amer. Acad. Arts 18: 186. 1883. Type: Guatemala. Mo[n]tagua, 1862, Salvin & Goodman s.n. (holotype: K; isotype: BM).

Cheilanthes tryonii T. Reeves, Brittonia 32: 504. 1980.

In *Cheilanthes*, this species has been called *C. tryonii* T. Reeves because use of the oldest applicable epithet (based on *Notholaena cinnamomea* Baker) was blocked by the earlier publication of *Cheilanthes cinnamomea* D. C. Eaton (Reeves 1980). With the transfer of this species to *Myriopteris,* we revert to the older epithet.

7) **Myriopteris clevelandii** (D. C. Eaton) Grusz & Windham, comb. nov. *Cheilanthes clevelandii* D. C. Eaton, Bull. Torrey Bot. Club 6: 33. 1875. Type: USA. California: Growing on a mountain about forty miles from San Diego at an elevation of about 2500 feet, Cleveland s.n. (holotype: YU; isotypes: GH, P, US).

8) **Myriopteris cooperae** (D. C. Eaton) Grusz & Windham, comb. nov. *Cheilanthes cooperae* D. C. Eaton, Bull. Torrey Bot. Club 6: 33. 1875. Type: USA. California: near

Santa Barbara, Mrs. Ellwood Cooper (syntype: YU); Sierra Valley, Lemmon s.n. (syntype: YU).

9) **Myriopteris covillei** (Maxon) Á. Löve & D. Löve, Taxon 26: 325. 1977. *Cheilanthes covillei* Maxon, Proc. Biol. Soc. Wash. 31: 147. 1918. Type: USA. California: Surprise Canyon, Panamint Mountains, 13 April 1891, 1550 meters, Coville & Funston 593 (holotype: US).

10) **Myriopteris cucullans** (Fée) Grusz & Windham, comb. nov. *Cheilanthes cucullans* Fée, Mém. Foug. 7: 39, t. 25, f. 4. 1857. Type: Mexico, ad vallem Mexicanum, Schaffner 82 [holotype: RB; isotypes: K, US (fragment)].

11) **Myriopteris fendleri** (Hook.) E. Fourn., Mex. Pl. 1: 125. 1872. *Cheilanthes fendleri* Hook., Sp. Fil. 2: 103, p. 107b. 1852. Type: USA. New Mexico, 1847, Fendler 1015 [holotype: K; isotypes: GH, MO, NY, US (fragment)].

12) **Myriopteris × fibrillosa** (Davenp.) Grusz & Windham, comb. nov. *Cheilanthes lanuginosa var. fibrillosa* Davenp., Bull. Torrey Bot. Club 12: 21. 1885. *Cheilanthes fibrillosa* (Davenp.) Davenp., Bull. Torrey Bot. Club 15: 225. 1888. Type: USA. California: San Jacinto Mountains, June 1882, Parish & Parish s.n. (holotype: GH).

13) **Myriopteris fimbriata** (A. R. Sm.) Grusz & Windham, comb. nov. *Cheilanthes microphylla* (Sw.) Sw. var. *fimbriata* A. R. Sm., Amer. Fern J. 70: 19, 21., f. 9–10. 1980.

Type: Mexico. Chiapas: Munic. Frontera Comalapa, 6–8 km east of Frontera Comalapa, Breedlove 39018 (holotype: DS).

Cheilanthes fimbriata (A. R. Sm.) Mickel & Beitel, Mem. New York Bot. Gard. 46: 112. 1988. *hom. illeg., non Cheilanthes fimbriata* Vis. Fl. Dalmat. 1. 42 t. 1 f. 1. 1842.

14) **Myriopteris gracilis** Fée, Mém. Fam. Foug. 5: 150, t. 29, f. 6. 1852. *Cheilanthes gracilis* (Fée) Mett. ex Riehl, Abh. Senckenberg. Naturf. Ges. 80. 1859. *hom. illeg. non Cheilanthes gracilis* (Michx.) Kaulf., Enum. Filic. 209. 1824. Type: USA. Missouri: Jefferson County, Habitat ad rupes circa Hillsboro, Americâ septentr., Riehl 529 (isotypes: MO, US).

Cheilanthes feei T. Moore, Index Fil., 38. 1857.

Myriopteris lanuginosa J. Sm. Hist. Fil. 280. 1875. (*non M. lanuginosa* (Mart. & Gal.) E. Fourn. Mexic. Pl. 1: 125. 1872.)

In *Cheilanthes*, this has been called *C. feei* T. Moore because use of the oldest applicable epithet (based on *Myriopteris gracilis* Fée) was blocked by the earlier publication of *Cheilanthes gracilis* (Michx.) Kaulf. With the transfer of this species to *Myriopteris*, we revert to the original name published by Fée in 1852.

15) **Myriopteris gracillima** (D.C. Eaton) J. Sm., Hist. Fil. 280. 1875. *Cheilanthes gracillima* D. C. Eaton, Rep. U.S. Mex. Bound. Botany 2: 234. 1859. Type: USA. Oregon: Cascade Mountains, 7000 feet of altitude, latitude 44º, Bigelow s.n. (lectotype: YU).

16) **Myriopteris intertexta** (Maxon) Grusz & Windham, comb. nov. *Cheilanthes covillei* Maxon subsp. *intertexta* Maxon, Proc. Biol. Soc. Wash. 31: 149. 1918. *Cheilanthes intertexta* (Maxon) Maxon in Abrams, Ill. Fl. Pacific States 1: 28. 1923. Type: USA. California: Santa Clara County, Santa Cruz Mountains, collected at the top of Black Mountain, 6 July 1903, Dudley s.n. (holotype: DS).

17) **Myriopteris jamaicensis** (Maxon) Grusz & Windham, comb. nov. *Cheilanthes jamaicensis* Maxon, Contr. U.S. Natl. Herb. 24: 51. 1922. Type: Jamaica. Below Cinchona, 28 February 1919, Harris 12905 (holotype: US; isotypes: GH, MO, NY).

18) **Myriopteris lanosa** (Michx.) Grusz & Windham, comb. nov. *Nephrodium lanosum* Michx. Fl. Bor.-Amer. 2: 270. 1803. *Cheilanthes lanosa* (Michx.) D. C. Eaton, Rep. U.S. Mex. Bound., Botany 2(1): 234. 1859. Type: USA. Tennassee (sic) et Carolinae septentrionalis (non designatus).

Myriopteris vestita (Sw.) J. Sm., Cul. Ferns 29. 1857. (fide C. Chr. 1906.) *Adiantum vestitum* Spreng., Anleit. Kenntn. Gew. 3: 122. 1804.

19) **Myriopteris lendigera** (Cav.) Fée, Mém. Fam. Foug. 5: 149. 1852 (as *M. lentigera)*. *Pteris lendigera* Cav., Descr. Pl. 268. 1801. *Cheilanthes lendigera* (Cav.) Sw., Syn. Fil. 128, 328. 1806. Type: Mexico. Hidalgo: Ixmiquilpan en la Nueva España, Nee s.n. [syntype: MA, US (fragment)]; Ecuador. Bolivar: junto á Guaranda en el Reyno de Quito, Nee s.n. (syntype: MA).

Cheilanthes minor Mart. & Gal. Mém. Act. Brux. 75, pl. 21, f. 1. 1842. *Myriopteris minor* (Mart. & Gal.) Fée, Mém. Fam. Foug. 5: 150. 1852.

Cheilanthes lanuginosa Mart. & Gal. Mém. Act. Brux. 75, pl. 20, f. 2. 1842. *Myriopteris lanuginosa* (Mart. & Gal.) E. Fourn. Mex. Pl. 1: 125. 1872.

Myriopteris villosa Fée, Mém. Fam. Foug. 5: 149. t. 28, f. 1. 1852.

Cheilanthes frigida Linden ex T. Moore, Gard. Chr. 772. 1857. *Myriopteris frigida* (Linden ex T. Moore) J. Sm. Cat. Cult. Ferns 28. 1857.

Myriopteris lendigera (Cav.) J. Sm., Cat. Cult. Ferns 28. 1857. *hom. illeg*.

Pomataphytum pocillatum M.E. Jones, Contributions to Western Botany 16: 12. 1930

20) **Myriopteris lindheimeri** (Hook.) J. Sm., Bot. Voy. Herald. 340. 1856. *Cheilanthes lindheimeri* Hook., Sp. Fil. 2: 101, t. 107a. 1852. Type: USA. Western Texas, 1847, Lindheimer 744 [lectotype: K; isolectotypes: GH, P (2 sheets), SD, US, YU].

21) **Myriopteris longipila** (Baker) Grusz & Windham, comb. nov. *Cheilanthes longipila* Baker, Ann. Bot. (Oxford) 5: 211. 1891. Type: Mexico. San Luis Potosi, 22º N. Lat., 6000–8000 ft., Parry & Palmer 989 [holotype: K; isotype: US (fragment)].

22**) Myriopteris longipila subsp. brevipila** (Mickel) Grusz & Windham, comb. nov. *Cheilanthes longipila* var. *brevipila* Mickel, Mem. New York Bot. Gard. 88: 198–199, f. 84N–Q, 87J–M. 2004. Type: Mexico. Guerrero: 2 km al SE de Amatitlán, 1600 m, 13 August 1994, Soto 1052 (holotype: NY; isotype: FCME).

23) **Myriopteris marsupianthes** Fée, Mém. Fam. Foug. 5: 149, t. 12A. f. 1. 1852. *Cheilanthes marsupianthes* (Fée) T. Reeves *ex* Mickel & A. R. Sm. Mem. New York Bot. Gard. 88: 201, f. 83M–P. 2004. Type: Mexico. Veracruz: Pic d'Orizaba, Martens & Galeotti 6256 (holotype: P; isotype: BR).

24) **Myriopteris maxoniana** (Mickel) Grusz & Windham, comb. nov. *Cheilanthes maxoniana* Mickel, Mem. New York Bot. Gard. 88: 201, f. 87A–D. 2004. Type: Mexico. Tamaulipas: San Lucas, Viereck 76 (holotype: US).

25) **Myriopteris mexicana** (Davenp.) Grusz & Windham, comb. nov. *Cheilanthes mexicana* Davenp., Bull. Torrey Bot. Club 15: 227. 1888. Type: Mexico. Chihuahua: on the verge of a high cliff near the summit of Potrero Peak (Santa Eulalia Mts.), October 1886, 7300 ft., Pringle 827 (holotype: GH; isotypes: MO, BR, DS, NY, P, UC, US, YU).

26) **Myriopteris mickelii** (T. Reeves) Grusz & Windham, comb. nov. *Cheilanthes mickelii* T. Reeves, Brittonia 32: 502, f. 1–5. 1980. Type: Mexico. Oaxaca: Distr. Yautepec, Mickel 4210 (holotype: NY; isotypes: MO, UC).

27) **Myriopteris microphylla** (Sw.) Grusz & Windham, comb. nov. *Adiantum microphyllum* Sw., Prodr. 135. 1788. *Cheilanthes microphylla* (Sw.) Sw., Syn. Fil. 127. 1806. Type: Jamaica, Swartz s.n. (holotype: S).

28) **Myriopteris moritziana** (Kunze) Grusz & Windham, comb. nov. *Cheilanthes moritziana* Kunze, Linnaea 23: 307. 1850. Type: Venezuela. Caracas: La Guayra, Moriz 263 (lectotype: B; isolectotype: GH).

29) **Myriopteris myriophylla** (Desv.) J. Sm., Bot. Voy. Herald, 340. 1856. *Cheilanthes myriophylla* Desv., Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 5: 328. 1811. Type: South America. Anon. s.n. (holotype: P).

Cheilanthes elegans Desv. Ges. Naturf. Freunde Berlin Mag. 5: 328. 1811. *Myriopteris elegans* (Desv.) J. Sm. Cat. Cult. Ferns 29. 1857.

Cheilanthes paleacea M. Martens & Galeotti, Mém. Fam. Foug. Mexique 76, pl. 21, f. 2. 1842. *Myriopteris paleacea* (M. Martens & Galeotti) Fée, Mém. Fam. Foug. 5: 149, t. 29, f. 6. 1852.

Myriopteris intermedia E. Fourn., Bull. Soc. Bot. Fr. 27: 328. 1880. *hom. illeg., non* Fée, Mém. Fam. Foug. 5: 149. 1852.

30) **Myriopteris newberryi** (D. C. Eaton) Grusz & Windham, comb. nov. *Notholaena newberryi* D. C. Eaton, Bull. Torrey Bot. Club 4: 12. 1873. *Cheilanthes newberryi* (D. C. Eaton) Domin, Biblioth. Bot. 20: 133. 1913. Type: USA. California: San Diego, Newberry s.n. (syntypes: Wood s.n., Brewer s.n., YU).

31) **Myriopteris notholaenoides** (Desv.) Grusz & Windham, comb. nov. *Pteris notholaenoides* Desv., Mém. Soc. Linn. Paris 6: 299. 1827. *Cheilanthes notholaenoides* (Desv.) Maxon *ex* Weath., Contr. Gray Herb. 114: 34. 1936. Type: Hispaniola, Anon. s.n. (holotype: P).

32) **Myriopteris × parishii** (Davenp.) Grusz & Windham, comb. nov. *Cheilanthes parishii* Davenp., Bull. Torrey Bot. Club 8: 61–62. 1881. Type: USA. California: San Diego County, W. J. Parish s.n. (holotype: GH; isotypes: GH, YU).

33) **Myriopteris parryi** (D. C. Eaton) Grusz & Windham, comb. nov. *Notholaena parryi* D. C. Eaton, Amer. Naturalist 9: 351. 1875. *Cheilanthes parryi* (D. C. Eaton) Domin, Biblioth. 85: 133. 1913. Type: USA. UT, Charles C. Parry 263 (holotype: YU; isotypes: GH, US, YU).

34) **Myriopteris peninsularis** (Maxon) Grusz & Windham, comb. nov. *Cheilanthes peninsularis* Maxon, Contr. U.S. Natl. Herb. 10: 496. 1908. Type: Mexico. Baja California, T. S. Brandegee s.n. (holotype: US).

35) **Myriopteris peninsularis subsp. insularis** (Weath.) Grusz & Windham, comb. nov. *Cheilanthes peninsularis* (Maxon) var. *insularis* Weath. Amer. Fern 21: 25. 1931. Type: Mexico. Socorro Island, Mason 1616 (holotype: CAS).

36) **Myriopteris pringlei** (Davenp.) Grusz & Windham, comb. nov. *Cheilanthes pringlei* Davenp., Bull. Torrey Bot. Club 10: 61, t. 34. 1883. Type: USA. Arizona, C. G. Pringle s.n. (holotype: GH; isotypes: DS, MO, NY, US, YU).

37) **Myriopteris pringlei subsp. moncloviensis** (Baker) Grusz & Windham, comb. nov. *Cheilanthes moncloviensis* Baker, Ann. Bot. (Oxford) 5: 210. 1891. *Cheilanthes pringlei* var. *moncloviensis* (Baker) Mickel, Mem. New York Bot. Gard. 88: 207–208, f. 79J–M. 2004. Type: Mexico. Coahila: Soledad, Edward Palmer 1378 (holotype: K; isotypes: MO, NY, US).

38) **Myriopteris rawsonii** (Mett ex. Kuhn) Grusz & Windham, comb. nov. *Cheilanthes rawsonii* Mett. ex. Kuhn, Filices Africanae 75. 1868. Type: Africa. Cape Province: Namaqualand, between Specktakel and Komaggas, Whitehead s.n. (holotype: BM; isotype: K).

39) **Myriopteris rufa** Fée, Mém. Fam. Foug. 8: 77. 1857. Type. Mexico. Veracruz: Volcan de Orizaba, Schaffner 83 (holotype: RB).

Cheilanthes eatonii Baker *in* Hook. & Baker, Syn. Fil. 140. 1867.

Cheilanthes castanea Maxon, Proc. Biol. Soc. Wash. 32: 111. 1919.

In *Cheilanthes*, this has been called *C. eatonii* Baker. Examination of putative type specimens of *Myriopteris rufa* housed at RB (digital image) and P indicates that the latter name very likely represents the same species as broadly defined by recent authors (e.g., Mickel and Smith 2004). Because *M. rufa* (published in 1857) has priority over *C. eatonii* (1867), we take up Fée's original name for this taxon in *Myriopteris*.

40) **Myriopteris scabra** (C. Chr.) Grusz & Windham, comb. nov. *Pellaea scabra* C. Chr., Index Filic. 483. 1906. Type: USA. Texas: crevices of rock on hills, Turkey Creek, 25 June 1849, Wright 824 (holotype: K; isotypes: GH, NY, US)

Cheilanthes aspera Hook., Sp. Fil. 2: 111t. 108 A. 1852. *hom. illeg. non* *Cheilanthes aspera* Kaulf. Linnaea 6(1): 186. 1831.

Cheilanthes horridula Maxon, Amer. Fern J. 8: 94. 1918.

In *Cheilanthes*, this has been called *C. horridula* Maxon because use of the oldest legitimate epithet (based on *Pellaea scabra* C. Chr.) was blocked by the earlier publication of *Cheilanthes scabra* H. Karst. (Maxon 1918). With the transfer of this species to *Myriopteris*, we revert to the older, exceedingly appropriate epithet.

41) **Myriopteris tomentosa** (Link) Fée, Mém. Fam. Foug. 5: 149. 1852. *Cheilanthes tomentosa* Link, Hort. Berol. 2: 42. 1833. Type: Mexico. Anon. s.n. (holotype: B; isotypes: PH, US (fragment)).

Cheilanthes bradburii Hook., Sp. Fil. 2: 97, t. 109, b. 1852. *Myriopteris bradburii* (Hook.) J. Sm. Hist. Fil. 280. 1875.

42) **Myriopteris viscida** (Davenp.) Grusz & Windham, comb. nov. *Cheilanthes viscida* Davenp., Bull. Torrey Bot. Club 6: 191. 1877. Type: USA. Arizona: Chiricahua Mountains, Lemmon s.n. [holotype: GH; isotype: US (fragment)].

43) **Myriopteris windhamii** Grusz, Amer. Fern Journ. 103: 112–117. 2013. Type: USA. Arizona: Huachuca Mountains, Windham 4165 (holotype: DUKE; isotypes: ARIZ, ASC, ASU, GH, MO, NMC, NY, TEX/LL, UNM, US, UT).

Cheilanthes villosa Davenp. *ex* Maxon, Proc. Biol. Soc. Wash. 31: 142. 1918.

In *Cheilanthes*, this has been called *C. villosa* Davenp. *ex* Maxon. Because transfer of the epithet *villosa* to *Myriopteris* is blocked by the earlier publication of *M. villosa* Fée (= *M. lendigera* fide Reeves 1979), we use the replacement name for this distinctive taxon published by Grusz (2013).

44) **Myriopteris wootonii** (Maxon) Grusz & Windham, comb. nov. *Cheilanthes wootonii* Maxon, Proc. Biol. Soc. Wash. 3: 146. 1918. Type: USA. Arizona: Santa Rita Mountains, Wooton s.n. (holotype: US).

45) **Myriopteris wrightii** (Hook.) Grusz & Windham, comb. nov. *Cheilanthes wrightii* Hook., Sp. Fil. 2: 87, t. 110A. 1858. Type: USA. Texas–New Mexico, Wright 823 (holotype: K; isotypes: GH, NY, US).

46) **Myriopteris yatskievychiana** (Mickel) Grusz & Windham, comb. nov. *Cheilanthes yatskievychiana* Mickel, Mem. New York Bot. Gard. 88: 212–213, f. 74F–K. 2004. Type: Mexico. Sonora: Sierra del Aliso, Alberto Búrquez M. 96-302 (holotype: MO).

47) **Myriopteris yavapensis** (T. Reeves *ex* Windham) Grusz & Windham, comb. nov. *Cheilanthes yavapensis* T. Reeves *ex* Windham, Contr. Univ. Michigan Herb. 19: 32. 1993. Type: USA. Arizona: Yavapai County, Windham 202 (holotype: UT; istotypes: ASC, ASU, US).

Names of uncertain application

Myriopteris cheiloglyphis Fée, Mém. Fam. Foug. 8: 77. 1857.

Excluded names

Myriopteris contracta (Kunze) Fée, Mém. Fam. Foug. 5: 149. 1852. = *Cheilanthes contracta* (Kunze) Mett. ex Kuhn

Myriopteris hirta (Sw.) J. Sm., Ferns Brit. and For. 174. 1866. = *Cheilanthes hirta* Sw.

Myriopteris induta (Kunze) Fée, Mém. Fam. Foug. 5: 149. 1852. = *Cheilanthes induta* Kunze

Myriopteris intermedia (Kunze) Fée, Mém. Fam. Foug. 5: 149. 1852. = *Cheilanthes hirta* Sw. fide Christensen (1906)

Myriopteris macleanii J. Sm., Hist. Fil. 280. 1875. = *Cheilanthes pilosa* Goldm. fide Christensen (1906)

Myriopteris scariosa (Sw.) Fée, Mém. Fam. Foug. 5: 149, t. 29, f. 6. 1852. = *Cheilanthes scariosa* Sw.

Myriopteris szovitzii (Fisch. and Meyer) J. Sm. Hist. Fil. 281. 1875. = *Cheilanthes persica* (Bory) Mett. ex Kuhn fide Christensen (1906)

CHAPTER III

PATTERNS OF DIVERSIFICATION IN THE XERIC-ADAPTED FERN GENUS *MYRIOPTERIS* (PTERIDACEAE)

Summary

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, I 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, I infer a phylogeny for the group and examine key morphological characters across this phylogeny. I 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.

Introduction

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 phylogenetic distant from *Cheilanthes* s. s. has recently been transferred to the

genus *Myriopteris* (Fig. 6, Chapter II). 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. 6, Chapter II; 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*, I 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, I also summarize the available data on reproductive mode and ploidy level for all species included in my 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 my molecular phylogenetic analyses (Table 2). 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). I 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 B), genomic DNA was extracted from silica-dried leaf fragments or air-dried herbarium specimens using the DNeasy plant mini kit (Qiagen, Valencia, California, U. S. A.) 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 3), 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, 45ºC 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 B).

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 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 in order 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; http://purl.org/phylo/treebase/phylows/study/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, I counted spore number per sporangium for all 29 fertile accessions included in my phylogenetic analyses , as well as for 22 additional individuals not included in the phylogeny (Table 2; Appendix B). 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.

Table 2. 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. aNote 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)	—			
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, Covillei & 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	—		—
***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. 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**		
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, Distrito 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		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			—
***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)	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 Taxo= 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

Table 3 **Primers used for DNA amplification and sequencing for all taxa included in this study.** *Asterisks indicate primers used for both the initial PCR amplification and for DNA sequencing; all others 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–R	TRNG1F*	GCGGGTATAGTTTAGTGGTAA	Nagalingum et al. 2007
trnG–R	TRNR22R*	GCGGGAATCGAACCCGCATCA	Nagalingum et al. 2007
trnG–R	TRNG63R	GCGGGAATCGAACCCGCATCA	Nagalingum et al. 2007
trnG–R	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	ESATPA412F*	GARCARGTTCGACAGCAAGT	Schuettpelz et al. 2006
atpA	ESTRNR46F*	GTATAGGTTCRARTCCTATTGGACG	Schuettpelz et al. 2006

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 listed in Table 4. The most-likely tree (lnL = -16,790.1213) resulting from the analysis of my combined three-locus data set is presented in Fig. 7. Taxon names displayed in Fig. 7 reflect placement within *Myriopteris* (Table 2); a list of synonyms in *Cheilanthes* is provided in Appendix C.

My 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. 7), 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. 7) as sister to a combined *covillei* + *lanosa clade* with low support.

Figure 7

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.

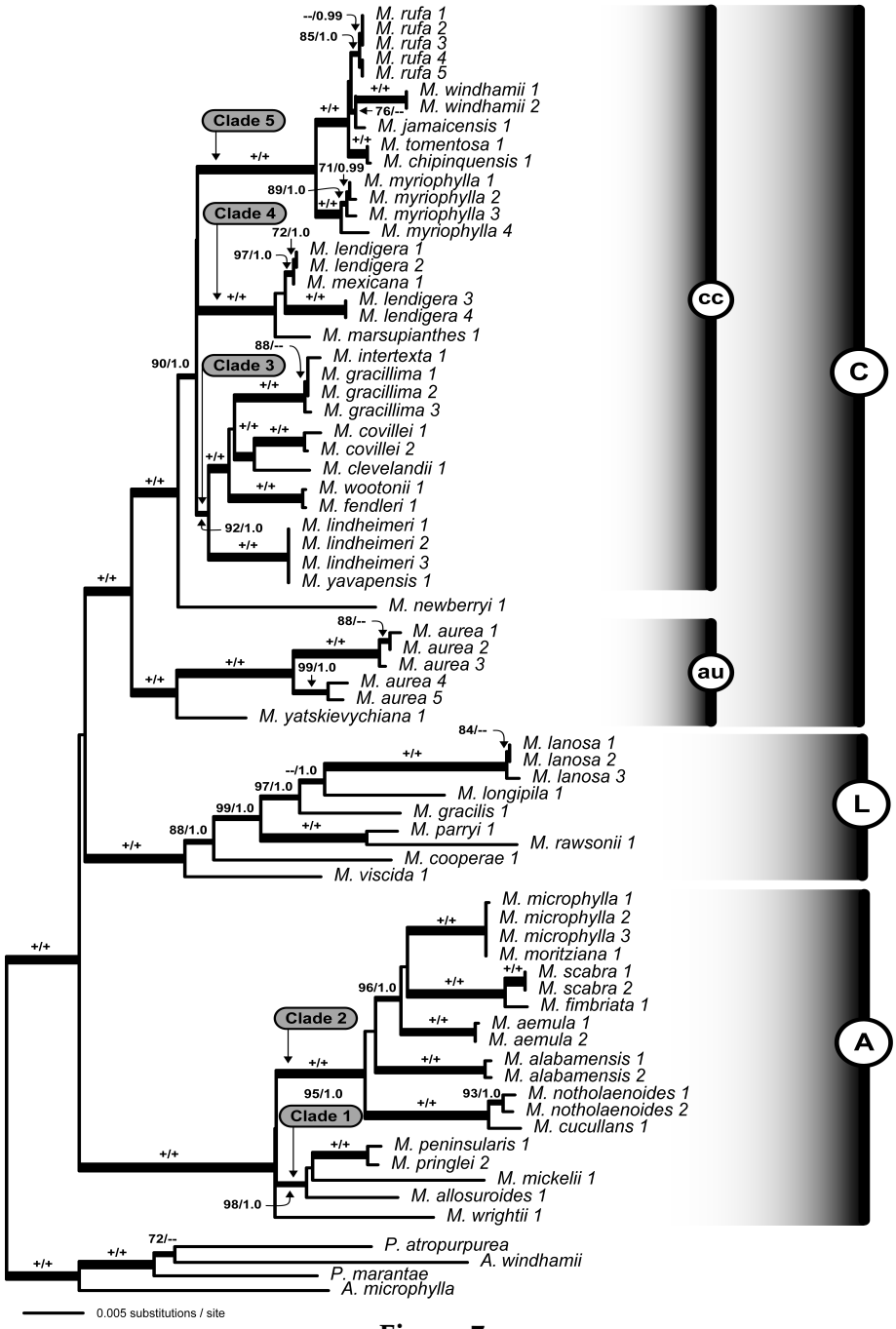

--/0.99
M. rufa 1
85/1.0
M. rufa 2
M. rufa 3
M. rufa 4
M. rufa 5
+/+
M. windhamii 1
+/+
M. windhamii 2
76/--
M. jamaicensis 1
+/+
M. tomentosa 1
+/+
M. chipinquensis 1
Clade 5
+/+
71/0.99
M. myriophylla 1
89/1.0
M. myriophylla 2
+/+
M. myriophylla 3
Clade 4
M. myriophylla 4
72/1.0
M. lendigera 1
97/1.0
M. lendigera 2
M. mexicana 1
+/+
+/+
M. lendigera 3
M. lendigera 4
M. marsupianthes 1
88/--
M. intertexta 1
90/1.0
Clade 3
M. gracillima 1
+/+
M. gracillima 2
M. gracillima 3
+/+
+/+
M. covillei 1
+/+
M. covillei 2
M. clevelandii 1
+/+
M. wootonii 1
+/+
M. fendleri 1
92/1.0
M. lindheimeri 1
+/+
M. lindheimeri 2
M. lindheimeri 3
M. yavapensis 1
+/+
M. newberryi 1
88/--
M. aurea 1
+/+
M. aurea 2
+/+
99/1.0
M. aurea 3
M. aurea 4
+/+
M. aurea 5
M. yatskievychiana 1
84/--
M. lanosa 1
+/+
M. lanosa 2
--/1.0
M. lanosa 3
97/1.0
M. longipila 1
99/1.0
M. gracilis 1
88/1.0
+/+
M. parryi 1
+/+
M. rawsonii 1
M. cooperae 1
M. viscida 1
M. microphylla 1
+/+
M. microphylla 2
M. microphylla 3
M. moritziana 1
+/+
+/+
M. scabra 1
96/1.0
+/+
M. scabra 2
M. fimbriata 1
Clade 2
+/+
M. aemula 1
M. aemula 2
+/+
+/+
M. alabamensis 1
M. alabamensis 2
95/1.0
93/1.0
+/+
M. notholaenoides 1
Clade 1
M. notholaenoides 2
M. cucullans 1
+/+
M. peninsularis 1
+/+
M. pringlei 2
M. mickelii 1
98/1.0
M. allosuroides 1
M. wrightii 1
72/--
P. atropurpurea
+/+
+/+
A. windhamii
P. marantae
A. microphylla
0.005 substitutions / site
cc
C
au
L
A

Figure 7

Table 4 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 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%)	36 (55%)	35 (53%)
Combined (72)	4,508	916	9.5	50 (75%)	47 (70%)	42 (63%)

THE ALABAMENSIS CLADE—This lineage (Clade A, Fig. 7) includes 13 of the 40 *Myriopteris* species sampled for this study. In the ML tree based on the 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. 7). 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 *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. 7), weakly resolved as sister to the *covillei clade* (Clade C, Fig. 7), 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 the MLBS analysis. My 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. 7) and maximally supported as sister to the Mohave/Sonoran Desert endemic, *M. parryi*.

THE COVILLEI CLADE—This lineage (Clade C, Fig. 7) 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. 7) 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. 7). The latter constitutes three well-supported monophyletic groups (Clades 3, 4, and 5, Fig. 7), 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. 7; Table 2) 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 2) 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 2) 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 Figures 8–10. The shape of ultimate segments (Fig. 8A) 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 8B 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. 8B), all representatives of the *covillei clade* (Clade C, Fig. 8B), and all but two sampled species of the *lanosa clade* (Clade L, Fig. 8B). Three of the four outgroup taxa (*Pellaea atropurpurea*, *Astrolepis windhamii*, and *Paragymnopteris marantae*) also have terete rachises. Within the *alabamensis clade* (Clade A, Fig. 8B), *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. 8C). 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. 8C) 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. 9A). Here, I separately map the type of indument found on the adaxial (Fig. 9B) and abaxial (Fig. 9C) surfaces of the ultimate segments for each taxon represented in the phylogeny.

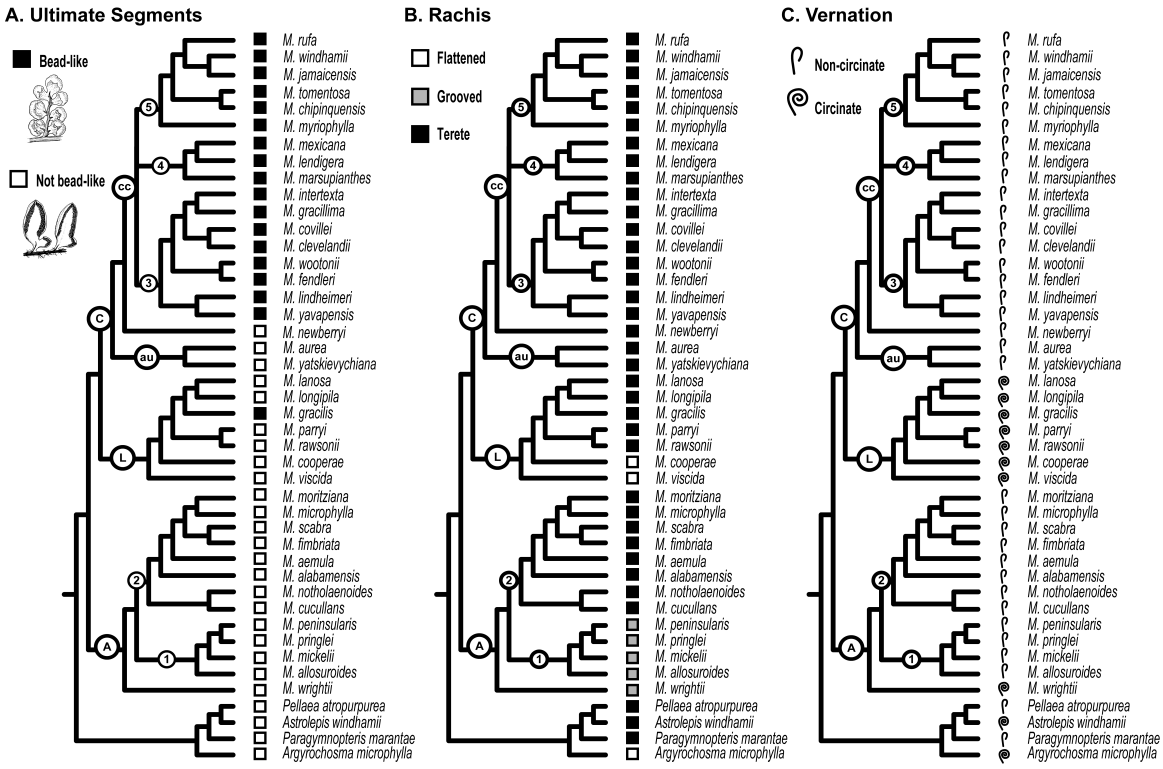


Figure 8

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.

I 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. 9B). 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. 9B). *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. 9B). With the exception of *M. fendleri*, which I 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 highly 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. 9C).

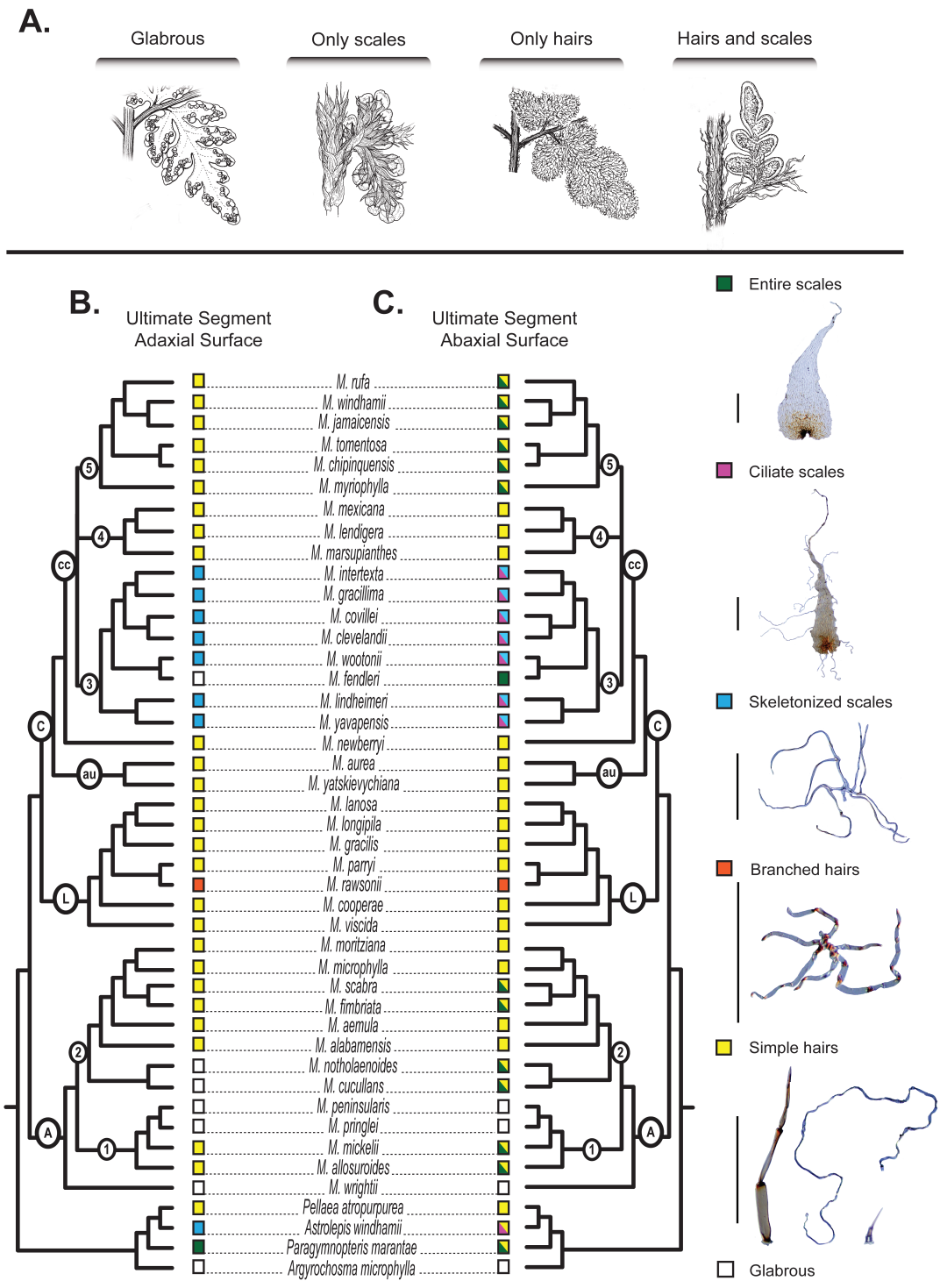

A.
Glabrous
Only scales
Only hairs
Hairs and scales
B.
Ultimate Segment
Adaxial Surface
C.
Ultimate Segment
Abaxial Surface
M. rufa
M. windhamii
M. jamaicensis
M. tomentosa
M. chipinquensis
M. myriophylla
M. mexicana
M. lendigera
M. marsupianthes
M. intertexta
M. gracillima
M. covillei
M. clevelandii
M. wootonii
M. fendleri
M. lindheimeri
M. yavapensis
M. newberryi
M. aurea
M. yatskievychiana
M. lanosa
M. longipila
M. gracilis
M. parryi
M. rawsonii
M. cooperae
M. viscida
M. moritziana
M. microphylla
M. scabra
M. fimbriata
M. aemula
M. alabamensis
M. notholaenoides
M. cucullans
M. peninsularis
M. pringlei
M. mickelii
M. allosuroides
M. wrightii
Pellaea atropurpurea
Astrolepis windhamii
Paragymnopteris marantae
Argyrochosma microphylla
5
4
cc
3
C
au
L
2
A
1
Entire scales
Ciliate scales
Skeletonized scales
Branched hairs
Simple hairs
Glabrous

Figure 9

Figure 9

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.

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. 9C). *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. 9C). 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 this phylogeny (Fig. 10). 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. 10. Based on my 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. My 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. 10), 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 my analysis being *M. yavapensis* in the *covillei clade* (Clade C, Fig. 10). *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 my analyses, is polyploid. Sexual taxa predominate in all ingroup clades except the isolated aurea group ('au', Fig. 10) 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.

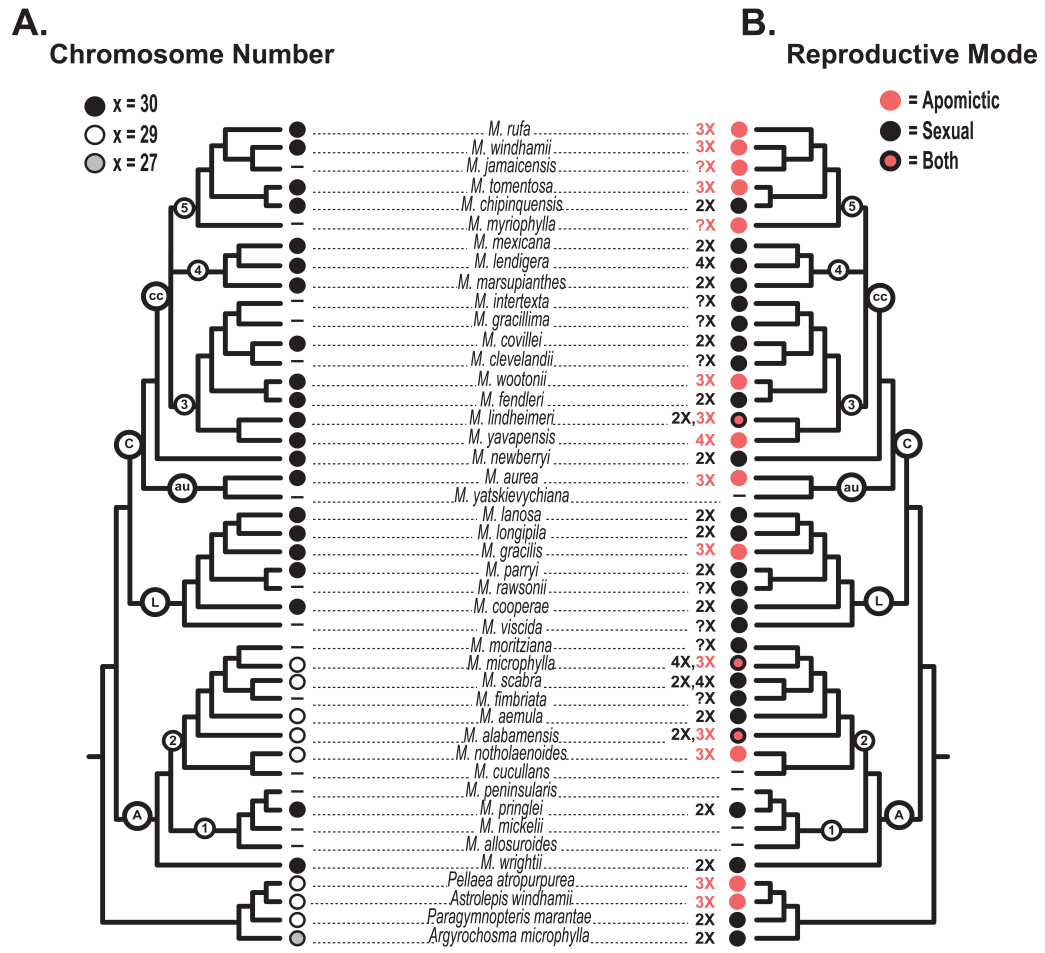


Figure 10

Mapping of cytological and reproductive characters in *Myriopteris*. A. Chromosome base numbers, gathered from existing chromosome counts (see Table 2), 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 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 '—'.

Discussion

Here, I explore evolutionary relationships among taxa belonging to the newly segregated genus, *Myriopteris* (Grusz 2013; Grusz and Windham 2013). My 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—My results agree with earlier studies (Kirkpatrick 2007; Rothfels et al. 2008; Windham et al. 2009; Eiserhardt 2011) in demonstrating that members of this group form a maximally supported clade (Fig. 7) only distantly related to *Cheilanthes* s. s. (Chapter II, Fig. 6). My maximum likelihood topology depicts three maximally-supported myriopterid clades (Clades A, L, C, Fig. 7), 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. 10). 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 this study, along with 22 additional taxa. The phylogenetic relationships presented by Eiserhardt et al. (2011) generally match those in my maximum likelihood tree (Fig. 7); their well-supported myriopterid clade comprises three major subgroups (equivalent to my *alabamensis*, *covillei*, and *lanosa clades*), and the lineages include *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 Clade 2 in this study, 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 my tree (Fig. 7) 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 my analyses. Although some other relationships portrayed by Eiserhardt et al. (2011) are at odds with my reconstruction (specifically their placement of "*C. newberryi*" within the equivalent of the core 'cc' clade of this study, Fig. 7), 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 this analysis, and his *insertae sedis* group comprises a subset of the taxa belonging to Clade 1, plus *Myriopteris wrightii* (Fig. 7). His subgenus *Physapteris* (C. Presl) Baker exactly corresponds to my core *covillei clade* ('cc', Fig. 7), 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* Baker." 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 my *lanosa clade* (Fig. 7).

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. 7). 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 these 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. 7).

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 this study, including all members of the core *covillei clade* ('cc', Fig. 8A) as well as *M. gracilis*, one of the more derived members of the *lanosa clade* (Clade L, Fig. 8A). Based on the distribution of bead-like ultimate segments across my 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. 8B), 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. 8B, 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. 7) 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. 8C) had hooked rather than coiled vernation. To augment these observations, I documented vernation type in all remaining species of *Myriopteris*. Non-circinate vernation, while not unique to *Myriopteris* (see outgroups, Fig. 8C), 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 my molecular phylogeny illustrates that indument type is also a phylogenetically informative character (Fig. 9B–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. 9B). 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*). I 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. My recoding of adaxial indument shown in Fig. 9B is based on my 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. 9B and 9C; 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. 9B and 9C). 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 my maximum likelihood phylogeny (Fig. 9B and 9C), I 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. 9B), as do many of the more derived species (Clades 4 and 5). Therefore, I 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 my 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. 10A).

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 my phylogeny (Fig. 10B), which reveals that 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), which 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. This 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, my 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, which 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. 7) 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 my counts of spore number per sporangium, *M. rawsonii* appears to be sexual, but its ploidy level remains unknown (Table 2; Fig. 10B).

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 this analysis (all apomictic) form two highly divergent sister clades ('au', Fig. 7), 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 my phylogenetic tree (Fig. 7), 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 very 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. 7). 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 very closely related to the wide-ranging Caribbean taxon *M. microphylla* (Clade 2, Fig. 7). 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.

CHAPTER IV

ARE LIP FERNS LIKE LIZARDS? EXAMINING PREMEIOTIC CHROMOSOME DUPLICATION AND GENOTYPIC DIVERSITY IN THE APOMICTIC DESERT FERN *MYRIOPTERIS LINDHEIMERI*

Introduction

During the last century, our understanding of the evolution and maintenance of sex across the tree of life has improved exponentially (e.g., Weismann 1904; Fisher 1930; Muller 1932, 1964; Crow and Kimura 1965; Hill and Robertson 1966; Maynard Smith 1968, 1971; Eshel and Feldman 1970; Lewontin 1971; Felsenstein 1974; Judson and Normark 1996; Archetti 2004, 2010; Kim and Orr 2005). In concert with this improved understanding of sexual reproduction has come an enhanced grasp of the complex and dynamic nature of asexual reproduction, and its role in organismal evolution (Darlington 1939; Mogie 1992; Schön et al. 2009). Even so, asexual organisms remain burdened by a widespread reputation for being "evolutionary dead ends" (Darlington 1939; Mather 1943, 1953; Clausen 1954; Stebbins 1950, 1958; Maynard Smith 1978). They are essentially clonal and lack genotypic diversity, which is critical for adaption by natural selection to ever-changing environments. And, while they are known to exhibit high levels of fixed heterozygosity, asexual populations are expected to generally lack genotypic diversity and be dominated by a single (or few) heterozygous genotype(s) (e.g., Hughes and Richards 1988; Delmotte et al. 2002).

Contrary to these expectations, however, apomictic lineages have been known to exhibit relatively high population-level genotypic diversity, and they often dominate broad ecological and geographical landscapes compared to their sexual relatives (Stebbins 1950; Bierzychudek 1985; Suomalainen et al. 1987). These observations have led investigators to speculate on the possible source(s) of this diversity (e.g., Gustafsson 1947; Stebbins 1950; Haskell 1953; Ellstrand and Roose 1987; Campbell and Dickinson 1990; Menken et al. 1995; Van Baarlen et al. 2000). Many investigators hypothesize that multiple origins, accumulation of new mutations, and/or hybridization with sexual relatives explain high levels of genetic variation in apomictic populations (e.g., Stebbins 1959; Evans, 1969; Mogie 1992; Van Dijk 2003; Verduijn et al. 2004; Whitton et al. 2008; Lu et al. 2010). Alternatively, a few have suggested that apomicts may generate the unexpectedly high number of variable genotypes witnessed in natural populations via subsexual processes (Darlington 1937; Gustafsson 1946; Klekowski 1973), such as independent assortment (IA), recombination, and gene conversion (Birky 1996; Mantovani 1998; Van Baarlen et al. 2000; Van der Hulst et al. 2000; Adolfsson and Bengtsson 2007).

Subsexual processes have been investigated in a variety of organisms, ranging from parthenogenetic lizards to apomictic orchids (e.g., Bi and Bogart 2006; Van Baarlen et al. 2000; Stenberg and Saura 2009; Lutes et al. 2010; Lu et al. 2010; Neaves and Baumann 2011). The role of such processes in generating genetic variation is especially well documented in *Ambystoma* salamanders, whereby apomictic reproduction involves an endomitosis that immediately precedes meiosis, thereby ultimately resulting in the production of unreduced gametes. Following endomitosis, each cell enters meiosis with a duplicated set of chromosomes (i.e., with twice the parental number of chromosomes; Macgregor and Uzzell 1964). This mechanism of pre-meiotic chromosome duplication is

especially important in the case of apomictic polyploids with an odd number of genomes (e.g., triploids, pentaploids, etc.). In these cases, pre-meiotic duplication facilitates the production of functional gametes in organisms that would otherwise produce largely nonfunctional aneuploid gametes due to the formation of univalents and multivalents during meiosis I (Fig. 11a). Pre-meiotic duplication allows regular bivalent formation in apomictic triploids, for example, because it results in an even number of homologous chromosomes (a duplicated triploid genome becomes hexaploid) as the cell enters meiosis. Most importantly, this pathway to chromosome pairing also provides an avenue for the production of genetically variable offspring via the potential for the pairing of non-sister chromosomes (i.e., non-identical homologues or homeologues) during meiosis (Bi and Bogart 2006).

Ferns are an ideal plant analogue for examining the role of subsexual processes in generating genetically variable offspring in apomictic lineages. Unlike the various forms of apomixis that are known to occur angiosperms (Asker and Jerling 1992; Mogie 1992), ferns—like most parthenogenetic animals—undergo a pre-meiotic duplication event via endomitosis, yielding unreduced gametes that reproduce asexually. This form of apomixis in ferns is referred to as meiotic obligate apogamy (DeBenedictis 1969; Walker 1984; Gastony and Windham 1989) and combines the production of unreduced spores via Döpp-Manton sporogenesis (= DMS; Döpp 1932; Manton 1950; Fig. 11a) with the apogamous production of sporophytes from somatic gametophyte tissue (Fig. 11b).

Klekowski (1972, 1973) was the first to recognize the potential implications of homeologous chromosome pairing in ferns. Yet, since then, there have been only a few studies that have thoroughly investigated this phenomemon empirically and, of those, most have focused on homeologous chromosome pairing in sexual tetraploid ferns, usually of allopolyploid origin (Klekowski and Hickok 1974; Hickok 1978a, 1978b;

Chapman et al. 1979). Only three studies have explored homeologous pairing in apogamous ferns, and they focus on the gametophytic offspring of only a single individual and make no attempt to assess the generality of this phenomenon in natural populations (Bierhorst 1975; Ishikawa et al. 2003; Ootsuki et al. 2012).

The goals of this study are therefore, to explore (1) whether DMS permits the pairing of homeologous chromosomes in apomictic polyploid ferns, leading to the production of *genetically variable* offspring, and (2) to document the extent to which this phenomenon occurs in natural populations.

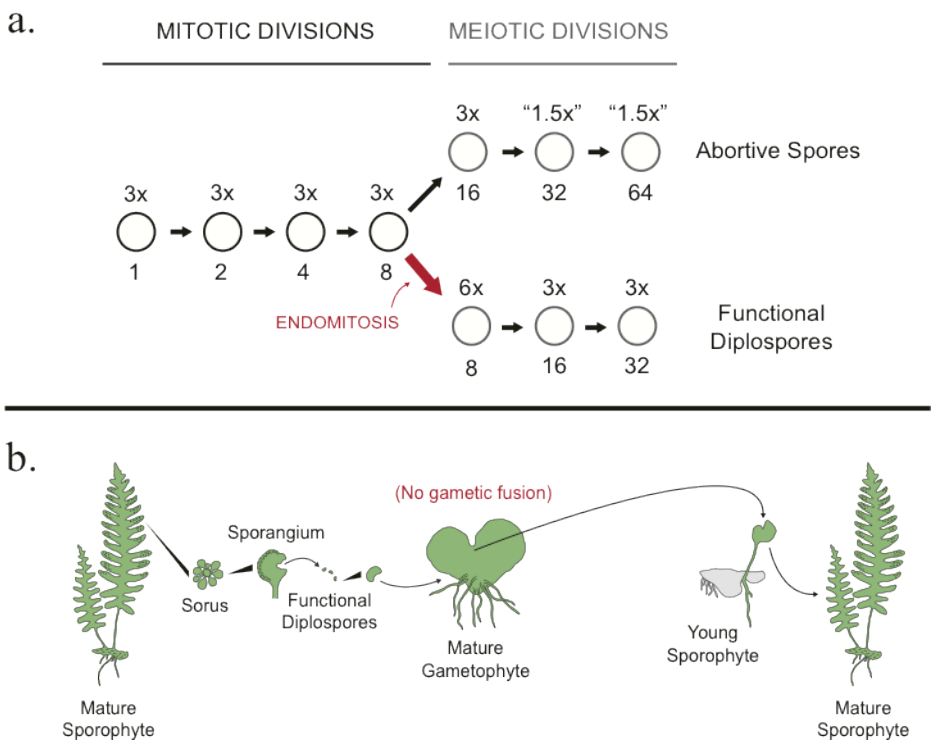


Figure 11

Meiotic obligate apogamy in ferns. (a) Döpp-Manton sporogenesis (DMS) is shown with ploidy level indicated above and number of cells indicated below. The top row of meiotic cells represents an apomictic triploid attempting "normal" meiosis, resulting in 64 abortive spores; the bottom row of cells represents an apomictic triploid undergoing DMS, resulting in 32 functional diplospores (modified from Gastony and Windham, 1989). (b) The production of sporophytes via apogamy. Diplospores are produced; they germinate, grow into mature gametophytes, and then produce sporophytes without the fusion of gametes.

Experimental Approach

Lip ferns of the desert southwest (formally *Myriopteris*) are classic in their ability to circumvent sex and undergo apomictic reproduction in extreme desert environments. Here, I examine one apomictic autotriploid from this group, Lindheimer's Lip Fern, i.e., *Myriopteris lindheimeri* (Hook.) Grusz & Windham. First, I developed microsatellite markers for *M. lindheimeri* and used these to survey individuals (both field collected and from herbarium material; Appendix D) for genetic variation across a large part the species range. I then used these microsatellite markers to identify highly heterozygous parental sporophytes by fragment analysis. From those highly heterozygous individuals, I sowed spores and allowed them to develop into mature gametophytes, until the first sporophyte leaves were produced. These offspring were then genotyped using the same microsatellite markers used for the parent sporophytes. By comparing the offspring genotypes to their respective parents, I was able to detect whether subsexual processes took place during DMS. Sampling individuals across the northern range of *M. lindheimeri* also allowed me to assess the generality of this phenomenon in natural populations and provided a snapshot of range wide genotypic diversity in this apomictic desert fern.

Materials and Methods

Microsatellite development. Genomic DNA of a single individual of diploid *M. lindheimeri* (*Schuettpelz 450*, collected from the Tonto National Forest, Pinal Co., AZ; voucher housed at DUKE) was extracted from silica-dried material using the DNeasy plant mini kit following the manufacturers protocol (Qiagen, Valencia, California, U. S.

A.). Genomic DNA was then run on two lanes (¼-plate = 24 wells) using the Roche 454 GS-FLX Titanium sequencing platform at the IGSP DNA Sequencing Facility, Duke University. Raw reads generated by 454 sequencing were then scanned for di-, tri-, tetra- and pentanucleotide microsatellite repeats using MSATCOMMANDER version 0.8.2 (Faircloth 2008). Traditional (unlabeled) primers were designed for a subset of the identified repeats using the standard settings in Primer3 (Rozen and Skaletsky 1999).

The selected repeat regions were then amplified following the protocol of Grusz et al. (2009), except that the annealing temperature was set to 60 ºC (to decrease non-specific primer binding). Amplicons were then visualized on a 1% agarose gel after being run for 35 minutes at 75 volts. Amplicons that produced a single bright band were then purified and sequenced following the protocol of Grusz et al. (2009). Clean sequence fragments (assumed to represent single-copy markers) were assembled in Sequencher 4.8 (Gene Codes Corporation, Michigan) and examined to confirm the presence of the anticipated microsatellite repeat. For single copy regions in which the anticipated repeat was present, new forward primers were designed with a 6-FAM fluorescently labeled CAG nucleotide tag (5'-CACGACGTTGTAAAACGAC-3') incorporated proximal to the primer annealing sequence, to be used for subsequent genotyping.

Each microsatellite marker was then tested on genomic DNA from the same individual for which 454 sequencing was completed (*Schuettpelz 450*). Genotyping reactions were conducted using 10X PCR buffer IV containing $MgCl_2$ (ABgene, Epsom, U. K.), combined with 2.4 mM dNTPs, 100 μg/ml BSA, 5 U/μl Taq polymerase, 2 μM reverse primer, 10 μM CAG-tagged forward primer (CAG tag: 5'-CACGACGTTGTAAA ACGAC-3'), 10 μM fluorescently-labeled CAG complementary primer, plus 1 μl DNA template for a 12 μl reaction. Genotyping reactions entailed an initial denaturation step

(94ºC for 7 min) followed by 10 denaturation, annealing, and elongation cycles (94ºC for 30 sec, 62ºC [-1ºC per cycle] for 30 sec) and 27 additional denaturation, annealing, and elongation cycles (94ºC for 30 sec, 51ºC for 30 sec, 72ºC for 30 sec) and a final elongation step (72ºC for 12 min).

Fragment analyses were run on a 3730xl DNA sequencer and the resulting data were visualized using GeneMarker 2.2.0 (SoftGenetics, State College, Pennsylvania). Polymorphic loci with unambiguous peaks were used for genotyping of *M. lindheimeri* accessions in subsequent analyses (Table 5).

***Survey of genetic variation in apomictic* M. lindheimeri.** An initial survey of genetic variation across eight microsatellite markers (Table 5) was conducted for 109 individuals distributed across the species range of *M. lindheimeri* (Fig. 12A; Appendix D). Four genetic diversity measures, including: % polymorphic loci, heterozygote frequency (over all loci), mean number of alleles per locus, and genetic diversity, were then summarized for all populations consisting of more than 3 individuals (Fig. 12B). From these 109 individuals, a subset of individuals that: (1) exhibited highly heterozygous genotypes, (2) were fertile, and (3) possessed mature sporangia, were selected for parent-offspring genotype comparisons.

Table 5 Microsatellite markers developed for use in *M. lindheimeri*.

Locus	Primer sequence	T_m (°C)	Motif	Allele size range (bp)	# Alleles
FYM3K	F: AGAGTGAAACCAGAAACCTGC	59.2	ATC	190-202	5
	R: GTGTGCCGCTTAAACAATGAAG	59.8			
HGGWA	F: ACCCACGCATGTAAACAGATTG	60.3	AAC	182–185	2
	R: ACCATTTCTGTGGGAGGTC	57.4			
HL9PJ	F: CTCACCAACTAAGCTCCTTGAC	59.4	CT	409–419	5
	R: CTCACCAACTAAGCTCCTTGAC	59.6			
HY3SM	F: TTGTCACTGTGCGACATGC	59.8	ATGC	345–359	3
	R: TCTTTCTAGCAATCTCAGAAGACC	58.9			
IQLI0	F: ACGCCAATCGATCTCAAGC	59.1	ACCTCC	184–202	3
	R: ACGCCAATCGATCTCAAGC	58.0			
JGM27	F: AGCGGGCCTATTCCAGATAC	59.8	AGC	258–267	3
	R: CTGTAGGTGGTGCGGAAAC	59.2			
JS90I	F: CTTAAAGCTGCCTGCGACC	59.9	CT	351–355	3
	R: GTTGCTGTCGGCTAAGGAC	59.3			
JW1YD	F: GATCGTCGGCCGGGAAG	60.7	CCG	194–200	3
	R: GATCGTCGGCCGGGAAG	59.9			

***Culturing apomictic offspring of* M. lindheimeri.** Spores were sown from a total of 41 highly heterozygous apomictic parent sporophytes of *M. lindheimeri* (Fig. 12B; Appendix D). The presence of apomixis in parent sporophytes was determined based on the number of spores produced per sporangium (32-spored sporangia were inferred to be apomictic; Tryon 1968; Barrington and Paris 1986; Gastony and Windham 1989; Sigel et al. 2011). From each apomictic parent, individual sporangia were removed using a dissecting pin and placed on Hevly's medium (pH 7; Hevly 1963) in a small petri plate. Each sporangium was ruptured using two needles and the spores contained therein were manually separated from one another and distributed evenly across the plate. This was done for multiple sporangia from each parent plant and, in later steps, care was taken to record the sporangium from which each spore was derived. Spores were then placed under artificial light on a 12-hour light-dark cycle and observed daily. Upon initial germination, each gametophyte was then transferred to its own isolated well in a 48-well petri plate (also on Hevly's medium, pH 7) to avoid any potential for interaction with gametophytes germinating nearby (Schneller et al. 1990).

As gametophytes developed they were examined periodically for the production of antheridia and archegonia; these reproductive organs are usually absent or abortive in apogamous ferns and I did not observe any functional reproductive structures. Once the gametophytes began to produce their first sporophyte leaves they were processed for DNA extraction and genotyping.

***Genotyping of apomictic progeny*.** A total of 847 offspring derived from 47 sporangia were genotyped for each microsatellite locus that was polymorphic in their respective parent plants (Table 6; Appendix D). Genotyping of offspring followed the same protocol as for the parent plants.

Spore diameter measurements. For each parent sporophyte that exhibited evidence of producing offspring via subsexual processes, ploidy level was assessed using spore diamter and known chromosome vouchers as a proxy following the method of Sigel et al. (2011. For each individual, spore number per sporangium was counted for 1–4 sporangia. The diameter of spores contained within those sporangia were measured and the mean and standard deviation for each sporangium was calculated.

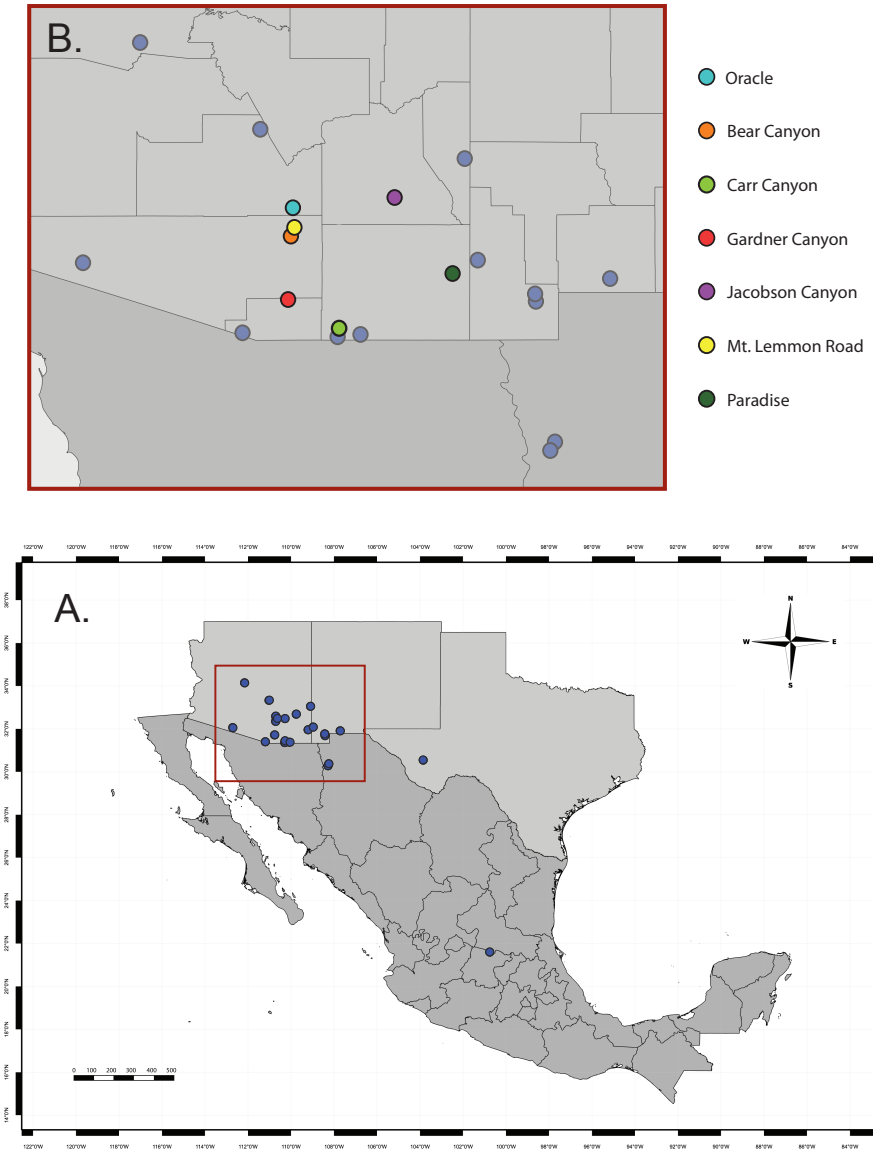


Figure 12

Distribution map of *M. lindheimeri* sampled for this study. A. Samples included in the range-wide survey of genetic variation in *M. lindheimeri*. B. Populations from which summary statistics of genetic diversity were collected.

Results

Microsatellite development. Sequencing of genomic DNA from *M. lindheimeri* (*Schuettpelz 450*) via 454 technology produced a total of 234,428 sequence reads. The survey of raw sequence reads for di-, tri-, tetra-, and pentanucleotide repreats performed with MSATCOMMANDER identified 25,295 sequences containing a total of 33,955 repeats, of which 19,666 were non-complementry repeats (Table 6). Given the surplus of repeat regions, I focused efforts toward primer development for repeats that fit the following criteria: long repeat length, the presence of flanking sequence on either side of the repeat in which to develop primers, and regions with di-, tri-, and tetranucleotide repeats.

Table 6. Summary of microsatellite repeats identified in *M. lindheimeri*.

Repeat Motif	*Minimum # Repeats*	*# Non-complementary Repeats*	*Length Range (bp)*
Dinucleotide	6	16809	12–380
Trinucleotide	4	2317	12–402
Tetranucleotide	4	501	16–368
Pentanucleotide	4	39	20–55
Total repeats: 19666			

***Survey of genetic variation in apomictic* M. lindheimeri.** My initial survey of genetic variation across the northern range of *M. lindheimeri* sporophytes (109 individuals; Fig. 12A) showed that in general, populations are highly polymorphic (Fig. 12B; Table 7; Appendix D), with percent polymorphic loci, P, ranging from 57.1% to

100%. For several populations, a minimal number of individuals were genotyped. As expected, these populations tend to have lower P than those with more samples (Table 7; e.g., collections from Oracle [*n* = 4], Bear Canyon [*n* = 6], and Mt. Lemmon Road [*n* = 8] compared to those from Paradise [*n* = 16] and Jacobson Canyon [*n* = 51]). Individuals sampled from Carr Canyon, even though more numerous than those sampled at other sites, had relatively lower values of P.

Table 7. Summary of genetic diversity measures for populations sampled across the northern range of *M. lindheimeri*. Populations correspond to those indicated in Fig. 12B. P = % polymorphic loci out of 7 or 8 loci (8^{th} locus failed to amplify for some populations); Het = heterozygote frequency over all loci; A = mean number of alleles per locus; G = genetic diversity = $1 - \Sigma G_i^2$, where G_i = frequency of the i^{th} genotype. For raw data see Appendix D.

Population	*n*	P	Het	A	G
Mt. Lemmon Road	4	87.5	0.55	2.5	0.9
Bear Canyon	6	85.7	0.36	1.9	0.3
Carr Canyon	10	66.7	0.30	2.6	0.2
Gardner Canyon	6	57.1	0.57	1.7	0
Jacobson Canyon	51	100	0.56	2.5	0.3
Mt. Lemmon Road	8	71.4	0.50	1.9	0.5
Paradise	16	100	0.42	1.9	0.5

Calculations of heterozygote frequency (per population over all loci; Het), indicate that with increased polymorphism at individual loci in a population generally comes an increase in Het (Table 7). Samples taken from Paradise (Table 7; Fig. 12B) deviate from this pattern; even though all markers are polymorphic (P = 100 %), heterozygote frequency is relatively lower than in other populations (Het = 0.42).

Calculations of genotypic diversity range from 0 (no diversity; Gardner Canyon) to 0.9 (distinct genotype in nearly every individual; Oracle); however, the Oracle population was poorly sampled, and samples from that population were taken somewhat further apart from one another than those in other canyons / populations (for specific localities, see Appendix D).

***Culturing and genotyping apomictic offspring of* M. lindheimeri.** Spores of *M. lindheimeri* consistently germinated ca. 10 days after sowing with the exception of those sown from older herbarium specimens (> 10 years post collection) whose spores took as long as two weeks to germinate, or did not germinate at all. Spores sown from chemical- / pestiside-treated herbarium specimens failed to germinate.

For each of the 41 polymorphic parent sporophytes from which spores were successfully germinated, an average of 20 (± 7.9; min. = 5, max. = 44) offspring were subsequently genotyped. The total number of offspring genotyped for each parent, and the marker(s) for which they were genotyped, are indicated in Table 8. Of the 41 parent plants, a total of 11 individuals produced offspring with microsatellite profiles that were distinct from their own (Table 6); offspring genotypes were, however, these were always a subset of the variation present in the parental genotype (Fig. 13). Most instances of genotypic variation between parents and their offspring were observed at the JW1YD locus, but were also seen (to a lesser extent) at FYM3K, HY3SM, JS90I, HL9PJ, and IQLI0. In some cases distinct offspring genotypes were seen for multiple offspring derived from the same sporangium (e.g., *Beck G9*, *Beck G1.2*, *Beck G8*, *Grusz 171.8*, *Franklin 4532*, and *Grusz 171.6*; Table 8). *Grusz 171.6* was noteworthy in having 5 offspring derived from the same sporangium with a distinct genotype from that of the parent plant (at JS90I; Table 8). In all cases, when offspring derived from the same sporangium exhibited different microsatellite profiles from their parent, those offspring all shared identical genotypes for that marker.

Table 8. Summary of genotyping results across polymorphic M. lindheimeri and their offspring. Individuals for which multiple sporangia were sampled have their total number of offspring per sporangium separated by a comma. Sporangia in which decay of heterozygosity was observed are indicated with a single asterisk. Black circles (●) indicate polymorphism detected in parental sporophyte for a given microsatellite marker; red circles (●) signify observed decay of heterozygosity in offspring generated by that individual. Red circles denoted with two (**) or three (***) asterisks signify whether two or three offspring underwent decay of heterozygosity, respectively; circles marked with two hats (^^) had five offspring exhibit decay of heterozygosity. Raw data (i.e., fragment lengths) are listed in Appendix D.

		Microsatellite Locus							
Voucher Information	**# Offspring Sampled**	**HGGWA**	**FYM3K**	**JW1YD**	**JGM27**	**HY3SM**	**JS90I**	**HL9PJ**	**IQLI0**
U.S.A, AZ: Cochise Co., Grusz 173.2	14	●		●	●	●	●		
U.S.A, AZ: Cochise Co., Grusz 173.3	21			●		●	●		
U.S.A, AZ: Cochise Co., Grusz 173.17	16, 11		●				●		
U.S.A, AZ: Cochise Co., Grusz Carr 1	27		●				●		
U.S.A, AZ: Cochise Co., Grusz Carr2	16		●				●		
U.S.A, AZ: Cochise Co., Grusz Carr 3	27*	●		●			●		
U.S.A., AZ: Graham Co., Beck G1.1	23			●		●	●	●	
U.S.A., AZ: Graham Co., Beck G1.2	17*, 22			●		●	●	●**	●
U.S.A., AZ: Graham Co., Beck G5	20, 24		●					●	
U.S.A., AZ: Graham Co., Beck G6	19	●	●				●		
U.S.A., AZ: Graham Co., Beck G7	18			●		●	●	●	
U.S.A., AZ: Graham Co., Beck G8	19*			●		●	●	●**	
U.S.A., AZ: Graham Co., Beck G9	20*			●		●	●	●	●**
U.S.A., AZ: Graham Co., Grusz 171.2	24*			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 171.4	14			●		●	●	●	

Vourcher Information	# Offspring Sampled	HGGWA	FYM3K	JW1YD	JGM27	HY3SM	JS90I	HL9PJ	IQLI0
U.S.A., AZ: Graham Co., Grusz 171.5	18			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 171.6	20*			●		●	●^^	●	
U.S.A., AZ: Graham Co., Grusz 171.7	12*			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 171.8	17			●		●	●	●***	
U.S.A., AZ: Graham Co., Grusz 171.12	24			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 171.13	24			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 171.14	23			●		●	●	●	
U.S.A., AZ: Graham Co., Grusz 178.2	17		●				●		
U.S.A., AZ: Graham Co., Grusz 178.6	7	●	●	●		●	●		
U.S.A., AZ: Graham Co., Grusz 178.8	18*	●	●	●		●	●		
U.S.A., AZ: Pima Co., Cottam 12886 (UT)	8*	●	●	●		●			
U.S.A., AZ: Pima Co., Grusz 177.1	14*		●				●		
U.S.A., AZ: Pima Co., Grusz 177.3	12, 16		●				●		
U.S.A., AZ: Pinal Co., Grusz 41B	17	●	●	●		●	●	●	●
U.S.A., AZ: Pinal Co., Grusz 45	20		●	●			●	●	
U.S.A., AZ: Pinal Co., Grusz 47	9		●				●	●	●
U.S.A., AZ: Santa Cruz Co., Franklin 4532 (NY)	11, 18, 18*		●	●**			●	●	
U.S.A., AZ: Santa Cruz Co., Franklin 5376 (NY)	20		●	●					
U.S.A., AZ: Santa Cruz Co., Grusz 175.2	25			●		●	●	●	
U.S.A., AZ: Santa Cruz Co., Grusz 175b.4	26			●		●	●	●	
U.S.A., NM: Hidalgo Co., Worthington 12682 (NY)	2, 21		●	●					
U.S.A., NM: Luna Co., Worthington 19958 (NY)	26		●	●					

Vourcher Information	# Offspring Sampled	HGGWA	FYM3K	JW1YD	JGM27	HY3SM	JS90I	HL9PJ	IQLI0
U.S.A., TX: Jeff Davis Co., Bridges s.n. (SRSC)	5		●						
MEX, Chihuahua, Spencer & Atwood 1176 (UT)	20					●			
MEX, San Luis Potosí, Rothfels 3066	13		●	●	●	●		●	●
Proportion of parents polymorphic:		**0.17**	**0.51**	**0.73**	**0.05**	**0.56**	**0.80**	**0.53**	**0.17**
Total offspring genotyped:		**91**	**30**	**626**	**27**	**456**	**716**	**460**	**111**
Total decay events observed:		**0**	**1**	**6**	**0**	**0**	**7**	**9**	**2**

Figure 13.

Subset of microsatellite profiles for apomictic *M. lindheimeri* sporophytes and their genetically distinct offspring. For all panels A–D, parental genotypes are shown above their offspring. A. JW1YD genotype for *Beck G9* and one offspring that is lacking the 196 bp parental allele. B. HL9PJ genotype for *Grusz 171.6* and one offspring individual that is lacking the 419 bp parental allele. C. HGGWA genotype for *Grusz Carr 3* and one offspring individual that is lacking the 185 bp parental allele. D. FYM3K genotype of *Grusz 177.1* and one offspring that is lacking the 200 bp parental allele.

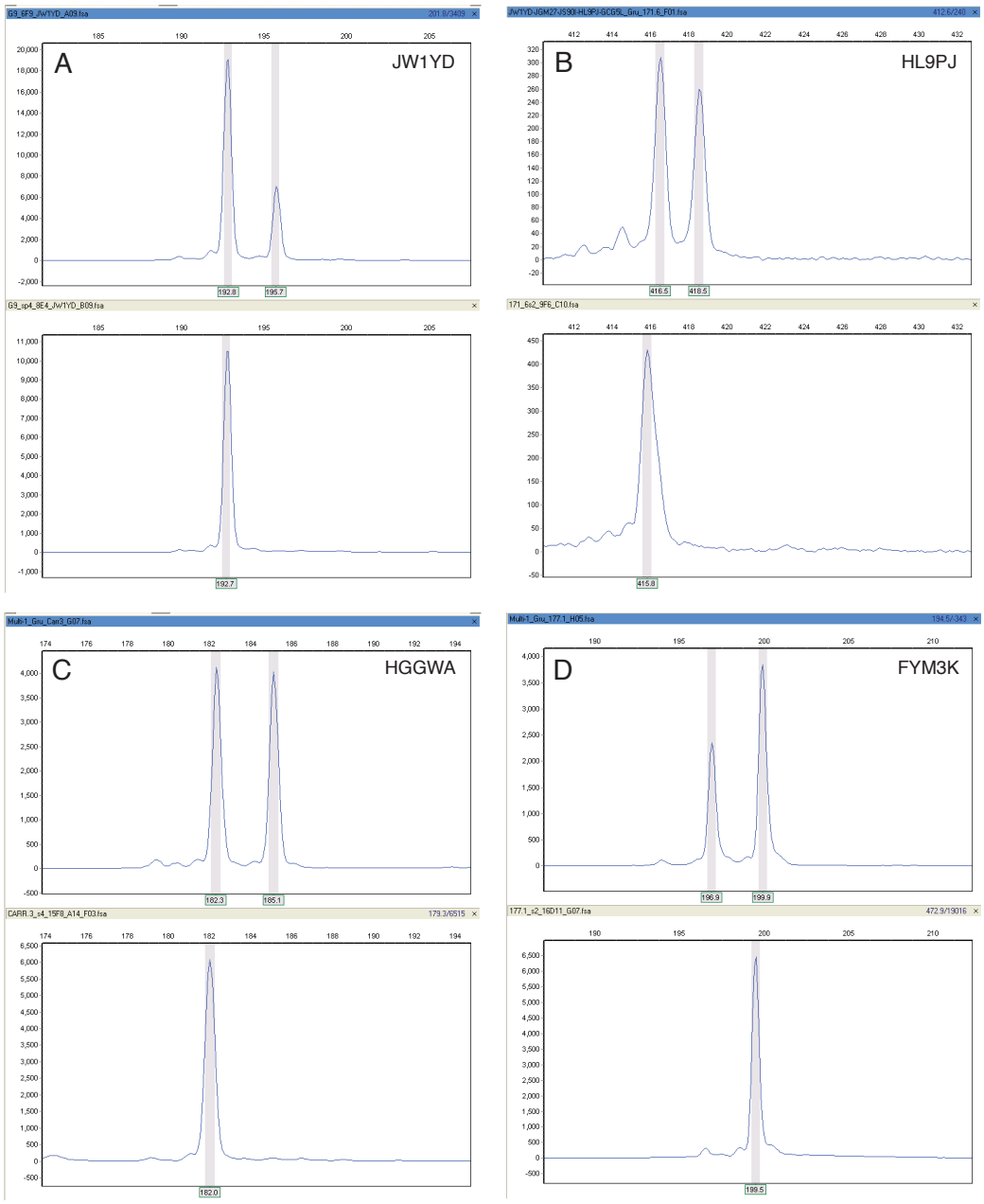


Figure 13

Spore diameter measurements. For each of the 11 parents that produced genetically distinct offspring, ploidy was assessed using spore diameter as a proxy (Sigel et al. 2011). Mean spore diameter for those individuals was then compared to the spore diameter of individual chromosome vouchers of *M. lindheimeri* and closely related taxa for which chromosome number had been previously documented (Fig. 14; Appendix D).

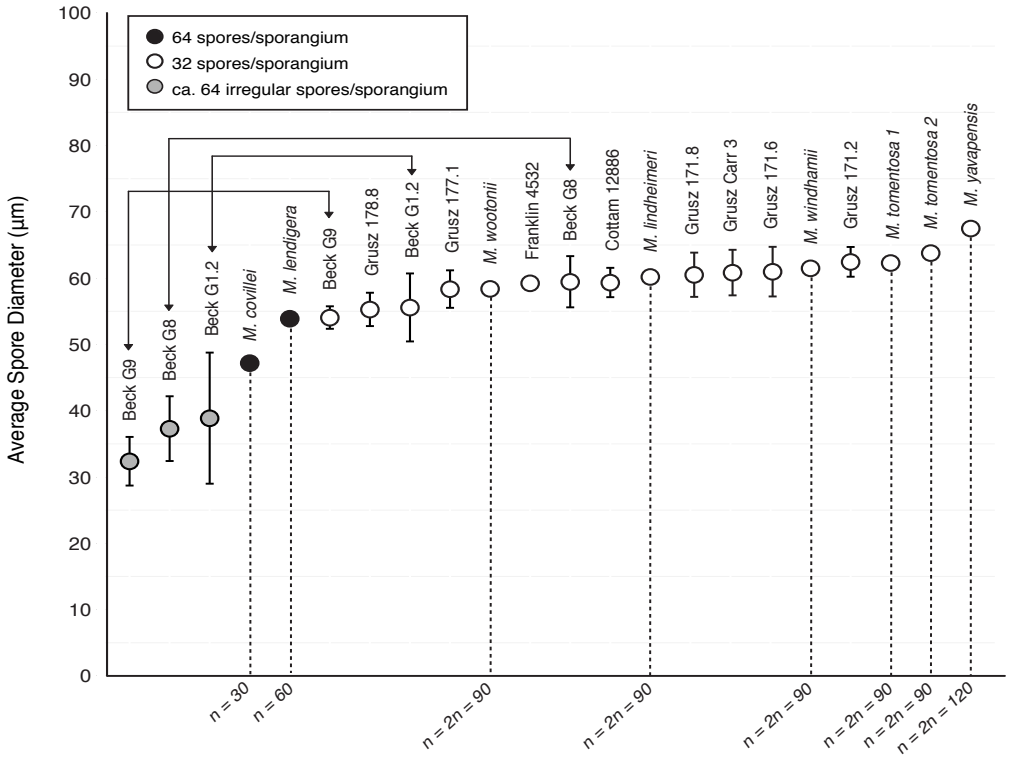


Figure 14

Summary of spore number per sporangium and spore diameter for sporophytes that produced genetically distinct offspring. Dots and whiskers signify mean ± one standard deviation. Filled dots = 64 normal spores per sporangium (black) or ca. 64 irregular spores per sporangium (gray); white dots = 32 spores per sporangium. Vouchers of known chromosome base number are designated with a dashed line. Individuals producing either 32 or ca. 64 irregular (i.e., abortive) spores per sporangium on the same plant have arrows connecting their two respective sporangial counts / measurements.

Discussion

Premeiotic chromosome doubling is, more often than not, the rule of reproduction for many parthenogenetic vertebrates. This process is well documented across the animal kingdom, but seems to have variable consequences in different parthenogenetic lineages. In some cases, as in the parthenogenetic whiptail lizards, premeiotic duplication is implicated to be a mechanism by which asexual lineages maintain heterozygosity. Lutes et al. (2010) demostrate that during meiosis in the allotriploid lizard *Aspidoscelis exsanguis*, sister chromosomes form bivalent pairs with high fidelity. They suggest that pairing of sister chromosomes during meiosis is a surefire mechanism for maintaining fixed heterozygosity in these parthenogenetic reptiles. MacGregor and Uzzell (1964) came to a similar conclusion many years earlier, based on their studies of parthenogenetic triploid salamanders of the *Ambystoma jeffersonianum* complex. They inferred that following endomitosis, sister chromosomes synapse with their identical homologues. Unlike studies in whiptail lizards, this conclusion did not hold true for long. Subsequent studies of *Ambystoma* allotriploids clearly documented the pairing of homeologous chromosomes following endomitosis in certain allotriploid lineages (Bi and Bogart 2006).

The only plant analogue to premeiotic doubling in parthenogenetic animals (to my knowledge) is Döpp-Manton sporogenesis in the ferns (DMS; Döpp 1939; Manton 1950; Gastony and Windham 1989). The outcome of DMS in ferns was first explored by Bierhorst (1975). Rather by accident, he stumbled upon an easily traceable, paracentric inversion that allowed him to observe homeologous pairing in a single apomictic individual of *Trichomanes pinnatum* Hedw. from French Guiana. A few years prior to Bierhorst's finding, Klekowski (1973) had argued that homeologous pairing could

provide an adaptive advantage in homosporous ferns by permitting the storage and release of genetic variability in the face of extreme inbreeding. He subsequently found empirical evidence for this hypothesis in a sexual tetraploid fern, *Ceratopteris thalictroides* (L.) Brongn., by cytologically /visually following the inheritance of a similar paracentric chromosomal inversion (Klekowski and Hickok 1974; Hickok 1978a, 1978b).

Exploration of homeologous (or non-sister) chromosome pairing was next investigated years later by Ishikawa et al. (2003). These authors genotyped 284 progeny of one apomictic allotriploid individual of *Dryopteris nipponensis* Koidz. at one isozyme locus, *Pgi-2*. Of those 284 progeny, they observed 5 instances of genetic segregation (from *abc* in the parent plant to *aab* (x3), *bbc* (x1), *and bcc* (x1) in the segregating offspring). Recently, Ootsuki et al. (2012) explored the same processes in the apogamous triploid fern, *Cyrtomium fortunei* J. Sm. These authors genotyped 732 progeny from one individual of *C. fortunei* at the nuclear marker *pgiC* and found 19 progeny revealing evidence of autosegregation. Based on these few studies, it seems safe to conjecture that premeiotic duplication in apomictic ferns does indeed—unlike in whiptail lizards (e.g., Fig. 13A)—provide a vehicle for the pairing of non-sister chromosomes, thereby generating genetically distinct (albeit more homozygous) offspring (Figs. 13A, B).

In each of the studies described above, the pairing of non-sister chromosomes after premeiotic duplication (and the occurrence of subsexual processes following that pairing) was only observed in one (or two) individuals; thus limiting assessment of the generality of this phenomemon in natural apomictic populations. To assess the generality of subsexual processes, I developed eight highly polymorphic microsatellite markers for Lindheimer's Lip Fern, *Myriopteris lindheimeri*. Using these markers, I identified 41 highly heterozygous individuals from across the species range to test for

the occurrence of subsexual processes. I then genotyped a total of 847 offspring of those parent plants (an average of 20 offspring per parent) at the eight microsatellite loci developed for this study (Table 5). The genotypes of parental sporophytes at those eight loci were then compared to the genotypes of their offspring:

H_0: Parent sporophyte genotype = Offspring gametophyte genotype (Fig. 13a)

H_1: Parent sporophyte genotype ≠ Offspring gametophyte genotype (Fig. 13b,c)

In ferns, each spore, and thus each gametophyte, is a single product of meiosis; therefore, if apomictic offspring genotypes are not identical to the parental sporophyte it can be concluded that subsexual processes are acting during DMS in these apomictic triploid ferns (Fig. 13b,c). It is also possible that random mutation may contribute to variation in the offspring, but for the purposes of this study random mutation is assumed to occur at extremely low levels (although mutation rate is not known in these ferns).

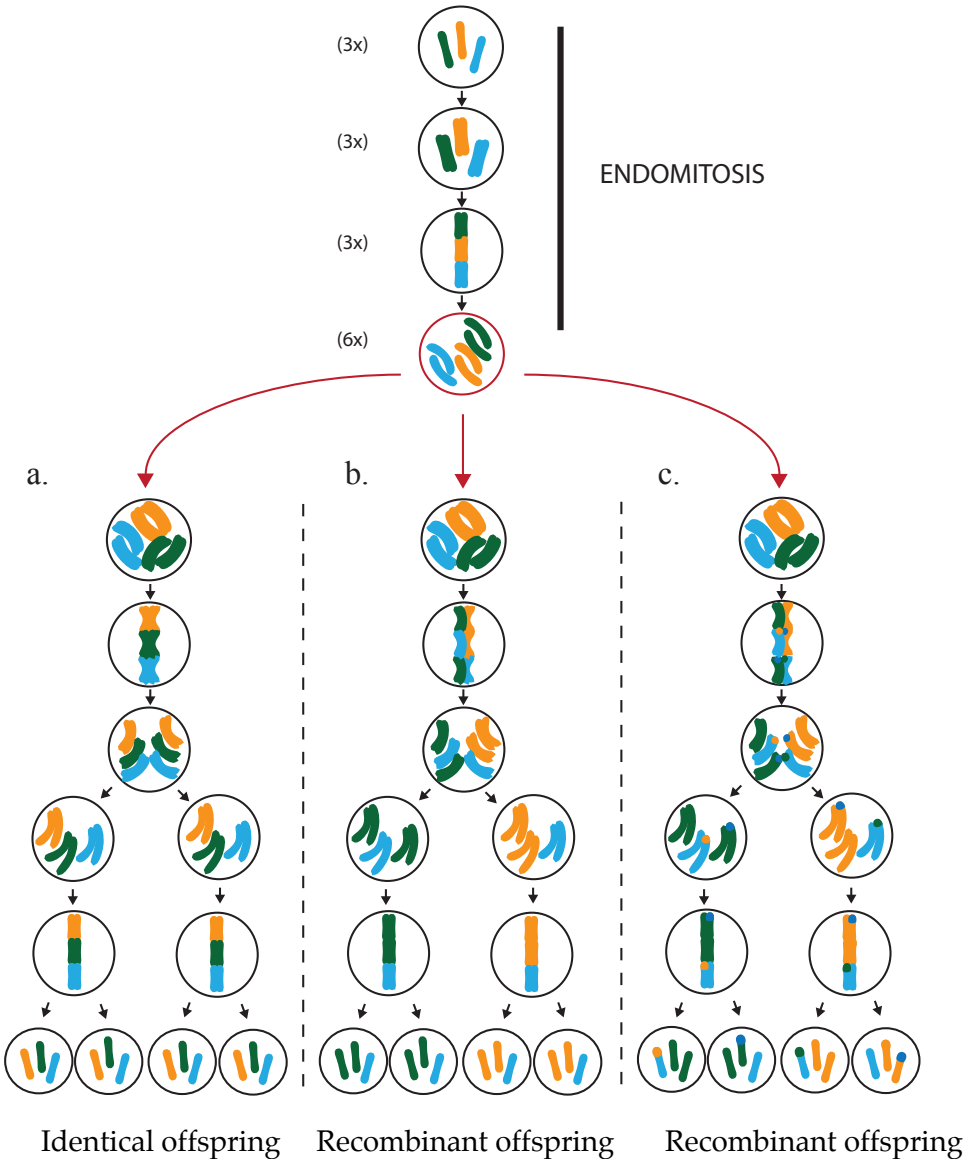


Figure 15

Possible outcomes of Döpp-Manton sporogenesis. Endomitosis prior to meiosis is shown in red. Three possible outcomes of Döpp-Manton sporogenesis include: (a) pairing of homologous duplicated chromosomes and lack of independent assortment (IA), resulting in meiotic products identical in ploidy level and genomic constitution to the parent plant; (b) pairing of nonhomologous/homeologous chromosomes and IA, resulting in genetically variable meiotic products; and (c) pairing of nonhomologous/homeologous chromosomes, recombination and IA leading to genetically variable meiotic products with unique chromosome haplotypes.

My results show that across the 847 progeny sampled, 25 instances of decay of heterozygosity were observed at the eight markers examined (Table 8; Appendix D), demonstrating that subsexual processes are acting in this apomictic lineage. It is noteworthy that, even given the shallow sampling of progeny from given individuals, 27% of parent sporophytes sampled showed evidence of subsexual processes. Among those, some individuals produced multiple individual offspring derived from the same sporangium with identical patterns of decay (e.g., at JW1YD in offspring of *Franklin 4532*; Table 8). As illustrated in Figure 15, we expect to find the reciprocal product of decay in offspring produced from the same sporangium (provided all spores from that sporangium germinate). This was not observed in the majority of segregating offspring of *M. lindheimeri*. One explanation for the lack of reciprocal decay is that most parents were diallelic at a given microsatellite marker (Appendix D). Had they been triallelic, it would have been an expectation to observe this decay, but in individuals with only two alleles decay may be present but masked by an initial double dose of one allele (e.g., *aab* produces *aaa* and *abb* via subsexual processes, only the former result of which is visible using microsatellite genotyping).

My survery of genetic variation among wild individuals (and in natural populations) of *M. lindheimeri* (Fig. 11A,B; Appendix D) showed that heterozygote frequency (Het; Table 7) was generally high in natural populations of this apomictic triploid. Unfortunately, I was not able to estimate allele frequencies, and hence H_e, because the majority of triploid heterozygotes observed only exhibited two alleles (therefore, not allowing me to distinguish which of those two alleles was present in higher frequency); I was unable to determine whether Het was higher than expected in a population undergoing random mating. It is true though, that asexual populations are

known for their tendency to produce an excess of heterozygotes. In tandem with this increase in fixed heterozygosity, asexual populations are expected to have relatively low levels of genotypic diversity (G; Table 7). This held true for populations of *M. lindheimeri*, especially in the Gardner Canyon population; with the exception of Oracle (G = 0.9), but this is probably best explained by sampling effect given that only 4 individuals were collected from that locality.

The population at Carr Canyon had low average heterozygosity relative to the other populations sampled; interestingly, one of the segregating apomicts observed in this study (*Grusz Carr 3*) came from that population. If autosegregation is common in this population, it may explain the low heterozygosity observed at Carr Canyon. The population at Jacobson Canyon did not have a particularly low average heterozygosity even though 7 of the 11 segregating individuals observed were collected from that site. A large number of samples from Jacobson Canyon were analysed because many collections from that population were highly heterozygous. Subsequent spore measurements of segregating apomicts from this site (Fig. 14) indicate that both triploid (*Beck G8*, *Grusz 171.8*, *Grusz 171.6*, *Grusz 171.2*) and *diploid* (*Beck G9*, *Beck G1.2*) apomicts occur at this location. These putatively diploid apomictic individuals produced two types of sporangia—some that produce 32 spores as a result of premeiotic duplication, and others that produce ca. 64 irregularly shaped spores (Fig. 14). Prior to this study, diploid apomicts were never observed in the Lip Ferns (*Myriopteris*), or in the larger, globally distributed cheilanthoid clade (comprised of xeric-adapted ferns among which apomixis is common). Based on these observations, Jacobson Canyon may represent a cradle of apomixis in a population with mixed reproductive modes.

Conclusion

My results show that—as seen in apomictic triploid *Ambystoma* salamnders—premeiotic chromosome duplication in apomictic *M. lindheimeri* is a source of genotypic diversity, both at the individual and population levels and that this process could very well be widespread in apomictic fern lineages that undergo DMS. Thus, Lip Ferns are not like (*Aspidoscelius*) lizards, at least when it comes to "mixing" things up.

FURTHER CONTRIBUTIONS

In addition to the research undertaken for the completion of my dissertation, I have also contributed to a variety of collaborations and their resulting peer-reviewed publications. These papers span a range of topics—from molecular and morphological systematics through to the evolution of fern genomes—each of which has served to expand our current understanding of fern evolutionary biology.

Grusz (2013) formally named the taxon previously referred to *Cheilanthes villosa* within the genus *Myriopteris*. This new species was named in honor of Michael D. Windham, in acknowledgement of his lifelong study of cheilanthoid ferns, as well as his many in-depth investigations into the origins of apomictic polyploid lineages.

Lagomarsino & al. (2012) explored the evolution of primary hemiepiphytism and gametophyte morphology in *Elaphoglossum amygdalifolium* (Dryopteridaceae). Because *E. amygdalifolium* holds the critical phylogenetic position as sister to the remaining 600+ species within the Dryopteridaceae, documenting its status as a primary hemiepiphyte provided a foundation for understanding epiphytism in general across this large, cosmopolitan fern family. This study was the first to document hemiepiphytism in the species, and the first of my publications to be completed with a student coauthor.

Wolf & al. (2011) was a collaborative paper on the evolution of chloroplast genes and genomes in ferns. This collaboration was spurred by analysis of next generation 454 sequencing data obtained for the completion of my dissertation research. Here, we published the first complete, annotated chloroplast genome of the apomictic triploid

fern, *Cheilanthes* (i.e., *Myriopteris*) *lindheimeri*. We also described broad evolutionary patterns and processes in fern plastid genomes, including a review of RNA editing and nucleotide substitution patterns across all currently available fern plastomes.

Pryer & al. (2010) utilizes DNA barcoding as a resource for unveiling a case of mistaken identity in the fern horticultural trade. We used three plast regions to confirm that indivuals marketed as *Cheilanthes wrightii* (= *M. wrightii*), which is native to deserts of the southwestern United States, are actually *Cheilanthes distans*, an Australian endemic. Our results reinforce the importance of DNA barcoding in the international horticultural industry.

Windham & al. (2009) provides an overview of the cheilanthoid fern phylogeny, which encompasses a monophyletic group containing nearly 500 spp. of mostly xeric-adapted ferns in the Pteridaceae. We recognize seven major clades within cheilanthoid ferns and present the informal name 'myriopterids' in reference to the genus now described (herein, Part 2) as *Myriopteris*.

Schuettpelz & al. (2008) describes primers for the amplification of a novel low-copy nuclear gene in ferns, *gapCp*. Historically, the exceptional lack of primers that successfully amplify low-copy nuclear genes in ferns has greatly inhibited our progress in understanding fern evolution. This broadly useful region is presented as a tool for phylogenetic analyses, particularly for the study of hybridization and polyploidy in leptosporangiate ferns.

Rothfels & al. (2008) was the first of many papers published as a result of our research into the molecular phylogeny and evolution of cheilanthoid ferns. This effort provided a monophyletic definition of *Notholaena* (Pteridaceae), one genus within the pellaeid ferns, which, combined, comprise the sister clade to *Myriopteris*.

APPENDIX A

Taxa sampled for DNA sequence data in this study, including voucher information and GenBank accession numbers. Taxon names are in bold italics followed by the nomenclatural authority. Numbers in bold are identification numbers used in this study followed by corresponding Fern DNA database numbers (http://www.pryerlab.net/DNA_database.shtml). These are followed by ploidy level (bold), then by collector and number (italics), location of voucher (herbarium acronym in parentheses), and finally by the loci sequenced (italics). Each consensus allele sequence of nuclear *gapCp* is identified, followed by the number of clones included in that consensus, the length of the sequence reported, and its corresponding GenBank accession number. Sequence data for plastid loci (*rbcL-atpB*, *trnG-trnR*, *trnP*^{UGG}*-petG*, and *rps4-trnS*^{GGA}) for each accession are followed by their corresponding GenBank accession numbers.

Cheilanthes covillei Maxon, **1:** 3485 **(diploid),** *Windham 2945* (UT), *gapCp*, allele 1, 4 clones, 600bp, FJ870860, allele 2, 3 clones, 600bp, FJ870861, *rbcL-atpB*, FJ870813, *trnG-trnR*, FJ870771, *trnP*^{UGG}*-petG*, FJ870791, *rps4-trnS*^{GGA}, FJ870835, **2:** 3150 **(diploid)**, *Schuettpelz 443* (DUKE), *gapCp*, allele 1, 7 clones, 600bp, FJ870857, allele 2, 4 clones, 596bp, FJ870858, *rbcL-atpB*, FJ870814, *trnG-trnR*, EU268697, *trnP*^{UGG}*-petG*, FJ870792, *rps4-trnS*^{GGA}, FJ870836, **3:** 3487 **(diploid)**, *Windham 3322* (DUKE), *gapCp*, allele 1, 9 clones, 600bp, FJ870862, *rbcL-atpB*, FJ870815, *trnG-trnR*, FJ870772, *trnP*^{UGG}*-petG*, FJ870793, *rps4-trnS*^{GGA}, FJ870837, **4:** 3156 **(diploid)**, *Schuettpelz 449* (DUKE), *gapCp*, allele 1, 6 clones, 600bp, FJ870859, *rbcL-atpB*, FJ870816, *trnG-trnR*, FJ870773, *trnP*^{UGG}*-petG*, FJ870794, *rps4-trnS*^{GGA}, FJ870838, **5:** 3845 **(diploid)**, *Windham 3436* (DUKE), *gapCp*, allele 1, 4 clones, 600bp, FJ870863, allele 2, 3 clones, 600bp, FJ870864, *rbcL-atpB*, FJ870817, *trnG-trnR*, FJ870774, *trnP*^{UGG}*-petG*, FJ870795, *rps4-trnS*^{GGA}, FJ870839; ***Cheilanthes fendleri*** Hooker, **1:**

3690 **(diploid)**, *Windham 3408* (DUKE), *gapCp*, allele 1, 4 clones, 602bp, FJ870866, allele 2, 2 clones, 602bp, FJ870867, *rbcL-atpB*, FJ870818, *trnG-trnR*, FJ870775, *trnP*^{UGG}*-petG*, FJ870796, *rps4-trnS*^{GGA}, FJ870840, **2:** 3177 **(diploid)**, *Schuettpelz 470* (DUKE), *gapCp*, allele 1, 8 clones, 602bp, FJ870865, *rbcL-atpB*, FJ870819, *trnG-trnR*, FJ870776, *trnP*^{UGG}*-petG*, FJ870797, *rps4-trnS*^{GGA}, FJ870841, **3:** 3691 **(diploid)**, *Windham 3410* (DUKE), *gapCp*, allele 1, 6 clones, 602bp, FJ870868, *rbcL-atpB*, FJ870820, *trnG-trnR*, FJ870777, *trnP*^{UGG}*-petG*, FJ870798, *rps4-trnS*^{GGA}, FJ870842; ***Cheilanthes lindheimeri*** Hooker, **1:** 3490 **(diploid)**, *Windham 97-015* (DUKE, UT), *gapCp*, allele 1, 4 clones, 601bp, FJ870879, allele 2, 1 clone, 601bp, FJ870880, *rbcL-atpB*, FJ870821, *trnG-trnR*, FJ870778, *trnP*^{UGG}*-petG*, FJ870799, *rps4-trnS*^{GGA}, FJ870843, **2:** 3157 **(diploid)**, *Schuettpelz 450* (DUKE), *gapCp*, allele 1, 3 clones, 601bp, FJ870872, allele 2, 3 clones, 601bp, FJ870873, *rbcL-atpB*, FJ870822, *trnG-trnR*, FJ870779, *trnP*^{UGG}*-petG*, FJ870800, *rps4-trnS*^{GGA}, FJ870844, **3:** 3692 **(diploid)**, *Spellenberg et al. 5056* (NMC), *gapCp*, allele 1, 3 clones, 601bp, FJ870881, allele 2, 2 clones, 600bp, FJ870882, *rbcL-atpB*, FJ870823, *trnG-trnR*, FJ870780, *trnP*^{UGG}*-petG*, FJ870801, *rps4-trnS*^{GGA}, FJ870845, **4:** 3205 **(triploid)**, *Schuettpelz 498* (DUKE), *gapCp*, allele 1, 5 clones, 601bp, FJ870877, allele 2, 5 clones, 601bp, FJ870878, *rbcL-atpB*, FJ870824, *trnG-trnR*, FJ870781, *trnP*^{UGG}*-petG*, FJ870802, *rps4-trnS*^{GGA}, FJ870846, **5:** 3196 **(triploid)**, *Schuettpelz 489* (DUKE), *gapCp*, allele 1, 9 clones, 601bp, FJ870874, allele 2, 3 clones, 600bp, FJ870875, allele 3, 5 clones, 601 bp, FJ870876, *rbcL-atpB*, FJ870825, *trnG-trnR*, FJ870782, *trnP*^{UGG}*-petG*, FJ870803, *rps4-trnS*^{GGA}, FJ870847, **6:** 3147 **(triploid)**, *Schuettpelz 440* (DUKE), *gapCp*, allele 1, 5 clones, 601bp, FJ870869, allele 2, 6 clones, 601bp, FJ870870, allele 3, 3 clones, 600bp, FJ870871, *rbcL-atpB*, FJ870826, *trnG-trnR*, FJ870783, *trnP*^{UGG}*-petG*, FJ870804, *rps4-trnS*^{GGA}, FJ870848; ***Cheilanthes newberryi*** (D.C. Eaton) Domin, **1:** 3827 **(diploid)**, *Metzgar 174* (DUKE), *gapCp*, allele 1, 4 clones, 582bp, FJ870883, *rbcL-atpB*, FJ870827, *trnG-trnR*, EU268685, *trnP*^{UGG}*-petG*, FJ870805, *rps4-trnS*^{GGA}, FJ870849; ***Cheilanthes wootonii*** Maxon,

1: 3195 **(triploid)**, *Schuettpelz 488* (DUKE), *gapCp*, allele 1, 3 clones, 602bp, FJ870884, allele 2, 3 clones, 602bp, FJ870885, allele 3, 5 clones, 601bp, FJ870886, *rbcL-atpB*, FJ870828, *trnG-trnR*, FJ870784, *trnP*^{UGG}*-petG*, FJ870806, *rps4-trnS*^{GGA}, FJ870850, **2:** 3693 **(triploid)**, *Windham 3409* (DUKE), *gapCp*, allele 1, 4 clones, 600bp, FJ870887, allele 2, 3 clones, 602bp, FJ870888, allele 3, 3 clones, 602bp, FJ870889, *rbcL-atpB*, FJ870829, *trnG-trnR*, FJ870785, *trnP*^{UGG}*-petG*, FJ870807, *rps4-trnS*^{GGA}, FJ870851, **3:** 3694 **(triploid)**, *Spellenberg & Mart 10407* (NMC), *gapCp*, allele 1, 4 clones, 600bp, FJ870890, allele 2, 8 clones, 602bp, FJ870891, allele 3, 2 clones, 602bp, FJ870892, *rbcL-atpB*, FJ870830, *trnG-trnR*, FJ870786, *trnP*^{UGG}*-petG*, FJ870808, *rps4-trnS*^{GGA}, FJ870852; ***Cheilanthes yavapensis*** T. Reeves ex Windham, **1:** 3489 **(tetraploid)**, *McGill 6156* (UT), *gapCp*, allele 1, 3 clones, 600bp, FJ870902, allele 2, 2 clones, 600bp, FJ870903, allele 3, 6 clones, 602bp, FJ870904, *rbcL-atpB*, FJ870831, *trnG-trnR*, FJ870787, *trnP*^{UGG}*-petG*, FJ870809, *rps4-trnS*^{GGA}, FJ870853, **2:** 3151 **(tetraploid)**, *Schuettpelz 444* (DUKE), *gapCp*, allele 1, 3 clones, 600bp, FJ870899, allele 2, 5 clones, 602bp, FJ870900, allele 3, 4 clones, 600bp, FJ870901, *rbcL-atpB*, FJ870832, *trnG-trnR*, FJ870788, *trnP*^{UGG}*-petG*, FJ870810, *rps4-trnS*^{GGA}, FJ870854, **3:** 3122 **(tetraploid)**, *Schuettpelz 415* (DUKE), *gapCp*, allele 1, 16 clones, 601bp, FJ870893, allele 2, 7 clones, 602bp, FJ870894, allele 3, 8 clones, 600bp, FJ870895, *rbcL-atpB*, FJ870833, *trnG-trnR*, FJ870789, *trnP*^{UGG}*-petG*, FJ870811, *rps4-trnS*^{GGA}, FJ870855, **4:** 3145 **(tetraploid)**, *Schuettpelz 438* (DUKE), *gapCp*, allele 1, 9 clones, 601bp, FJ870896, allele 2, 2 clones, 602bp, FJ870897, allele 3, 3 clones, 600bp, FJ870898, *rbcL-atpB*, FJ870834, *trnG-trnR*, FJ870790, *trnP*^{UGG}*-petG*, FJ870812, *rps4-trnS*^{GGA}, FJ870856.

APPENDIX B

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); KF961750; 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* (PRE); KF961877; KF961756; KF961818; (1) 41. 2: *no DB #*, *Goldblatt 7014* (PRE); ——; (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 (Johnson et al. 2012); ——.

APPENDIX C

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 & Windham
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

APPENDIX D

List of vouchers used in microsatellite analyses and spore measurements. All vouchers are of *M. lindheimeri* unless otherwise noted. Format follows: *Collector #* (voucher home herbarium): latitude longitude, population (where applicable); microsatellite genotype at HGGWA; FYM3K; JW1YD; JGM27; HY3SM; JS90I; HL9PJ; IQLI0. Missing data are indicated with an em dash. Chromosome vouchers follow the format: *Taxon*: *Collector #* (voucher home herbarium); *chromosome base number*.

Atwood 21622 (BYU): N30.283 W108.283; 182; 197, 200; 193; 261; 355; 351, 353, 355; 411; 184, 196. *Beck G1.1* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G1.2* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; 184, 196. *Beck G2* (DUKE): N32.683 W109.763, Jacobson Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Beck G3* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G5* (DUKE): N32.683 W109.763, Jacobson Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Beck G6* (DUKE): N32.683 W109.763, Jacobson Canyon; 197, 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Beck G7* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G8* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G9* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; 184, 196. Beck G10 (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G11* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; —; —; —. Beck G12 (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. Beck G13 (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261;

345, 355; 351, 353, 355; 417, 419; —. *Beck G14* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Beck G15* (DUKE): N32.683 W109.763, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Bridges s.n.* (SRSC): N30.541 W103.851; Fort Davis; 182; 197, 200; 193; 261; 355; 351, 353, 355; 411; 184, 196. *Cottam 12886* (UT): N32.066 W112.716; 200, 203; 193, 196; 261; 355, 359; 351, 353, 355; 409, 411, 415; 184, 202. *Franklin 4532* (NY): N31.383 W110.083; 182; 190, 200; 193, 196; 261; 355; 353, 355; 409, 411, 415; 196. *Franklin 5376* (NY): N31.4 W111.2; 182; 190, 200; 193, 196; 261; 355; 353, 355; 409, 411, 415; 196. *Grusz Carr 1* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 2* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 3* (DUKE): N31.439 W110.286; Carr Canyon; 182, 185; 200; 193, 196; 261; 355; 355; 417; —. *Grusz Carr 4* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 5* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 6* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 7* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 8* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz Carr 9* (DUKE): N31.439 W110.286; Carr Canyon; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 19A* (DUKE): N33.051 W109.097, Jacobson Canyon; 197, 200; 193; 261; 355; 353, 355; 411; 184, 196. *Grusz 21B* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 344, 355; 351, 353, 355; 409, 415; 184, 196. *Grusz 22A* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 344, 355; 351, 353, 355; 409, 415; 184, 196. *Grusz 41B* (DUKE): N32.584 W110.721, Oracle; 197, 200, 202; 193, 196; 261; 355, 359; 353, 355; 411, 415; 184, 196. *Grusz 45* (DUKE): N32.584 W110.721, Oracle; 190, 200; 193, 196;

261; 355; 353, 355; 409, 411, 415; 196. *Grusz 47* (DUKE): N32.584 W110.721, Oracle; 190, 200; 193; 261; 355; 353, 355; 411, 413; 196, 202. *Grusz 171.1* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.2* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; 184, 196. *Grusz 171.3* (DUKE): N32.684 W109.761, Jacobson Canyon; 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Grusz 171.4* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.5* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.6* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.7* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.8* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.9* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.10* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.11* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.12* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.13* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. Grusz 171.14 (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.15* (DUKE): N32.683 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.16* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.17* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355;

417, 419; —. *Grusz 171.18* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; —; 261; 345, 355; —; —; —. *Grusz 171.19* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.20* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.21* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; —; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.22* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; —; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.23* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; —; —; —. *Grusz 171.24* (DUKE): N32.684 W109.761, Jacobson Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 171.25* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; —; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.26* (DUKE): N32.684 W109.761, Jacobson Canyon; —; —; 261; —; —; —; —. *Grusz 171.27* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.29* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.30* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.31* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 171.32* (DUKE): N32.684 W109.761, Jacobson Canyon; 200; 193, 196; 261; —; 351, 353, 355; 417, 419; —. *Grusz 171.33* (DUKE): N32.684 W109.761, Jacobson Canyon; —; 193, 196; 261; —; 351, 353, 355; 417, 419; —. *Grusz 173.1* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193, 196; 261; 355; 353, 355; 417; —. *Grusz 173.2* (DUKE): N31.959 W109.212, Paradise; 182, 185; 200; 193, 196; 261, 270; 348, 355; 353, 355; —; —. *Grusz 173.3* (DUKE): N31.959 W109.212, Paradise; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 173.4* (DUKE): N31.959 W109.212, Paradise; 182, 185; 200; 193, 196; 261, 270; 348, 355; 353, 355; —; —. *Grusz 173.5* (DUKE):

N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 173.6* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193, 196; 261; 355; 353, 355; 417; —. *Grusz 173.7* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.8* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.9* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.10* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.12* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.13* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.15* (DUKE): N31.959 W109.212, Paradise; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.16* (DUKE): N31.959 W109.212, Paradise; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.16* (DUKE): N31.959 W109.212, Paradise; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.17* (DUKE): N31.959 W109.212, Paradise; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 173.14* (DUKE): N31.959 W109.212, Paradise; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 174.1* (DUKE): N31.363 W110.298; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 174.2* (DUKE): N31.363 W110.298; 182; 197, 200; 193; 261; 355; 353, 355; —; —. *Grusz 175.1* (DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175.2* (DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175.4* (DUKE): N31.716 W110.77; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175b.1* (DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175b.2* (DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175b.3* (DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 175b.4*

(DUKE): N31.716 W110.77, Gardner Canyon; 182; 200; 193, 196; 261; 345, 355; 351, 353, 355; 417, 419; —. *Grusz 177.1* (DUKE): N32.354 W110.723, Bear Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 177.2* (DUKE): N32.354 W110.723, Bear Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 177.3* (DUKE): N32.354 W110.723, Bear Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 177.4* (DUKE): N32.354 W110.723, Bear Canyon; —; 193; 261; 355; 353, 355; 417; —. *Grusz 177.5* (DUKE): N32.354 W110.723, Bear Canyon; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 178.1* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 178.2* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 178.3* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 178.4* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200; 193; 261; 355; 353, 355; 417; —. *Grusz 178.5* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Grusz 178.6* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Grusz 178.7* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Grusz 178.8* (DUKE): N32.47822 W110.72094, Mt. Lemmon Road; 197, 200, 203; 193, 196; 261; 355, 359; 353, 355; 417; —. *Manning 969* (SRSC): N30.545 W103.856; Fort Davis; 182; 200; 190, 193; 261; 355; 351, 353, 355; 411; 196. *Metzgar 128* (DUKE): N32.09 W108.974; 197, 200; 193, 196; 261, 270; 347, 355, 363; —; —; —. *Rothfels 3066* (DUKE): N21.597 W100.757; 182, 185; 194, 197; 193, 196; 258, 261, 267; 355; 353; 409, 415; 184, 196. *Schuettpelz 440* (DUKE): N; —; 193, 196; 261; —; 353, 355; —; 186, 199. *Schuettpelz 450* (DUKE): N33.33 W11.032; 200, 203; 193; 261, 279; 355; —; —; —. *Schuettpelz 450* (DUKE): N33.33 W11.032; 200, 203; 193; 261, 279; 355; —; —; —. *Schuettpelz 458* (DUKE): N31.437 W110.316; Carr Canyon; —; —;

193; 261; 355; —; —; —. *Schuettpelz 498* (DUKE): N32.354 W110.72, Bear Canyon; 200; 193, 196; 261, 270; 347, 355; 353, 355; —; —. *Spencer & Atwood 1176* (UT): N30.373 W108.242; 182; 200; 193; 261; 344, 355; —; 411, 415; 196, 202. *Windham 97-015* (DUKE): N32.591 W110.721, Oracle; 197, 200, 203; 193; 261; 355; —; —; —. *Worthington 12682* (NY): N31.77 W108.43; 182; 197, 200; 193; 261, 270; 355; 351, 353, 355; 411; 184, 196. *Worthington 19958* (NY): N31.914 W107.72; 182; 197, 200; 193; 261, 270; 355; 353, 355; 409, 411, 415; 184, 196. *Worthington 20617* (UTEP): N31.7 W108.423; 182; 190, 200; 193; 261; 355; 351, 353, 355; 411; 184, 196. *Myriopteris covillei*: *Windham 343* (ASC); *n = 30*. *Myriopteris lendigera*: *Windham 304* (UT); *n = 60*. *M. lindheimeri*: *Windham & Yatskievych 426* (UT); *n = 2n = 90*. *M. windhamii*: *Yatskievych 84-08* (IND, UT); *n = 2n = 90*. *M. wootonii*: *Windham & Yatskievych 266* (UT); *n = 2n = 90*. *M. yavapensis*: *Windham 202* (ASC, ASU, US, UT; type collection); *n = 2n = 120*.

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Refereed Publications

11. Grusz, A. L., M. D. Windham, G. Yatskievych, L. Huiet, G. J. Gastony, and K. M. Pryer. (accepted). Patterns of diversification in the xeric-adapted fern genus *Myriopteris* (Pteridaceae). *Systematic Botany*.

10. Grusz, A. L. and D. W. Freshwater. 2014. Studies of Costa Rican Gelidiales (Florideophyceae): II. Two Pacific taxa including *Gelidium microglossum* sp. nov. *Pacific Science* 68: 97–110.

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5. Pryer, K. M., E. Schuettpelz, L. Huiet, A. L. Grusz, C. J. Rothfels, T. Avent, D. Schwartz, and M. D. Windham. 2010. DNA barcoding exposes case of mistaken identity in the fern horticultural trade. *Molecular Ecology Resources* 10: 979–985.

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2. Schuettpelz, E. A. L. Grusz, M. D. Windham, and K. M. Pryer. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. *Systematic Botany* 33: 621–629.

1. Rothfels, C. J., M. D. Windham, A. L. Grusz, G. J. Gastony, and K. M. Pryer. 2008. Toward a monophyletic *Notholaena* (Pteridaceae): resolving patterns of evolutionary convergence in xeric-adapted ferns. *Taxon* 57: 712–724.